

**UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL  
UFRGS**

**PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA  
CELULAR E MOLECULAR - PPGBCM**

**Carolina Baldisserotto Comerlato**

**Probiótico LAB18S: um estudo genômico,  
proteômico e das interações com os  
prebióticos e com o microbioma intestinal  
humano.**

**Porto Alegre**

**2020**

**Carolina Baldisserotto Comerlato**

**Probiótico LAB18S: um estudo genômico,  
proteômico e das interações com os  
prebióticos e com o microbioma intestinal  
humano.**

Tese submetida ao Programa de Pós-Graduação em  
Biologia Celular e Molecular da Universidade Federal  
do Rio Grande do Sul como requisito para a obtenção  
do grau de Doutor.

Orientador: Prof. Dr. Adriano Brandelli

**Porto Alegre**

**2020**

## **INSTITUIÇÕES E FONTES FINANCIADORAS**

### **Instituições:**

Universidade Federal do Rio Grande do Sul (UFRGS);

Laboratório de Bioquímica e Microbiologia Aplicada – ICTA (UFRGS);

Centro Nacional de Pesquisa em Energia e Materiais (CNPEM);

Laboratório Nacional de Biociências (LNBio);

University of Ottawa;

Ottawa Institute of Systems Biology - Dr. Figeys' Lab.

### **Fontes financiadoras:**

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) -  
408864/2016-8;

Programa de Bolsas da Coordenação de Aperfeiçoamento de Pessoal de Nível  
Superior - Brasil (CAPES);

Emerging Leaders in the Americas Program (ELAP) – Canadá.

*Dedico esse trabalho aos meus pais,*

*Paulo e Lilhane.*

*Que me ensinaram o caminho da honestidade e da persistência.*

## AGRADECIMENTOS

A Deus, pai de amor e bondade, por me oferecer tantas oportunidades de crescimento moral e intelectual.

Ao meu orientador, Prof. Adriano Brandelli, pela oportunidade, incentivo e ensinamentos. Sou grata pela confiança que me dedicou e pela paciência de sempre.

Ao Prof. Daniel Figeys por aceitar me co-orientar na última parte desse estudo e por ter me dado essa oportunidade. Assim como agradeço ao Xu Zhang, Krystal e Mila não só por todo apoio técnico, mas também pelo carinho em que me acolheram no laboratório e no Canadá.

Aos meus queridos colegas de laboratório que compartilharam comigo tanto dias bons quanto ruins, que ouviram meus desabafos e que me incentivaram a continuar. Obrigada por compartilharem comigo seus conhecimentos e por terem me amparado em tantas etapas do meu trabalho.

À Nathi, Maria, Luís, Pri e Cristian por me recepcionarem tão bem no início do doutorado e por terem sido verdadeiros amigos. Levo vocês no coração.

A meus pais, Paulo e Lilhane, que sempre dedicaram tantos cuidados a mim abdicando muitas vezes deles mesmos. Obrigada pelo exemplo de honestidade, pelo amor incondicional e por terem guiado meus passos para o caminho correto desde sempre. Vocês sempre me ensinaram que o conhecimento é o único bem que ninguém poderia me tirar e essa tese é com certeza dedicada a vocês.

A minha irmã Ana que mesmo do outro lado do oceano sempre se preocupou comigo. Mana, és um exemplo de dedicação e coragem, te amo muito.

As minhas grandes amigas Fefe, Vane e Eve pelo carinho nesses muitos anos de amizade e por terem me dado tanto apoio durante essa caminhada. Sem vocês na minha vida com certeza ela seria mais difícil.

Aos amigos que fiz no Canadá, Fellipe, Jonathan, Luís, Liliam e Laurinha por todos os momentos únicos que vive, pela compreensão e convivência nesses seis meses. Obrigada por terem sido minha família canadense.

Aos queridos amigos Vaniza e Diomar por serem meus pais em Porto Alegre e me ensinarem tanto sobre a vida e sobre ser uma pessoa melhor.

## SUMÁRIO

<b>ABREVIATURAS .....</b>	<b>08</b>
<b>LISTA DE FIGURAS E DE TABELAS.....</b>	<b>09</b>
<b>RESUMO.....</b>	<b>10</b>
<b>ABSTRACT .....</b>	<b>11</b>
<b>1. INTRODUÇÃO .....</b>	<b>12</b>
1.1 Probióticos .....	12
1.1.1 Definição .....	12
1.1.2 Critérios de Seleção e Segurança .....	13
1.2 Prebióticos.....	17
1.2.1 Definição .....	17
1.2.2 Frutooligossacarídeo (FOS) .....	18
1.2.3 Galactooligossacarídeo (GOS) .....	20
1.3 Simbióticos .....	21
1.4 Mecanismos de ação dos probióticos e prebióticos .....	22
1.5 Importância clínica dos probióticos e prebióticos .....	26
1.5.1 Atividade Antipatogênica dos probióticos .....	27
1.5.2 Atividade Antidiabética .....	28
1.5.3 Atividade Antiobesidade .....	29
1.5.4 Atividade Anticâncer .....	30
1.5.5 Doenças intestinais .....	32
<b>2. OBJETIVOS .....</b>	<b>35</b>
2.1 Objetivo geral.....	35
2.2 Objetivos específicos.....	35
<b>3. DESCRIÇÃO DOS ARTIGOS CIENTÍFICOS .....</b>	<b>36</b>
ARTIGO CIENTÍFICO 1 .....	38
ARTIGO CIENTÍFICO 2 .....	65
ARTIGO CIENTÍFICO 3 .....	136
ARTIGO CIENTÍFICO 4 .....	192
<b>4. DISCUSSÃO .....</b>	<b>209</b>
<b>5. CONCLUSÕES.....</b>	<b>217</b>
<b>6. REFERÊNCIAS BIBLIOGRÁFICAS .....</b>	<b>219</b>

<b>7. APÊNDICES .....</b>	<b>235</b>
<b>9. CURRÍCULO VITAE .....</b>	<b>238</b>

## ABREVIATURAS

DNA: ácido desoxirribonucleico

OMS: Organização Mundial de Saúde

FAO: *Food and Agriculture Organization*

QPS: *Qualified Presumption of Safety*

EFSA: *European Food Safety Authority*

FDA: *Food and Drug Administration*

GRAS: *Generally recognized as safe*

ACNFP: *New Foods and Processes Advisory Committee*

CIM: Concentração inibitória mínima

MLST: Tipagem de sequências Multilocus

FOS: frutooligossacarídeo

GOS: galactooligossacarídeo

HMO: oligossacarídeos do leite humano

GALT: leucócitos nos tecidos linfóides associados ao intestino

IgA: Imunoglobulina A

SNC: sistema nervoso central

AGCCs: ácidos graxos de cadeia curta

DM2: diabetes mellitus tipo 2

ADI: arginina deiminase

DII: doença inflamatória intestinal

SII: síndrome do intestino irritável



## **LISTA DE FIGURAS**

**FIGURA 1.** Proposta de esquema de decisão para a avaliação de segurança de candidatos probióticos de *Enterococcus* para aplicações em alimentos / rações

**FIGURA 2.** Principais mecanismos de ação dos probióticos

## **LISTA DE TABELAS**

**TABELA 1.** Métodos recomendados para a avaliação de segurança de *Enterococcus* (espécies não-QPS).

## RESUMO

A microbiota intestinal exerce um papel fundamental na saúde humana e o aumento de evidências apoia o papel benéfico dos microrganismos probióticos na manutenção da saúde intestinal. Neste estudo foram abordados estudos ômicos para melhor compreender a interação do probiótico com o hospedeiro. O genoma do probiótico *Enterococcus durans* LAB18S foi avaliado através do sequenciamento e diversos genes potencialmente associados a propriedades probióticas, tais como capacidade de adesão, viabilidade a pH baixo, tolerância ao sal biliar, atividade antimicrobiana, utilização do substrato prebiótico e genes envolvidos no metabolismo do selênio. A fim de se verificar a relação simbiótica entre prebióticos e probióticos foram avaliadas a capacidade de carboidratos em estimular o crescimento e a diferença na expressão proteica do LAB18S cultivado em FOS, GOS e glicose. A análise proteômica mostrou que tanto FOS quanto GOS foram utilizados como fonte de carbono pela bactéria probiótica, além de estimularem o microrganismo a produzir diferentes proteínas e / ou diferentes níveis de expressão proteica. O isolado LAB18S foi, então, cultivado em condições aeróbias e anaeróbias usando FOS e GOS, a fim de estimar a ação do oxigênio na expressão de proteínas. O proteoma e o secretoma da célula inteira foram analisados e os resultados mostraram que o LAB18S em simbiose com FOS apresentou resultados proteômicos mais promissores. Enzimas clinicamente importantes para o tratamento de câncer, L-asparaginase e arginina desiminase, foram superexpressas quando o isolado foi cultivado em FOS. Além disso, a ausência de oxigênio induziu o probiótico a produzir proteínas relacionadas à multiplicação celular, integridade e resistência da parede celular e desintoxicação. Por fim, a interação das proteínas secretadas pelo probiótico *E. durans* LAB18S em simbiose com FOS e GOS com o microbioma intestinal humano de um indivíduo saudável foi analisada através de uma cultura *ex vivo*. A metaproteômica mostrou alterações na expressão proteica do microbioma e os resultados revelaram que as comunidades microbianas mudaram com a presença de diferentes concentrações do secretoma do LAB18S. Esses resultados sugerem que o probiótico em simbiose com os prebióticos possuem efeitos benéficos atuando na modulação das atividades funcionais da microbiota intestinal humana.

**Palavras-chave:** *Enterococcus durans*, probiótico, prebiótico, FOS, GOS, microbioma intestinal humano, genômica, proteômica.

## ABSTRACT

Intestinal microbiota plays a key role in human health and the growing evidence supports the beneficial role of probiotic microorganisms in maintaining intestinal health. This study performed omic studies to better understand the interaction of the probiotic with the host. The probiotic genome of *Enterococcus durans* LAB18S was evaluated by sequencing and several genes potentially associated with probiotic properties, including adhesion capacity, low pH viability, bile salt tolerance, antimicrobial activity and prebiotic substrate utilization were verified, as well as genes involved in selenium metabolism. In order to verify evidence of the symbiotic relationship between prebiotics and probiotics, the ability of carbohydrates to stimulate growth and to evaluate the difference in protein expression of LAB18S grown in FOS, GOS and glucose was evaluated. Proteomic analysis showed that both FOS and GOS were used as carbon sources by probiotic bacteria, besides stimulating the microorganism to produce different proteins and / or different levels of protein expression. LAB18S isolate was then cultivated under aerobic and anaerobic conditions using FOS and GOS in order to evaluate oxygen action on protein expression. The whole cell proteome and secretome were analyzed and the results showed that LAB18S in symbiosis with FOS has more promising proteomic results. Clinically important enzymes for the treatment of cancer, L-asparaginase and arginine deiminase, were overexpressed when the isolate was grown in FOS. In addition, the absence of oxygen induced the probiotic to produce proteins related to cell multiplication, cell wall integrity and resistance, and detoxification. Finally, the interaction of proteins secreted by probiotic *E. durans* LAB18S in symbiosis with FOS and GOS with the human intestinal microbiome of a healthy individual was analyzed by *ex vivo* culture. Metaproteomics evaluated changes in protein expression of the microbiome and the results revealed that microbial communities changed with the presence of different concentrations of the LAB18S secretome. These results suggest that probiotic in symbiosis with prebiotics have beneficial effects acting on the modulation of functional activities of the human intestinal microbiota.

**Keywords:** *Enterococcus durans*, probiotic, prebiotic, FOS, GOS, human intestinal microbiome, genomic, proteomic.

# 1 INTRODUÇÃO

## 1.1 PROBIÓTICOS

### 1.1.1 Definição

O termo probiótico foi mencionado pela primeira vez no início da década de 50, por Werner Kollath, para definir a importância das substâncias bioativas que se mostraram essenciais para o desenvolvimento de uma vida saudável (GASBARRINI *et al.*, 2016). Em 2000 o governo argentino solicitou à Organização das Nações Unidas para Alimentação e Agricultura (FAO) um painel de especialistas para avaliar as propriedades nutritivas e de saúde dos probióticos nos alimentos. A definição de probiótico manteve a essência das definições históricas oferecidas nas décadas anteriores e foi intencionalmente ampla, para abranger uma variedade maior de microrganismos, hospedeiros, benefícios e tipos de produtos (HILL *et al.*, 2014). Porém, esse conceito foi corrigido gramaticalmente em 2014, para a definição mais válida e aceita de probióticos, proposta pela FAO: “probióticos são microrganismos vivos que, quando administrados em quantidades adequadas, conferem um benefício à saúde do hospedeiro” (FAO / WHO, 2006).

Os primeiros conceitos definiam as funções probióticas por contribuírem beneficemente para o intestino do hospedeiro (PARKER, 1974) ou por equilibrar e melhorar as propriedades da microbiota endógena (HAVENAAR & HUIS, 1992). Atualmente, o conceito de probióticos evoluiu e a definição seria que os efeitos desses organismos não são apenas mediados pela microbiota, e sim que outros tipos de mecanismos estejam associados (SANDERS *et al.*, 2019). Essa ideia de que os probióticos funcionam de maneira que podem

agir além de somente afetar a microbiota endógena abre portas para uma gama mais ampla de possibilidades para incentivar a inovação no campo.

### **1.1.2 Critérios de seleção e segurança**

De acordo com a lista da *Qualified Presumption of Safety* (QPS) da *European Food Safety Authority* (EFSA), as espécies de *Enterococcus* não são recomendadas para essa lista (EFSA *et al.*, 2017). A QPS é uma suposição de segurança baseada em evidências razoáveis na qual os microrganismos são avaliados e no caso de não levantarem preocupações de segurança, recebem o “status QPS”. Além do status QPS, a *Food and Drug Administration* (FDA) implementou uma série de requisitos designados *Generally recognized as safe* (GRAS) que devem ser seguidos para que um produto químico ou uma substância adicionada a alimentos sejam considerados seguros. O gênero *Enterococcus* ainda não obteve o status GRAS (HUYS *et al.*, 2013), mas alguns membros são utilizados como probióticos e na produção de aditivos alimentares para prevenir a diarreia ou melhorar o crescimento em animais (FRANZ *et al.*, 2011). Os avanços mais recentes em epidemiologia molecular baseado em *fingerprints* moleculares, estudos fenotípicos e análises genômicas completas forneceram evidências adicionais de que os isolados nosocomiais de *Enterococcus* são genotipicamente diferentes dos isolados comensais (MONTEALEGRE *et al.*, 2016; BONACINA *et al.*, 2017; JUNG *et al.*, 2017). Por exemplo, a espécie *E. faecium* foi subdividida em três diferentes clados: o clado A1 associado ao hospital, raramente encontrado em indivíduos saudáveis; o clado A2 associado a animais; e o clado B associado à comunidade, comumente encontrado em indivíduos saudáveis e raramente causa infecções (MONTEALEGRE *et al.*, 2016).

Embora outras evidências sejam necessárias, os avanços descritos acima apoiam o pedido de novas recomendações sobre a estrutura legislativa probiótica, a fim de distinguir entre cepas de *Enterococcus* seguras e potencialmente prejudiciais. No entanto, organizações como a EFSA, o *New Foods and Processes Advisory Committee*, ACNFP e a *Food Standards Agency* permitiram o uso de certas cepas de enterococos como aditivo alimentar e suplementos com base em uma cuidadosa avaliação (ACNFP, 1996; FRANZ *et al.*, 2011; LAUKOVÁ, 2011; EFSA, 2012a). Para a avaliação de novos probióticos candidatos pela EFSA, o genoma completo da cepa deve estar disponível (BRODMANN *et al.*, 2017). Atualmente, isolados de *E. faecium* e *E. faecalis* são os únicos utilizados como probióticos ou aditivos alimentares (FRANZ *et al.*, 2011). O uso de outras espécies de enterococos está sujeito a pouca ou nenhuma regulação, apesar do crescente número de estudos que elucidam o potencial probiótico de algumas espécies, como *E. mundtii*, *E. durans* e *E. hirae* (NAMI *et al.*, 2014; PIENIZ *et al.*, 2014, GUPTA & TIWARI, 2015, VAN ZYL *et al.*, 2016).

A primeira instrução relacionada à segurança da linhagem probiótica é a identificação do grupo ou classe de risco a que o microrganismo pertence com sua respectiva referência (*Center for Disease Control / EUA, European Food Safety Authority (EFSA), OMS*). O grupo de risco orientará sobre os principais problemas de segurança relacionados ao microrganismo. O gênero *Enterococcus* é alocado no grupo de risco 2, que inclui microrganismos que abrigam fatores de virulência (EC, 2000). Assim, eles podem atuar como um reservatório para disseminação da resistência a antibióticos e genes de virulência através da cadeia alimentar (JAHAN *et al.*, 2015). A suscetibilidade à ampicilina e aos fatores e marcadores de virulência IS16, *hylEfm* e *esp* são considerados relevantes para a avaliação da segurança (EFSA, 2012a). Os métodos recomendados para a avaliação de segurança do *Enterococcus* estão resumidos na Tabela 1.

**Tabela 1.** Métodos recomendados para a avaliação de segurança de *Enterococcus* (espécies não-QPS).

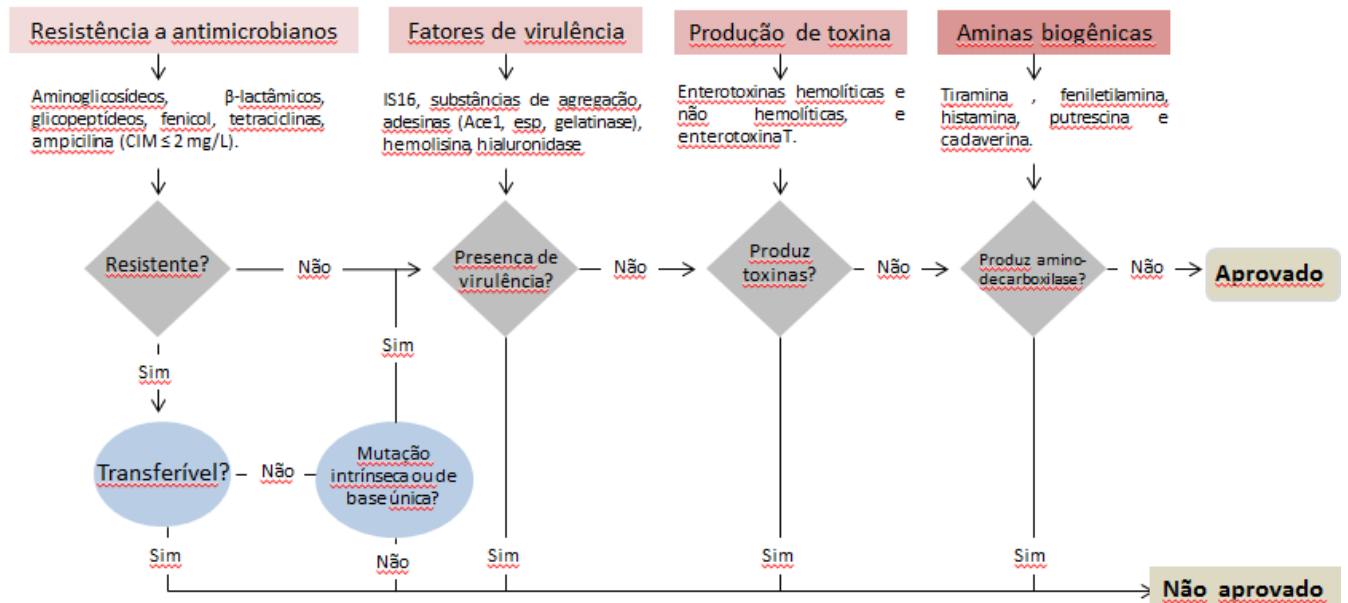
Testes	Métodos	Referências
<b>TESTES DE SUSCEPTIBILIDADE A ANTIMICROBIANOS</b>		
<b>Suscetibilidade a antimicrobianos de relevância clínica:</b> (ampicilina, vancomicina, gentamicina, canamicina, estreptomina, eritromicina, clindamicina, tetraciclina, cloranfenicol)	Concentrações inibitórias mínimas (CIMs) (mg / L ou µg / mL; Teste de Suscetibilidade: EUCAST / CLSI, padrão ISO)	EFSA, 2012b; LAULUND <i>et al.</i> , 2017
<b>DETECÇÃO DE MARCADORES DE VIRULÊNCIA ASSOCIADOS A ISOLADOS HOSPITALARES</b>		
<i>IS16</i>	PCR	WERNER <i>et al.</i> , 2011
<i>Esp</i>	Técnicas de hibridização	HENDRICKX <i>et al.</i> , 2007
<i>hylEfm</i>	PCR	RICE <i>et al.</i> , 2003
<b>OUTRAS CONSIDERAÇÕES</b>		
*Avaliação genotípica	Tipagem de sequências Multilocus (MLST) DNA fingerprint PCR	TEO <i>et al.</i> , 2011
<b>Operons da Vancomicina</b> ( <i>vanA</i> , <i>vanB</i> , <i>vanC</i> , <i>vanD</i> , <i>vanE</i> , <i>vanG</i> , <i>vanM</i> , <i>vanL</i> , <i>vanN</i> )		
<b>Genes de adesão à superfície</b> ( <i>efaAfs</i> , <i>efaAfm</i> )		EATON & GASSON, 2001
<b>Genes de Citolisina</b> ( <i>cylLL</i> , <i>cylLs</i> , <i>cylM</i> , <i>cylB</i> , <i>cylA</i> )		
<b>Gene de agregação de proteínas</b> ( <i>agg</i> )		
<b>Metalopeptidase extracelular</b> ( <i>gelE</i> )		NAKAYAMA <i>et al.</i> , 2002
*Avaliação fenotípica		
<b>Atividade hemolítica</b>	Ensaio de atividade hemolítica em placas de ágar sangue com 5% de sangue de ovelha ou cavalo	SEMEDO <i>et al.</i> , 2003
<b>Hidrólise da gelatina</b>	Ensaio de atividade da gelatinase em placas de ágar Todd-Hewitt (TH) contendo 3% de gelatina.	QIN <i>et al.</i> , 2000
<b>DETECÇÃO DE AMINAS BIOGÊNICAS</b>		
Histamina		
Putrescina	Cromatografia líquida de alta pressão HPLC	EFSA Panel on Biological Hazards, 2011
Feniletilamina		
Cadaverina		
Detecção de microorganismos positivos para aminoácidos descarboxilase	PCR quantitativo em tempo real	LANDETE <i>et al.</i> , 2007
<b>PRODUÇÃO DE TOXINAS</b>		

<b>Potencial citotóxico</b>	Teste de citotoxicidade em células Vero	LAULUND <i>et al.</i> , 2017
Genoma Completo (quando disponível)	Sequenciamento de nova geração (NGS)	EFSA, 2012a

Fonte: adaptado de HANCHI *et al.*, 2018.

A segurança é avaliada através de testes microbiológicos (hemólise, produção de gelatinase, determinação de antibiograma), testes moleculares (genes de resistência a antimicrobianos e fatores de virulência) e detecção da produção de toxinas (para cepas não-QPS). Finalmente, uma avaliação da falta de infectividade pela cepa candidata em animais imunocomprometidos acrescentaria uma medida de confiança na segurança (FAO / WHO, 2006). A Figura 1 ilustra um esquema de decisão proposto com base nos regulamentos da EFSA para a avaliação de segurança de candidatos probióticos para enterococos que levam a aplicações de alimentos / rações.

**Figura 1.** Proposta de esquema de decisão para a avaliação de segurança de candidatos probióticos de *Enterococcus* para aplicações em alimentos / rações.



Fonte: adaptado do painel da EFSA em BIOLOGICAL HAZARDS (2011), EFSA (2012a) e LAULUND *et al.* (2017).

## 1.2 PREBIÓTICOS



### 1.2.1 Definição

O conceito de prebiótico é mais recente do que o de probiótico sendo, inicialmente, proposto por GIBSON & ROBERFROID (1995). A atual definição científica de prebiótico foi proposta em 2016 pela *International Scientific Association for Probiotics and Prebiotics* (ISAPP): “um substrato que é utilizado seletivamente pelos microrganismos hospedeiros e confere um benefício à saúde” (GIBSON *et al.*, 2017). Assim, o conceito abrange três partes essenciais: uma substância, um efeito fisiológico e um mecanismo.

Substâncias prebióticas são predominantemente baseados em carboidratos, mas outras substâncias, como polifenóis e ácidos graxos poliinsaturados, podem exercer os mesmos efeitos, sendo a maioria utilizada em ingredientes de alimentos (GIBSON *et al.*, 2017). Os prebióticos mais comumente estudados são as fibras solúveis inulina, frutooligossacarídeos (FOS), galactooligossacarídeos (GOS) e, mais recentemente, oligossacarídeos do leite humano (HMOs). Esses compostos possuem estrutura química estável a variações de fatores como a temperatura e o pH (YOUNIS *et al.*, 2015). Além disso, esses são resistentes à ação de enzimas hidrolíticas e são de fácil fermentação pelos microrganismos endógenos intestinais promovendo o crescimento de probióticos, como *Bifidobacterium* sp. e *Lactobacillus* sp. (HUTKINS *et al.*, 2016). Assim como os probióticos, os prebióticos também possuem uma atuação que vai muito além da microbiota intestinal, possuindo atuação em doenças como a obesidade (HUME *et al.*, 2017), osteoporose, doenças cardiovasculares e diabetes tipo 2 (FLORES-MALTOS *et al.*, 2016).

## 1.2.2 Frutooligossacarídeo (FOS)

O frutooligossacarídeo é um dos prebióticos mais estudados (BALI *et al.*, 2015). O FOS, além de possuir propriedades prebióticas, ajuda a reduzir o nível de colesterol, inibindo o crescimento de bactérias nocivas e melhorando a absorção de minerais no intestino. Esse composto é formado por uma fração de glicose seguida de uma de frutose variando de 2 a 60 frações por ligações glicosídicas dos tipos  $\beta$  (2-1) ou  $\beta$  (2-6) (CASCI & RASTALL, 2006; CAMPBELL *et al.*, 1997). Esse composto, assim como a maioria dos prebióticos, não é digerido no intestino delgado, mas é metabolizado no ceco em ácido graxo de cadeia curta e L-lactato, além de outras moléculas bioativas benéficas para a saúde humana (HUTKINS *et al.*, 2016).

Dentre os inúmeros benefícios de FOS estão o aumento da absorção de minerais como  $Mg^{+2}$  e  $Ca^{+2}$  e a diminuição do nível de ácidos graxos no intestino (MONTET & RAY, 2016). O aumento da absorção de cálcio, como resultado da ingestão de FOS, foi demonstrado, o que potencialmente aumenta a densidade mineral óssea (COXAM, 2007). A biossíntese de frutanos ocorre em plantas e fungos, além de bactérias, e o mercado comercial dos prebióticos está, atualmente, dominado por inulina (polímero de frutose), FOS, GOS e IMO. A inulina é um polímero da frutose que são ligados por ligações  $\beta$  (2-1), com uma unidade de glicose unida terminalmente através da ligação  $\alpha$  (1-2). Enzimas denominadas inulinases clivam as ligações  $\beta$  (2-1) gerando o FOS (SILVA *et al.*, 2013). A inulina e o FOS são prebióticos extensivamente explorados e vários estudos sugerem que o uso de frutanos na dieta estimula mais efetivamente os microrganismos do intestino. O FOS é comercialmente usado como ingrediente alimentar e disponível no mercado sob vários

nomes comerciais, tais como Neosugar, NutraFlora®, Meioligo® e Actilight® (BALI *et al.*, 2006).

O FOS possui algumas propriedades interessantes para a indústria de alimentos como a solubilidade em água, higroscopia, reduzido valor calórico, é um prebiótico encontrado no meio-ambiente e o sabor é bastante semelhante à sacarose, porém aproximadamente 30% menos doce (ROBERFROID & SLAVIN, 2000). Durante décadas, os pesquisadores estão envolvidos em estratégias para produção de FOS. Frutanos foram extraídos com sucesso de fontes vegetais como *Cichorium intybus* (chicória), *Allium cepa* (cebola) e *Helianthus tuberosus* (alcachofra de Jerusalém) para produção de FOS. No entanto, o isolamento e o processamento de FOS a partir dessas fontes naturais são caros e desafiadores (ITAYA *et al.*, 2007).

O FOS é uma fonte de carbono preferencial para probióticos. Dentre as propriedades desse oligossacarídeo estão o aumento do crescimento da microbiota intestinal benéfica e o combate a organismos patogênicos. Além disso, a ingestão regular e adequada de FOS propicia efeitos benéficos em caso de problemas associados a distúrbios gastrointestinais, obesidade, diarreia, osteoporose, doenças ateroscleróticas, cardiovasculares e diabetes tipo 2 (FLORES-MALTOS *et al.*, 2016). FOS é recomendado para pacientes que sofrem de diarreia aguda, que é um problema comum em crianças, além de estimular a absorção de água e eletrólitos na mucosa intestinal (GUO *et al.*, 2016; PATEL & GOYAL, 2012). Muitas vezes se faz a combinação de diferentes prebióticos para obter melhores resultados, visto que um estudo investigou que a mistura de frutooligossacarídeo e galactooligossacarídeo era útil no controle de sintomas da fenilcetonúria em lactentes (PATEL & GOYAL, 2012). O consumo de FOS também mostrou que esse atua na redução de genotoxinas e da enzima  $\beta$ -glucuronidase, que gera carcinogênicos no intestino e, portanto, regulam a incidência de

câncer de cólon (HUTKINS *et al.*, 2016; DOMINGUEZ *et al.*, 2014). O FOS também é útil em controle de doenças inflamatórias do intestino, como doença de Crohn e doença de colite ulcerativa (CHEN *et al.*, 2016). Os frutooligossacarídeos são aditivos alimentares funcionais prebióticos do status GRAS. Portanto, atualmente está incluído em fórmulas e produtos alimentícios para bebês e são utilizados para melhorar o perfil de ácidos graxos livres do queijo (CELLIGOI *et al.*, 2015).

### 1.2.3 Galactooligossacarídeo (GOS)

Galactooligossacarídeos são oligossacarídeos contendo galactose com ligações  $\beta$  (1-3) e  $\beta$  (1-4) entre os monômeros. O GOS pode ser sintetizado utilizando monossacarídeos ou dissacarídeos como substrato (por exemplo, lactose) e um biocatalisador, a  $\beta$ -galactosidase. A reação para a produção de GOS é chamada de reação de transgalactosilação e pode ocorrer apenas na presença de um catalisador (VERA *et al.*, 2016).

A sua propriedade bifidogênica é comprovada sendo que possui uma ação mais acentuada sobre *Bifidobacterium sp.* (MONTEAGUDO- MERA *et al.*, 2016). Além disso, é resistente a enzimas digestivas e fermentado por microrganismos específicos. Esse prebiótico também não é digerível e pode tolerar grandes variações de temperatura e pH e, portanto, é o aditivo preferido em produtos alimentícios (VERA *et al.*, 2016).

O GOS é um prebiótico de origem animal, visto que o leite de vaca é rico em lactose cuja isomerização e transglicosilação formam lactulose e GOS, respectivamente. As galactosidases de *Lactobacillus reuteri* L103 e L461 foram consideradas como biocatalisadores adequados para converter a lactose em GOS (SPLECHTNA *et al.*, 2006). Esse composto também é usado no leite de bebês como ingredientes funcionais para efeitos

promotores da saúde. Baixos níveis de GOS (0,24 g / 100 ml) ajudarão a proliferar a microbiota do leite materno. A mistura de 90% de galactooligossacarídeos de cadeia curta, juntamente com 10% de frutooligossacarídeos de cadeia longa, é utilizada no leite humano para mimetizar a distribuição do tamanho molecular dos oligossacarídeos naturais (BHATIA *et al.*, 2015). A adição de GOS aos iogurtes produz consistências mais suaves e cremosas. Além disso, a microbiota do iogurte não é capaz de quebrar esse prebiótico e, portanto, o GOS chega ao intestino não digerido (SANGWAN *et al.*, 2011). A produção comercial de GOS foi estabelecida usando  $\beta$ -galactosidase derivada de *Aspergillus oryzae* (MATSUMOTO *et al.*, 1989) e *Cryptococcus leurentii* OKN-4 (OHTSUKA *et al.*, 1988).

### 1.3 SIMBIÓTICOS

O desenvolvimento da pesquisa microbiana levou à formação dos simbióticos que nada mais são que a fusão de probióticos e prebióticos e possuem a função de melhorar a sobrevivência e a implantação de microrganismos vivos no intestino (GIBSON & ROBERFROID, 1995). Os benefícios sinérgicos dos simbióticos são promovidos de forma mais eficiente quando ambos os probióticos e prebióticos trabalham juntos no sistema vivo. Existem evidências científicas crescentes de que relação simbiótica entre prebióticos e probióticos contribui significativamente para a saúde. O interesse comercial em alimentos funcionais contendo simbióticos aumentou devido à consciência dos benefícios para a saúde intestinal, prevenção de doenças e terapia. Atualmente, a pesquisa nesta área é focada no desenvolvimento de novos alimentos promotores da saúde, bem como na seleção de novas culturas demonstrando uma maior capacidade de colonizar o intestino humano, juntamente com a capacidade de digerir novas formas de prebióticos. Algumas das composições

simbióticas mais comuns atualmente são com inulina, FOS e GOS, como prebióticos e bifidobactérias (do gênero *Bifidobacterium* sp.) como probiótico (TUFARELLI & LAUDADIO, 2016).

Muitos estudos mostraram que vários efeitos benéficos dos probióticos, prebióticos e simbióticos são muito mais eficazes quando administrados em conjunto (SCAVUZZI *et al.*, 2014, OOI & LIONG, 2010). Portanto, estudos voltados para o desenvolvimento de novas combinações entre esses compostos são vitais para explorar mais possibilidades de melhorar a saúde nutricional e benefícios clínicos.

#### **1.4 MECANISMOS DE AÇÃO DOS PROBIÓTICOS E PREBIÓTICOS**

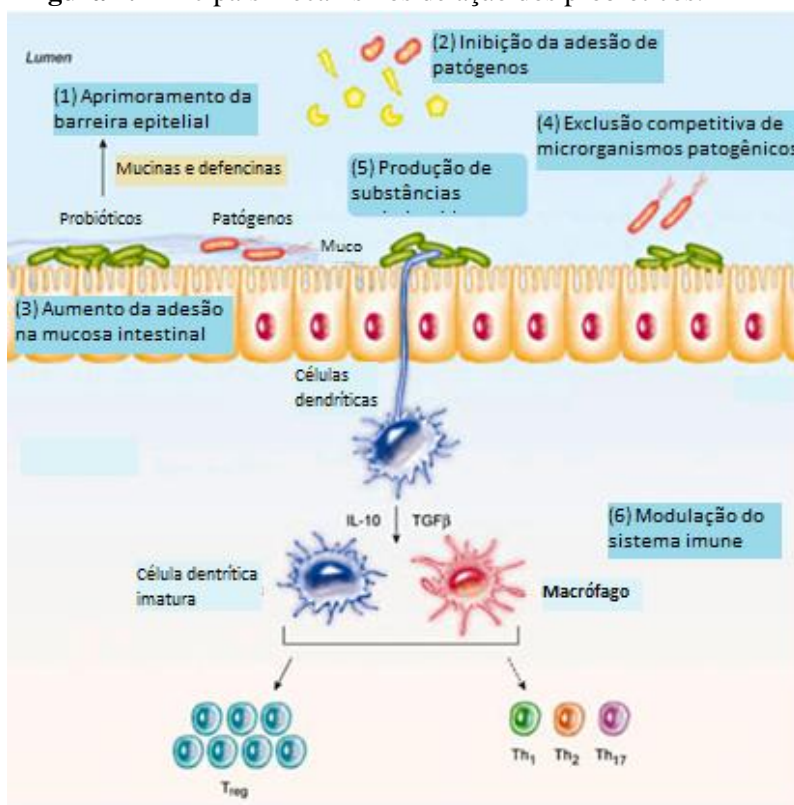
Uma pergunta comum é de que maneira os probióticos funcionam? A heterogeneidade da microbiota intestinal e as diferentes formulações de produtos dessa natureza mostram que não existe uma resposta única. Por esse motivo, existem benefícios gerais atribuídos aos probióticos, com um mecanismo compartilhado para criar um ambiente intestinal mais favorável, e apoiar um trato digestivo e um sistema imunológico saudável (HILL *et al.*, 2014). Esta conclusão foi baseada em estudos de meta-análise de alta qualidade, relacionados à diarreia infecciosa, diarreia associada a antimicrobianos, motilidade intestinal, síndrome do intestino irritável e outras condições. Porém, não significa que os mecanismos sejam os mesmos para cada condição, nem que mecanismos exclusivos tenham sido provados. Há muitas maneiras do hospedeiro responder ao uso desses compostos (SEGERS & LEBEER *et al.*, 2014). Um aspecto intrigante das cepas probióticas é a capacidade de algumas conferirem efeitos distantes ao local de administração. Isto pode ocorrer através da transferência dos microrganismos, por exemplo, do intestino para as

glândulas mamárias de mulheres lactantes (ARROYO *et al.*, 2010). Ou, pode ser devido à produção de moléculas que são adsorvidas através do intestino (BARBONETTI *et al.*, 2013), ou que influenciam os compostos hospedeiros direta ou indiretamente.

No caso de locais onde os probióticos não tenham alcance, como o cérebro, as moléculas produzidas pelos microrganismos benéficos parecem atingir esse órgão através do sistema do nervo vago (LYTE, 2011; FORSYTHE *et al.*, 2014). Potencialmente, essa é a área mais intrigante da pesquisa com probióticos, já que se relaciona com o órgão que controla grande parte do que fazemos. Parece factível que mudar radicalmente a microbiota intestinal através do transplante ou administração de cepas probióticas produzindo fatores específicos, não só poderia alterar o humor e a memória (MESSAOUDI *et al.*, 2011), mas influenciar o que comemos e quando (TRINDER *et al.*, 2015), e quão lentamente ou rapidamente mudanças patológicas podem ocorrer.

Os probióticos afetam o ecossistema intestinal estimulando os mecanismos imunes da mucosa, interagindo com microrganismos comensais ou potencialmente patogênicos, gerando produtos metabólicos finais, como ácidos graxos de cadeia curta (AGCCs), e se comunicando com as células do hospedeiro através de sinais químicos (Figura 2). Estes mecanismos podem conduzir ao antagonismo de patógenos potenciais, a melhorar o ambiente intestinal, fortalecer a barreira intestinal, à regulação negativa da inflamação e à regulação positiva da resposta imune a desafios antigênicos (MUJAGIC *et al.*, 2017).

**Figura 2.** Principais mecanismos de ação dos probióticos.



Fonte: BERMUDEZ-BRITO *et al.*, 2012.

O aprimoramento da barreira epitelial através da ação de cepas probióticas foi observado, principalmente, por estudos em linhagens celulares em que várias cepas probióticas de *Lactobacillus* e *Bifidobacterium* demonstraram aumentar a expressão de proteínas do tipo *tight junction* (LA FATA *et al.*, 2018). Outra maneira pela qual as cepas probióticas podem melhorar a função de proteção da barreira é através da expressão positiva de genes de secreção de muco, reduzindo assim a ligação do patógeno às células epiteliais (YAN *et al.*, 2013). A inflamação da regulação descendente é também considerada como um fator que melhora a função de barreira (SANDERS *et al.*, 2019). É importante observar que, embora algumas cepas probióticas tenham a capacidade de melhorar a função de barreira, esse processo nem sempre ocorre em todas as coortes por razões ainda não totalmente compreendidas (STADLBAUER *et al.*, 2015).



O antagonismo através da produção de substâncias antimicrobianas e a competição com patógenos por adesão ao epitélio e por nutrientes são mecanismos que estão diretamente associados ao seu efeito sobre outros microrganismos. Esses mecanismos são importantes na profilaxia e no tratamento de infecções e na manutenção do equilíbrio da microbiota intestinal do hospedeiro. A capacidade das cepas probióticas de coagregarem pode levar à formação de uma barreira protetora que previne bactérias patogênicas da colonização do epitélio (SANDERS *et al.*, 2019). Bactérias probióticas podem ser capazes de aderir às células epiteliais, bloqueando assim os patógenos. Além disso, a adesão de microrganismos probióticos às células epiteliais pode desencadear uma cascata de sinalização, levando à modulação imunológica. Alternativamente, a liberação de alguns componentes solúveis pode causar uma ativação direta ou indireta (através de células epiteliais) de células imunológicas. Este efeito desempenha um papel importante na prevenção e tratamento de doenças contagiosas, bem como na inflamação crônica do trato digestório ou de parte dele. Os resultados de estudos *in vitro* indicam o papel de substâncias de baixo peso molecular produzidas por microrganismos probióticos (por exemplo, hidroperóxido e ácidos graxos de cadeia curta) na inibição da replicação de patógenos. Por exemplo, algumas bactérias podem ser capazes de produzir bacteriocinas, substâncias de baixo peso molecular (peptídeos antibacterianos), bem como as de alto peso molecular (bacteriocinas classe III) e alguns antibióticos (OELSCHLAEGGER *et al.*, 2010).

Os mecanismos de ação dos prebióticos têm por objetivo principal estimular a composição da microbiota intestinal e sua atividade metabólica. É provável que fornecer uma fonte de energia que apenas espécies específicas do microbioma intestinal possam utilizar tenha um impacto maior na composição e no metabolismo do mesmo em comparação com outros fatores. Alguns desses outros fatores que podem ser estimulados pelos

prebióticos são: modulação do metabolismo lipídico, capacidade de absorção aumentada do cálcio, efeito sobre o sistema imunológico e modificação da função intestinal (VAN LOO *et al.*, 2005). A estrutura molecular dos prebióticos determina seus efeitos fisiológicos e os tipos de microrganismos que são capazes de usá-los como fonte de carbono e energia no intestino (GIBSON *et al.*, 2017). Foi demonstrado que, apesar da variedade de carboidratos que exibem a atividade prebiótica, o principal efeito da sua administração é uma contagem aumentada de bactérias benéficas (GASBARRINI *et al.*, 2016). O mecanismo de ação de prebióticos em funções imunológicas permanece não compreendido. Alguns modelos de mecanismos de ação têm sido propostos, tais como: regulação da ação de enzimas hepáticas lipogênicas, influência no aumento da produção de ácidos graxos de cadeia curta, como o ácido propiônico, modulação da produção de mucina, aumento na contagem de linfócitos e / ou leucócitos nos tecidos linfoides associados ao intestino (GALTs) e no sangue periférico e aumento da secreção de IgA pelos GALTs estimulando a função fagocítica dos macrófagos intra-inflamatórios (DE PRETER *et al.*, 2011).

## **1.5 IMPORTÂNCIA CLÍNICA DOS PROBIÓTICOS E PREBIÓTICOS**

O uso de probióticos para benefícios clínicos à saúde é uma área que vem crescendo na pesquisa, mas que ainda precisa ser mais bem explorada. São exemplos de algumas das principais propriedades dos probióticos: atividades antipatogênicas, antidiabéticas, antiobesidade, anti-inflamatórias, anticâncer, antialérgicas e angiogênicas, e seus efeitos sobre o cérebro e sistema nervoso central (SNC).

### 1.5.1 Atividade antipatogênica dos probióticos

Essa é uma das principais atividades benéficas dos probióticos, pois os peptídeos bioativos produzidos por esses microrganismos não perturbam a composição da complexa população microbiana intestinal, como é o caso dos antimicrobianos. Em um estudo recente de ISEPPI *et al.* (2019), os isolados de *Lactobacillus paracasei* ssp. *paracasei* LP5 e *Lactobacillus brevis* LP9 apresentaram atividade antimicrobiana significativa contra *S. agalactiae* e *L. monocytogenes*. Sendo verificada a presença das bacteriocinas BacLP5 e BacLP9 pertencentes a bacteriocinas de classe II. Outros estudos evidenciaram a influência dos probióticos na sobrevivência de alguns isolados patogênicos e observaram a atividade de ácidos graxos de cadeia curta (AGCCs). Os AGCCs ajudaram a equilibrar o pH no lúmen do cólon, o que é fundamental na expressão de numerosas enzimas bacterianas e no metabolismo de compostos bioativos e carcinogênicos no intestino (TEJERO-SARINENA *et al.*, 2013; KAREEM *et al.*, 2014). ISLAM (2016) sugere uma ampla variedade de compostos com atividade antimicrobiana, produzidos por probióticos, como bacteriocinas, ácidos graxos, acetaldeídos, peróxido de hidrogênio (H<sub>2</sub>O<sub>2</sub>) e peptídeos. Bacteriocinas e peptídeos possuem uma ação na membrana das células alvo, aumento a sua permeabilidade e despolarizando o seu potencial de membrana e, assim, levando a ruptura e morte celular (SIMOVA *et al.*, 2009). Os ácidos orgânicos, como ácidos lático e acético, também podem ser eficientes contra patógenos, visto que eles reduzem o pH do meio e inibem o crescimento de muitos microrganismos (KAREEM *et al.*, 2014). Além de produzir compostos bioativos que afetam diretamente os patógenos, os probióticos também estimulam as vias de defesa antipatogênicas do hospedeiro, como por exemplo, o estímulo ou ativação da via envolvida na produção de defensinas que são peptídeos antimicrobianos catiônicos produzidos em

vários tipos de células, incluindo células de Paneth nas criptas do intestino delgado e células epiteliais intestinais (FIGUEROA-GONZALEZ *et al.*, 2011).

### **1.5.2 Atividade antidiabética**

A Organização Mundial da Saúde (OMS) acredita que uma em cada onze pessoas no mundo tem diabetes (WHO, 2016). Esse número só cresce, pois segundo a OMS, o diabetes será a sétima principal causa de morte em 2030 (ALWAN *et al.*, 2011). No entanto, pesquisadores fizeram progressos na compreensão da importância dos simbióticos na cura desse distúrbio (IQBAL *et al.*, 2014). A DM2 pode estar associada à composição da microbiota intestinal humana e induzir um estado inflamatório de baixo grau. Além disso, a composição da microbiota intestinal também desempenha um papel significativo no desenvolvimento de condições pré-diabetes, como a resistência à insulina. Em pacientes com essa doença observa-se uma taxa mais elevada dos filos Bacteroidetes e Firmicutes, o que se correlaciona positivamente com a concentração de glicose no plasma (ROAGER *et al.*, 2019). Estudos mostram evidências crescentes de que alimentos funcionais, como prebióticos, probióticos e simbióticos, podem ser utilizados na prevenção e tratamento do diabetes (MEDINA-VERA *et al.*, 2019).

Os AGCCs são produzidos por vários microrganismos do microbioma humano e agem como fonte de energia para o epitélio do cólon além de influenciarem as vias de sinalização do hospedeiro que modulam o apetite e a inflamação. Uma deficiência nos AGCCs intestinais está associada a DM2. ZHAO *et al.* (2018) observaram que uma dieta rica em fibras promoveu o crescimento de organismos produtores de AGCCs em pacientes diabéticos. O manejo do DM2 pela modulação dos hormônios intestinais, como é o caso do

polipeptídeo inibitório gástrico e do peptídeo-1 semelhante ao glucagon, através de intervenções probióticas e prebióticas se apresenta como outra estratégia eficiente. Nesse contexto, os hormônios estão relacionados à homeostase da glicose, o que resulta em neutralizar o distúrbio causado pela resistência periférica à insulina ou falha da produção da mesma em células- $\beta$  (ZHAO *et al.*, 2018)

### **1.5.3 Atividade antiobesidade**

A obesidade, cuja incidência está aumentando em todo mundo, é uma doença que provoca o acúmulo anormal ou excessivo de gordura e pode prejudicar diretamente a saúde (BISCHOFF *et al.*, 2017). A etiologia da obesidade tem sido associada a diversos fatores como: dieta, meio ambiente, educação e genética, por exemplo. Recentemente foi observado que as características do microbioma intestinal desempenham um papel importante nessa doença (GERARD, 2016), apresentando o microbioma como um alvo terapêutico. Estudos que utilizam o transplante do microbioma intestinal de camundongos obesos em camundongos livres de germes podem replicar o fenótipo obeso (KARIMI *et al.*, 2015). Os filos Bacteroidetes gram-negativos e os Firmicutes Gram-positivos dominam o microambiente intestinal e pesquisas recentes provaram que a obesidade está associada ao aumento Bacteroidetes ao longo do tempo, concomitantemente a uma redução de Firmicutes (RIVA *et al.*, 2017).

Nos últimos anos vem se estudando a relação dos probióticos com a obesidade. Isolados como o *Lactobacillus gasseri* BNR17, mostraram propriedades de inibição do aumento do tecido adiposo, assim como, *Lactobacillus casei*, *Lactobacillus acidophilus* e *Bifidobacterium longum* também foram relatados como tendo efeitos hipocolesterolêmicos

(KARIMI *et al.*, 2015). Em um recente estudo de ÇELIK & ÜNLÜ SÖĞÜT (2019), foi avaliada a expressão e a secreção de uma adipocina, que são proteínas sinalizadoras secretadas pelo tecido adiposo, através do efeito da suplementação probiótica em ratos Wistar obesos. A suplementação com probióticos reduziu o ganho de peso e contribuíram nos níveis de glicose em jejum, insulina, resistência à insulina, triglicerídeos, marcadores inflamatórios e citocinas.

#### **1.5.4 Atividade anticancer**

Segundo a OMS, o câncer tem acometido pessoas em todo o mundo e aproximadamente 9.6 milhões de pessoas foram estimadas a morrer de câncer em 2018, sendo que de 30 a 50 % desses casos poderiam ser prevenidos. Além disso, foi estimado um custo de 1.16 trilhões de dólares anuais com essa doença em 2010 (WHO, 2017). Nos últimos dez anos, intensas pesquisas sobre câncer envolvendo genômica, proteômica e patologia molecular aumentaram o conhecimento nessa área. Concomitantemente, muitos fármacos que usam nanotecnologia e biotecnologia (nanocápsulas) foram descobertos, porém ainda existem efeitos colaterais associados a eles. Os medicamentos de fontes naturais que conferem efeitos anticarcinogênicos têm sido foco de interesse, como é o caso dos probióticos (GAYATHRI & RASHMI, 2016).

Os isolados *Lactobacillus fermentum* NCIMB-5221 e -8829 são probióticos e possuem alta capacidade na supressão das células cancerígenas colorretais, além de promoverem o crescimento normal das células epiteliais do cólon através da produção de AGCCs (ácido ferúlico). Esta capacidade também foi comparada com outros probióticos, *L. acidophilus* ATCC 314 e *L. rhamnosus* ATCC 51303, ambos previamente caracterizados com atividade

anticancerígena (KAHOULI *et al.*, 2016). Os três AGCCs mais abundantes detectados em fezes humanas são o acetato, o propionato e o butirato possuindo um papel importante para o corpo humano. O butirato é o AGCCs mais importante para a saúde, pois é a maior fonte de energia para células do intestino, além de possuir atividade anticâncer através da habilidade de induzir apoptose em células cancerígenas do cólon (RÍOS-COVIÁN *et al.*, 2016).

Alguns estudos observaram que alterações nos componentes da superfície celular de lactobacilos podem alterar as respostas imunoregulatórias de células dendríticas e da mucosa intestinal. O uso de lactobacilos geneticamente modificados que regulam negativamente as respostas inflamatórias pode ser uma importante ferramenta no combate a doença inflamatória intestinal e ao câncer de cólon (KHAZAIE *et al.*, 2012). Os prebióticos também possuem um papel importante na ação anticâncer, sendo o seu consumo capaz de reduzir genotoxinas e enzimas  $\beta$ -glicuronidases que geram carcinógenos no intestino, e, assim, regulando a incidência de câncer de cólon.

Já se tem conhecimento que os isolados probióticos são capazes de produzir enzimas que possuem propriedades anticâncer, tais como: L-asparaginase (*ansA*) e arginina deiminase (*arcA*) (AMER *et al.*, 2012; VIMAL & KUMAR, 2017). A L-asparaginase é utilizada para o tratamento da leucemia linfoblástica, já que as células cancerígenas necessitam de grandes quantidades do aminoácido L-asparagina, mais especificamente as células de tumor linfático. A administração de L-asparaginase diminui a asparagina extracelular limitando o crescimento e até matando as células tumorais, enquanto as células normais podem sintetizar L-asparagina e, portanto, são menos afetadas pelo tratamento com L-asparaginase (NARTA *et al.*, 2007). Essa enzima está comercialmente disponível e foi obtida a partir das cepas recombinantes de *Aspergillus niger* ou *Aspergillus oryzae* com base na tecnologia de

clonagem (XU *et al.*, 2016). As fontes microbianas são preferidas, pois além de ser mais econômico na produção, também são fáceis de modificar e otimizar (VIMAL & KUMAR, 2017).

Outra enzima com propriedades anticâncer produzida por microrganismos é a arginina deiminase (ADI). A via anaeróbica dessa enzima é a mais utilizada para degradação da arginina (ZÚÑIGA *et al.*, 2002). Essa rota converte L-arginina em L-ornitina, amônia e dióxido de carbono com a produção de um mol de ATP por mol de L-arginina consumida (SCHULZ *et al.*, 2014). A arginina deiminase tem sido considerada como um potencial agente anticancerígeno (ZÚÑIGA *et al.*, 2002). ADI tem sido investigada como inibidora da proliferação celular em linhagens de células tumorais como, melanomas avançados (OTT *et al.*, 2013), câncer de pulmão (WALTS *et al.*, 2016) e câncer colorretal (FUNAYAMA *et al.*, 2017). Essa enzima já foi identificada, purificada e caracterizada a partir de bactérias, archaea e alguns eucariotos, excluindo células de mamíferos. Alguns exemplos de bactérias produtoras de arginina deiminase são os *Lactobacillus* (DE ANGELIS *et al.*, 2002), os *Lactococcus* (KIM *et al.*, 2007) e os *Enterococcus* (BARCELONA-ANDRES *et al.*, 2002).

### **1.5.5 Doenças intestinais**

Os probióticos têm sido extensivamente estudados no que se referem a doenças gastrointestinais, tais como: gastroenterites, diarreia associada ao uso de antimicrobianos, doença inflamatória intestinal e síndrome do intestino irritável (PARKER *et al.*, 2018; MCKENZIE *et al.*, 2016). Embora os prebióticos e probióticos possam manipular a população bacteriana, eles também podem beneficiar o hospedeiro através do ambiente intestinal por meio de interações diretas com patógenos potenciais e / ou sistema



imunológico. Uma revisão de 2017 com 31 ensaios clínicos direcionados para prevenção de diarreia associada a *Clostridium difficile* concluiu que os probióticos alcançaram uma redução no risco de 60 % (GOLDENBERG *et al.*, 2017).

A doença inflamatória intestinal (DII) é caracterizada por uma inflamação crônica do trato gastrointestinal, tendo dois principais subtipos (doença de Crohn e a colite ulcerativa), as quais não possuem nenhuma terapia médica de cura (BENCHIMOL *et al.*, 2011). Esse tipo de inflamação possui interações complexas entre fatores genéticos, epigenéticos, microbioma intestinal e sistema imune do hospedeiro. Através da disbiose, ou seja, desequilíbrio da microbiota intestinal, e da alteração das vias metabólicas bacterianas podem ocorrer a origem e progressão da DII (HABERMAN *et al.*, 2015; SERBAN, 2015). A produção de AGCCs por bactérias probióticas são utilizados como principal fonte de energia para as células epiteliais intestinais. A produção de butirato por bactérias tem um papel importante na integridade da barreira epitelial, sendo a redução nos níveis de butirato encontrados em amostras fecais de pacientes com DII (LI *et al.*, 2015). Um estudo de meta-análise, realizado em 2017, analisou 27 ensaios que demonstraram que os probióticos, na sua maioria, são efetivos em pacientes com colite ulcerativa e doença de Crohn. As combinações de bactérias probióticas em doença de Crohn pareceram ser mais eficientes (GANJI-ARJENAKI & RAFIEIAN-KOPAEI, 2017).

A síndrome do intestino irritável (SII) é uma doença comum com prevalência entre 5 a 20 % na população geral, e causa desordem funcional do intestino (LOVELL & FORD, 2012). Essa síndrome é mais prevalente em mulheres jovens e a causa dessa desordem funcional ainda não está clara. Existem terapias farmacológicas para SII, mas que muitos pacientes não respondem bem a elas (JOHANSON & KRALSTEIN, 2007; OLAFSDOTTIR *et al.*, 2012). A modulação da microbiota intestinal pode ser uma opção de tratamento, visto

que os probióticos têm sido bastante estudados na SII (MOAYYEDI *et al.*, 2010). Algumas bactérias benéficas parecem ter propriedades anti-inflamatórias (O' MAHONY *et al.*, 2005), ou a habilidade de modular a hipersensibilidade do intestino (KAMIYA *et al.*, 2006). Os prebióticos também possuem seu papel nessa doença, eles podem estimular o crescimento e a atividade dessas bactérias probióticas desencadeando na melhora do paciente (VERDU *et al.*, 2006).

## **2 OBJETIVOS**

### **2.1 Objetivo geral**

Avaliar as características probióticas do isolado *Enterococcus durans* LAB18S, sua relação simbiótica com diferentes prebióticos, assim como a sua interação com o microbioma intestinal humano.

### **2.2 Objetivos específicos**

- Investigar, através do sequenciamento do genoma, genes potencialmente associados a características probióticas, assim como genes envolvidos no metabolismo do selênio;
- Verificar evidências da relação simbiótica entre prebióticos e probióticos avaliando a capacidade de carboidratos em estimular o crescimento;
- Analisar a diferença na expressão proteica do LAB18S cultivado em FOS, GOS e glicose;
- Analisar, através de cultura *ex vivo*, a interação das proteínas secretadas pelo probiótico *E. durans* LAB18S em simbiose com FOS e GOS com o microbioma intestinal humano de um indivíduo saudável.

### 3 DESCRIÇÃO DOS ARTIGOS CIENTÍFICOS

O isolado *Enterococcus durans* LAB18S foi isolado de queijo Minas frescal e pertence à coleção do Laboratório de Bioquímica e Microbiologia Aplicada da UFRGS. Estudos fenotípicos prévios com esse isolado foram realizados a fim de verificar características probióticas. Através desse estudo pode-se observar que esse isolado exibiu algumas propriedades probióticas *in vitro*, tais como, atividade antimicrobiana contra bactérias Gram-positivas e Gram-negativas, habilidade antioxidante, capacidade de sobreviver em condições gastrointestinais simuladas e a capacidade de crescer em meio enriquecido por selênio, acumulando esse elemento na sua biomassa. A partir desses resultados, nosso grupo de pesquisa mostrou interesse em aprofundar os conhecimentos sobre esse candidato à probiótico realizando, primeiramente, uma abordagem genômica. O sequenciamento do DNA foi realizado e os resultados dessa análise compõem o artigo intitulado “Genomic analysis of *Enterococcus durans* LAB18S, a potential probiotic strain isolated from cheese” submetido na revista *Archives in Microbiology*.

Após a análise dos resultados do genoma do LAB18S, observamos que o isolado *E. durans* possuía a capacidade de metabolizar alguns oligossacarídeos como o FOS. A partir disso, decidimos avaliar se a ação de diferentes fontes de carbono com propriedades prebióticas estimulavam o crescimento e / ou a produção de proteínas relacionadas à esses genes encontrados no genoma. Assim, escolhemos, para realização dos testes, dois oligossacarídeos muito utilizados pela indústria que são o frutooligossacarídeo (FOS) e o galactooligossacarídeo (GOS) que mostram excelentes resultados sinérgicos com os probióticos. Com esse objetivo encontramos os resultados no artigo intitulado “Proteomic

study of *Enterococcus durans* LAB18S growing on prebiotic oligosaccharides” que foi aceito para a publicação na revista *Food Microbiology*.

Através da oportunidade de ir para o Canadá, no laboratório do professor Daniel Figeys, analisamos a interação do LAB18S com o microbioma intestinal humano em um sistema de cultivo *ex vivo*, desenvolvido no laboratório do professor Figeys. Primeiro, investigamos a ação do oxigênio na expressão de proteínas do LAB18S em simbiose com FOS e GOS, através de análises proteômicas, e os resultados deste estudo estão no artigo “Comparative proteomic analysis reveals metabolic variability of probiotic *Enterococcus durans* during aerobic and anaerobic cultivation” submetido na revista *Molecular Nutrition & Food Research*.

Em seguida, avaliamos as proteínas secretadas pelo LAB18S crescido em simbiose com FOS e GOS no microbioma intestinal de um indivíduo saudável. Assim, conseguimos perceber diferença nas comunidades microbianas intestinais relacionadas ao crescimento microbiano e à expressão de proteínas, tanto nas diferentes concentrações utilizadas do secretoma do LAB18S, quanto no estímulo de FOS e GOS. Esses resultados preliminares estão no artigo intitulado “Metaproteomics reveals the action of proteins secreted by probiotic *E. durans* LAB18S in an *ex vivo* culture of the human gut microbiome”, esse artigo será submetido posteriormente e ainda está em fase de construção sendo necessárias análises adicionais.

## ARTIGO CIENTÍFICO 1

---

### **Genomic analysis of *Enterococcus durans* LAB18S, a potential probiotic strain isolated from cheese**

Submetido no periódico Archives of Microbiology em 15 de setembro de 2019, fator de impacto 1.607

**Genomic analysis of *Enterococcus durans* LAB18S, a potential probiotic strain isolated from cheese.**

Carolina Baldisserotto Comerlato<sup>a</sup>

Janira Prichula<sup>b</sup>

Francieli Maboni Siqueira<sup>c</sup>

Ana Carolina Ritter<sup>a</sup>

Ana Paula Muterle Varela<sup>d</sup>

Fabiana Quoos Mayer<sup>d</sup>

Adriano Brandelli<sup>a\*</sup>

<sup>a</sup> Laboratório de Bioquímica e Microbiologia Aplicada, Departamento de Ciência de Alimentos, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

<sup>b</sup> Laboratório de Cocos Gram-positivos, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil

<sup>c</sup> Laboratório de Bacteriologia Clínica, Departamento de Patologia Clínica Veterinária, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

<sup>d</sup> Laboratório de Biologia Molecular, Departamento de Diagnóstico e Pesquisa Agropecuária, Secretaria da Agricultura Pecuária e Irrigação do RS, Eldorado do Sul, Brazil.

\* Corresponding author: Dr. A. Brandelli, ICTA-UFRGS, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brazil; e-mail: [abrand@ufrgs.br](mailto:abrand@ufrgs.br)

## Abstract

The gut microbiota exerts a fundamental role in human health and increased evidence supports the beneficial role of probiotic microorganisms in the maintenance of intestinal health. In this study, the genome of *E. durans* LAB18S was sequenced and evaluated for genes that can be relevant for probiotic activity and are involved in selenium metabolism. Genome sequencing was performed using the Illumina MiSeq System. A variety of genes potentially associated with probiotic properties, including adhesion capability, viability at low pH, bile salt tolerance, antimicrobial activity, and utilization of prebiotic substrate fructooligosaccharide were identified. Six genes involved in selenium metabolism were predicted. Analysis of the SECIS element showed twelve known selenoprotein candidates. LAB18S was the only food isolate showing absence of plasmids, virulence and antimicrobial resistance genes, when compared with other 30 *E. durans* genomes. The results of this study provide evidence supporting the potential of *E. durans* LAB18S as alternative for probiotic formulations.

**Keywords:** *Enterococcus*; probiotic; prebiotic; genome; selenoproteins;  $\beta$ -galactosidase



## Introduction

*Enterococcus* genus belongs to the class of lactic acid bacteria (LAB) of the phylum Firmicutes, showing the ability to survive under various environmental conditions (Byappanahalli et al. 2012). This bacterial genus is an important component of the intestinal microbiota of humans and animals and is found in food products, mainly dairy foods. Many enterococci isolated from fermented dairy products proven to be natural probiotics and have been considered beneficial and safe to the host (Franz et al. 2011).

Currently, the role of probiotic bacteria in health and functionality of human and livestock animal intestines has been greatly emphasized. The intestinal microbiome has a great importance in human health, promoting intestinal homeostasis, development of the immune system, protection against pathogens and stimulating the production of micronutrients and energy (Clemente et al. 2012).

Some *in vitro* tests are recommended to characterize a microorganism with probiotic potential, including adherence to human and/or mucosal epithelial cells, antimicrobial activity against pathogens, ability to decrease the adhesion of pathogens and stimulate the hydrolysis of bile salts (Hill et al. 2014). These tests have become the dogma for probiotic characterization, but phenotypic characterization is not enough to provide a full description of probiotic microorganisms. Thus, the study of genomic data obtained by high-throughput DNA sequencing tools may provide novel useful information, expanding the current knowledge on probiotic strains. Genomic analysis may be useful to identify genes related to probiotic properties and to find additional molecules and metabolic routes that contribute to the specific activity of a probiotic strain (Li et al. 2018). These genes can codify proteins associated with survival to gastrointestinal tract transit, such as bile salt hydrolases,

production of antimicrobial substances like bacteriocins, and beneficial enzymes, such as phytase, inulinase and  $\beta$ -galactosidase (Ladero et al. 2013; Bonacina et al. 2017).

Selenium (Se) is a trace element known primarily for its functions in redox homeostasis as a promising chemo-preventive agent for cancer (Hatfield et al. 2006) and because it has beneficial effects associated with probiotic bacteria (Galano et al. 2013). The major biological form of Se is selenocysteine (Sec, the 21<sup>st</sup> amino acid), which is co-translationally inserted into selenoproteins by recoding the UGA codon (Hatfield and Gladyshev 2002). In bacteria, the mechanism of Sec biosynthesis and its insertion into proteins requires an in-frame UGA codon, a Sec insertion sequence element (SECIS). SECIS is a hairpin structure within the selenoprotein mRNA immediately downstream of the Sec codon encoding the UGA codon (Zhang and Gladyshev 2005).

Although genome sequences of some *Enterococcus* species like *E. faecalis* and *E. faecium* have been described (Bonacina et al. 2017; Zhong et al. 2017), poor information is available for *E. durans* (Li et al. 2018). The *E. durans* LAB18S was previously isolated from a typical Brazilian soft cheese and exhibited some desirable probiotic properties *in vitro*, such as antimicrobial activity, antioxidant ability and survival to simulated gastrointestinal tract conditions (Pieniz et al. 2014). In addition, the capacity to growth in selenium enriched medium, accumulating this element in the biomass, which were evidenced in both culture supernatants and intracellular extracts (Pieniz et al. 2017). Further research is needed to prove its potential health benefits and its application as a probiotic lineage in the industry. Thus, the aim of this study was to characterize the genome of *E. durans* LAB18S strain, searching for relevant genes associated with probiotic properties and selenoproteins.

## Materials and Methods

### Genomic DNA preparation and high-throughput sequencing

*E. durans* LAB18S was isolated from soft cheese, belonging to the collection of the Laboratory of Applied Microbiology and Biochemistry (Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil). The strain was maintained as frozen stock cultures in Brain Heart Infusion (BHI, Oxoid) containing 20% (v/v) glycerol. The bacterium was grown in MRS broth at 37°C at mid log phase.

*E. durans* LAB18S total DNA was extracted with phenol-chloroform following usual procedures and purified using a Genomic DNA Clean & Concentrator (Zymo Research). The quality and quantity of the DNA were assessed by spectrophotometry analysis using NanoDrop (Thermo Scientific™) and fluorometry (Qubit™; Invitrogen), respectively. DNA fragment libraries were further prepared with 50 ng of DNA using a Nextera XT DNA sample preparation kit and sequenced using an Illumina® MiSeq System (2x250 paired-end reads with the Illumina v2 reagent kit), manufacturer's instructions.

After quality checking with FastQC software, reads were trimmed with Geneious software (version 10.2.3). The paired-end sequence reads were then assembled by *de novo* assembly using SPAdes 3.9.0 (Bankevich et al. 2012), and Geneious software version 10.2.3 followed by template-assisted assembly to the reference *E. durans* KLDS6.0933 (NZ\_CP012366).

## **Gene prediction and bioinformatics analysis**

Annotation NCBI Prokaryotic Genome Annotation Pipeline (PGAAP) was employed to identify coding sequences (CDS) based on the best-placed reference protein set. Similarly, to aid the gene prediction and annotation, *E. durans* genome were performed by RAST (Rapid Annotation Subsystem Technology) webservice. Genes of interest had their annotation refined manually. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession NCVP000000000. The version described in this paper is version NCVP010000000.

Genes involved in the biosynthesis of secondary metabolites were analyzed in silico using the antiSMASH algorithm (<http://antismash.secondarymetabolites.org/>). We then used bSECISearch to predict candidates for bacterial SECIS elements and their ORFs in order to analyze the genome of *E. durans* LAB18S for full complement of selenoprotein genes (Zhang and Gladyshev 2005).

## **Comparative analysis**

Antimicrobial resistance genes were identified using ResFinder 3.2 (Zankari et al. 2012) following the thresholds 60% identity over a length of 60% coverage, respectively. VirulenceFinder (Joensen et al. 2014) and PlasmidFinder (Caratolli et al. 2014) were used to predict potential virulence genes and plasmids, respectively. Identification thresholds were set at 60% identity over a minimum length of 60% for PlasmidFinder, and 85% identity over a length of 60% for VirulenceFinder.

Core genome Single Nucleotide Polymorphism (SNP) tree were performed using Parsnp v1.2 program included in Harvest (Treangen et al. 2014). A total of 31 *E. durans* genomes, one draft genome from this study and 30 genomes from previous studies obtained from the NCBI database were used (supplementary Table S1). Core genome SNPs of *E. durans* were identified, the reference genome was randomly selected using the parameter ‘-r!’ and recombination regions were used (Treangen et al. 2014). An approximately maximum likelihood tree was constructed from concatenated SNPs using FastTree2 (Price et al. 2010), and interactive Tree Of Life (iTOL) v4 software (Letunic and Bork 2019) were used to visualization and edition of the phylogenomic tree.

## **Results**

### **Structure and general features of *E. durans* LAB18S genome**

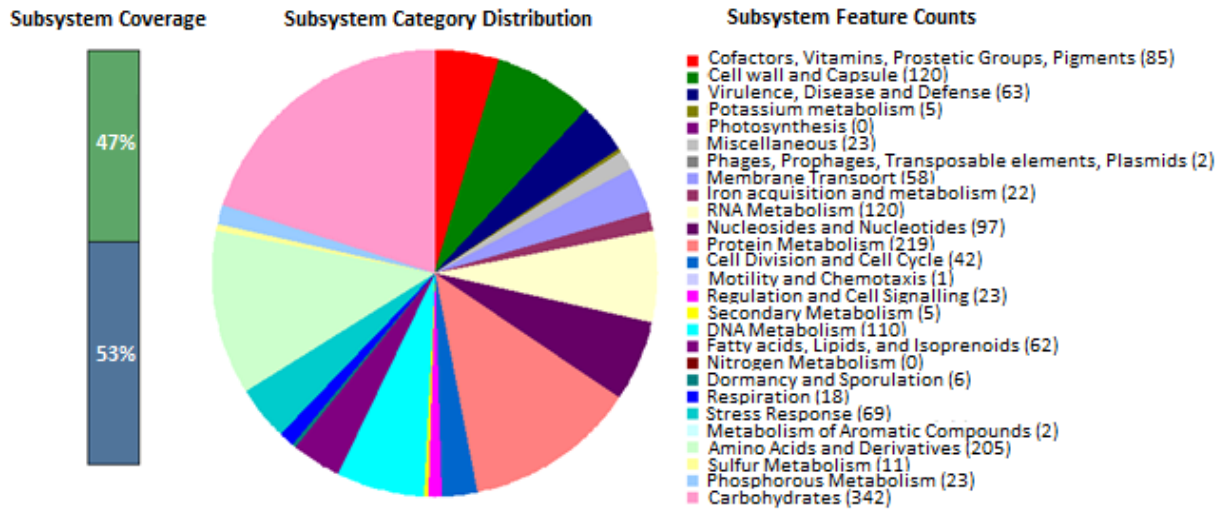
The genome sequence of *E. durans* LAB18S was obtained using the Illumina® MiSeq system, and compared with the complete genome sequence of *E. durans* KLDS6.0933 (GenBank accession number CP012366.1). The general genomic properties of *E. durans* LAB18S are presented in Table 1. The complete genome is composed of a chromosome with GC content of 38% and contains 2,579 CDSs, 108 RNAs and 180 pseudogenes (Table 1). By assembling the genome, a total of 82 contigs were obtained and a mean coverage of 31.7 x giving reliability to the results. Comparatively, the reference strain (*E. durans* KLDS6.0933) has 2,867,028 bp and the *E. durans* LAB18S genome is slightly larger with additional 329 bp.

**Table 1.** General genome features of *E. durans* LAB18S compared with *E. durans* KLDS6.0933

Feature	<i>E. durans</i> LAB18S	<i>E. durans</i> KLDS6.0933
Size (bp)	2,867,357	2,867,028
GC content (%)	38.1	37.8
Predicted genes	2669	2737
Protein coding genes (CDS)	2579	2333
Pseudogenes	180	323
rRNA	36	18
tRNA	68	68
ncRNA	4	4

Through the RAST webservice, the genes were grouped into subsystems that are shown in supplementary Figure S1. In brief, there are 126 genes for cell wall and capsule; 342 genes for carbohydrate transport and metabolism, which contains 17 genes related with fructooligosaccharides (FOS) and raffinose utilization; 63 genes for virulence, disease and defense, which contains adhesion, bacteriocins, resistance to antibiotics and toxic compounds, invasion and intracellular resistance genes; 2 genes for phages and prophages; 58 for membrane transport; 219 for protein metabolism; 6 for dormancy and sporulation and 69 for stress response.

**Figure S1.** Genes of the *E. durans* LAB18S genome grouped into subsystems by RAST webservice.



Some genes identified during annotation allowed a high quality evaluation not only of the gene functions, but also of the genomic context. Genes that characterize a probiotic isolate and genes involved in selenium metabolism were predicted.

### Genes associated with probiotics properties

The genome of *E. durans* LAB18S obtained in this study was mined to find genetic elements associated with probiotic properties. *E. durans* LAB18S genome showed several genes that may be related with probiotic activity (Table 2). It encodes an S-layer protein (LIU RS11695), and two fibronectin-binding proteins (LIURS07910 and LIU RS10480), which may contribute to bacterial adherence. Besides, this genome carries an exopolysaccharide (EPS) cluster that could be produced by probiotics and improve its adhesion properties and its persistence in the gut. In addition, it also contains genes that can be associated to viability at lower pH ( $\text{Na}^+/\text{H}^+$  antiporters) and bile salt tolerance (Table 2).

The potential for carbohydrate utilization was also analyzed and genes for fructooligosaccharide (FOS) and disaccharides utilization were found. Besides, the  $\beta$ -galactosidase (BGL) gene was identified in the genome (Table 2).

Secondary metabolite analysis revealed the presence of genes associated with colicin V, enterocin A, and the small bacteriocin microcin J25 (Table 2). Furthermore, two genes of toxin-antitoxin proteins, namely RelE and Zeta-toxin, were also identified. The BLAST algorithm was used to align the deduced colicin V sequence of *E. durans* LAB18S with colicin V and colicin V production protein CvpA from other genera and species. This sequence is quite conserved among different species of *Enterococcus*, *Bacillus* and *Carnobacterium* and strain LAB18S (supplementary Figure S2).



**Table 2.** Genes associated with potential probiotic properties of *E. durans* LAB 18S.

Protein	Gene	Function
<i>Maintenance in the gastrointestinal tract</i>		
S-layer protein	<i>lbs</i>	Improves adhesion properties and persistence in the gut
Fibronectin-binding protein	<i>prtF</i>	Improves adhesion properties and persistence in the gut
Heat-shock protein 33	<i>hsp33</i>	Improves persistence in the gut
EPS cluster	<i>epsABCDE</i>	Improves adhesion properties and persistence in the gut
Na <sup>+</sup> /H <sup>+</sup> antiporter	<i>nhaC</i>	Improves viability at low pH
Cyclopropane-fatty-acyl-phospholipid synthase	<i>Cfa</i>	Key protein in bile salt tolerance
<i>Bacteriocins and toxin-antitoxins</i>		
Microcin cluster	<i>micJ25</i>	Low molecular mass bacteriocins produced under stress conditions
Enterocin A immunity protein	<i>entI</i>	Putative protection against the effect of bacteriocin enterocin A
Colicin V precursor	<i>cvaC</i>	Kills sensitive cells by disrupting their membrane potential
Zeta-toxin	<i>pSM19035</i>	Inhibits cell wall biosynthesis
Toxin RelE	<i>relE</i>	Cleaves translating mRNA in the ribosomal A-site upon aminoacid starvation
<i>Resistance to heavy metals</i>		

Multi-copper oxidase	<i>cueO</i>	Provides copper tolerance
Copper-transporting efflux system	<i>cusCFBA</i>	Mediates resistance to copper and silver
Cation efflux system protein CzcA	<i>czcA</i>	Provides resistance to cobalt, zinc and cadmium
Mercuric reductase	<i>merA</i>	Provides resistance to mercury
<i>Carbohydrate utilization</i>		
Raffinose operon regulatory protein	<i>rafR</i>	Metabolism of fructooligosaccharides (FOS) and raffinose
Lactose operon	<i>lacZYA</i>	Metabolism of lactose and galactose
Maltodextrin phosphorylase	<i>malP</i>	Metabolism of maltodextrin and $\alpha$ -1,4-glucans
4-alpha-glucanotransferase	<i>malQ</i>	Starch metabolism

---

**Figure S2.** Multiple sequence alignment between colicin V from *E. durans* LAB18S and other colicin V and CvpA sequences from other genera and species retrieved by using the BLAST algorithm. The black color indicates highly conserved residues and red indicates less conserved ones.

```

Col V, E. durans LAB18S 1  MLSLLILFILLIAFFSGARRGFAMQVVYTI GYVLSFFAAQHFKQLADHLELYI PYPAVTSTSRMVFDDQAISFRLDEA 79
Col V, E. durans 1  MLSLLILFILLIAFFSGARRGFAMQVVYTI GYVLSFFAAQHFKQLADHLELYI PYPAVTSTSRMVFDDQAISFRLDEA 79
CvpA Family, E. hirae 1  MLSLLILFILLIAFFSGARRGFAMQVVYTI GYLSFMAAQHFKDLASRLELYI PYPAVTSTSRMVFDDQTFPSFRLDEA 79
CvpA Family, Bacilli 1  MLSLLIIIFLLIAFFSGAKRGFALQVVYTV GYLVSPVAQHFKQLANHLELYI PYPAVTPTSDLVFFDQTFISFLDQA 79
CvpA Family E. mundtii 1  MLSLLILFILLIAFFSGASRGFALQGIYLI GYFVSLAAQTYKTLASHLQLYI PYPAVTANSNLVFFDQAISFRLDEA 79
CvpA Family E. faecalis 1  MLTLLILFILLIAFFSGARRGFAMQAVTV GYVISPFAAQHFKPLANHLRLYI PYPAVTPDSQMAFFDQARSLSLDQA 79
Col V, E. faecium 1  MLSLLILFILLIAFFSGARRGFALQIIIFAI GYVLSFIAAQHFKPLASHLELYI PYPAVTPTSKLAFDQVFAFHLDEA 79
CvpA Family, C. inhibens 1  mLMVTVIIVLLLAIGAYSGARRGLILQLVLT IGYFISYLLAGKYQTLGSHLELIV PYPASASESQVFVYNQALGFDDGA 80
CvpA Family, C. viridans 1  mLMTVLIVLILAMGAYSGARRGLVQLQVFT IGYFVSYLLARNYQLGSHLELIV PYPASATESSQVFVYDQALGFNDGA 80

Col V, E. durans LAB18S 80  FYAGVAFLLILLAGALITRFIGIFAHSLTYV PVLQVDWLAGGVLSVVVAYVTIFLLLSLLTLVPVDFIQNQFSGNSLAR 159
Col V, E. durans 80  FYAGVAFLLILLAGALITRFIGIFAHSLTYV PVLQVDWLAGGVLSVVVAYVTIFLLLSLLTLVPVDFIQNQFSGNSLAR 159
CvpA Family, E. hirae 80  FYAGVAFLLILFIGLLTRFIGIFVHSLTYIPI LKQVDWLAGGILSLIVAYVTIFLLLSLLTFVPEVDIVQKQFSGNSLAR 159
CvpA Family, Bacilli 80  FYAGIAFLIILMAGWLITRFIGIFVHSLTYI PVLKQVDWLAGGILSVVVTFVMIPLLSLLS FVPSDFIQNQFRSSGLAR 159
CvpA Family E. mundtii 80  FYAGVAFLLIILFIGWLVTRFIGVFAHGLTFI PVLKQLDWVAGGILSVIITYISLFLVLRLLTFIPVGF IQNQFNNGNLLAT 159
CvpA Family E. faecalis 80  FYAGVAFLAIFAAGWLITRFIGVFLHGLTYV PVLQADWLAGGILSMVVAYVIFMLLSLLMMVPLDSIQNLFPKSNGLPR 159
Col V, E. faecium 80  FYAGTAFLLIILLIGWLLTRFVGVFVHGLTY VPILRQVDWLAGGILSLIMAYVTIFLILQLLAFVPLD----- 146
CvpA Family, C. inhibens 81  FYNGVAFILILFVGWLTIRFVGGLLNSLTFI PVLKQLNALGGAILNVI VSYVAIFLVLFLMTMVPIDAIQEAFNTSWLAR 160
CvpA Family, C. viridans 81  FYNGVAFITILFVGWLTIRFVGGLLNAVTLI PVIKQLNTLGGALLNVI VSYIAIFLVLFLMTMVPVDAIQESFNNSWLAR 160

Col V, E. durans LAB18S 160  FIVEKTPLLSDKIHDLWVTNIInq 183
Col V, E. durans 160  FIVEKTPLLSDKIHDLWVTNIInq 183
CvpA Family, E. hirae 160  FIVDQTPFLTNKIHDLWITNVIN- 182
CvpA Family, Bacilli 160  YIVENTPILTNKIYDLWITRVIG- 182
CvpA Family E. mundtii 160  FMVERTPILANKIYDLWVTQVIN- 182
CvpA Family E. faecalis 160  FIVENTPVLSNKIYDLWITRIIG- 182
Col V, E. faecium -----
CvpA Family, C. inhibens 161  TIVEDTPVISAQLYNLWIETSLK- 183
CvpA Family, C. viridans 161  TIVEDTPVISAQLYNWISSLK- 183

```

## Genes related to selenoproteínas

*E. durans* LAB18S genome contains seven genes involved in selenium metabolism (Table 3). Five genes encode typical selenoproteins, namely glutathione peroxidase (*gpX*), thioredoxin reductase (*trxB1*, *trxB2*), glycine reductase complex selenoprotein B (*grdB*), and peroxiredoxin (*prX*). Another two genes are related with selenium metabolism: L-seryl-tRNA selenium transferase (*selA*) and YggS family pyridoxal phosphate (*yggS*).

**Table 3.** Selenoprotein related genes predicted in *E. durans* LAB 18S genome.



















Protein	Gene	Function
<i>Selenoproteins</i>		
Glutathione peroxidase	<i>gpX</i>	Catalyzes the reduction of H <sub>2</sub> O <sub>2</sub> ; protection against oxidative stress
Thioredoxin reductase	<i>trxB1, trxB2</i>	NADPH-depended oxidoreductase activity
Glycine reductase complex	<i>grdB</i>	Active protein in the peroxidase reaction
Peroxiredoxin	<i>prX</i>	Antioxidant enzyme that uses thioredoxin (Trx) to recharge after reducing H <sub>2</sub> O <sub>2</sub>
<i>Other selenium-related proteins</i>		
L-seryl-tRNA selenium transferase	<i>selA</i>	Converts seryl-tRNA(Sec) to selenocysteiny-tRNA (Sec) required for selenoprotein biosynthesis
Selenocysteine-specific elongation factor	<i>selB</i>	Translation factor necessary for the incorporation of selenocysteine into proteins
YggS family pyridoxal phosphate	<i>yggS</i>	Decomposes selenocysteine to alanine and elemental Se or H <sub>2</sub> Se during selenium metabolism

In the analysis of the selenocysteine insertion sequence (SECIS) element, 1,274 hits were identified as candidates of bacterial SECIS (bSECIS)-like elements. These hits were divided into homologs of previously known selenoproteins (40 sequences) and candidates of selenoproteins (1,234 sequences). Then, optimal bSECIS elements and their predicted ORFs were presented with weight scores greater than the cutoff (weight score > 30) and 26 of known selenoproteins and 765 unknown bSECIS elements were detected. For better selenoprotein candidates, we run Blast search (tblastn + blastx) at NCBI to filter out false positives and the result was 12 bSECIS elements involved with selenium.

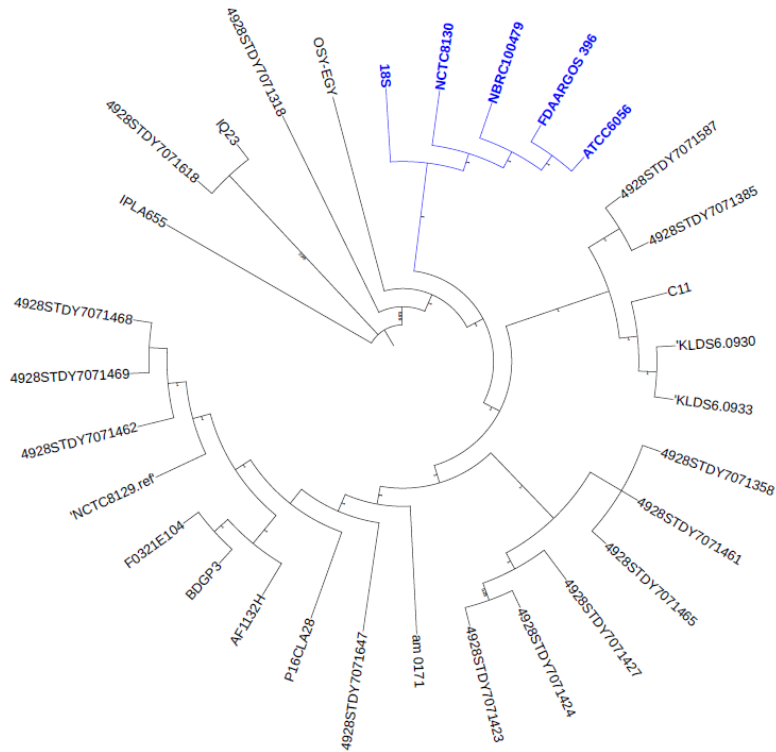
Virulence genes were not found in the *E. durans* genomes compared in this study. Antimicrobial resistance was checked against the ResFinder database and genes associated with tetracycline resistance, namely *tet(M)* and *tet(O)*-like were found in seven genomes of *E. durans*, mostly from fecal origin (Table 4). Only three enterococci under study did not present any plasmids, including *E. durans* LAB18S. Besides the absence of plasmids, these three strains also showed no virulence and antimicrobial resistance genes. Among these isolates, only *E. durans* LAB18S has a food origin and is therefore an excellent candidate for use as probiotics. The 31 genomes of *E. durans* were clustered into a phylogenetic tree (supplementary Figure S3). *E. durans* LAB18S has been clustered with isolates NCTC8130, FDAARGOS\_396 and ATCC 6056, which are of fecal origin, and NRBC10079, which lacks source information. None of these isolates showed antimicrobial resistance or virulence genes. The presence of plasmids was found in these isolates, excepting for LAB18S.

**Table 4.** Comparative analysis of the presence of virulence genes, antimicrobial resistance genes and plasmids of 31 *E. durans* genomes.

Species	Strain	Origin	Resistance		Virulence	Plasmids								
			<i>tet</i> (M)	<i>tet</i> (O)		<i>rep1</i>	<i>rep2</i>	<i>rep4</i>	<i>rep11</i>	<i>rep18</i>	<i>repUS1</i>	<i>repUS15</i>		
<i>Enterococcus durans</i>	NCTC8129	Unknown												
<i>Enterococcus durans</i>	NCTC8130	Unknown												
<i>Enterococcus durans</i>	P16CLA28	Cloaca ( <i>Gallus gallus</i> )												
<i>Enterococcus durans</i>	F0321E104	Feces ( <i>Bos taurus</i> )												
<b><i>Enterococcus durans</i></b>	<b>18S</b>	Frescal cheese												
<i>Enterococcus durans</i>	KLDS6.0930	Water												
<i>Enterococcus durans</i>	KLDS6.0933	Water												
<i>Enterococcus durans</i>	IQ23	Cheese												
<i>Enterococcus durans</i>	AF1132H	Feces ( <i>Homo sapiens</i> )												
<i>Enterococcus durans</i>	ATCC6056	Feces ( <i>Homo sapiens</i> )												
<i>Enterococcus durans</i>	IPLA655	Cheese												
<i>Enterococcus durans</i>	C11	Kimchi												
<i>Enterococcus durans</i>	OSY-EGY	Egyptian hard Cheese												
<i>Enterococcus durans</i>	am_0171	Feces ( <i>Homo sapiens</i> )												
<b><i>Enterococcus durans</i></b>	<b>BDGP3</b>	Feces ( <i>Drosophila melanogaster</i> )												
<i>Enterococcus durans</i>	4928STDY7071618	Feces ( <i>Homo sapiens</i> )												

<i>Enterococcus durans</i>	4928STDY7071587	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	4928STDY7071465	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	4928STDY7071468	Feces ( <i>Homo sapiens</i> )		
<b><i>Enterococcus durans</i></b>	<b>4928STDY7071461</b>	<b>Feces (<i>Homo sapiens</i>)</b>		
<i>Enterococcus durans</i>	4928STDY7071424	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	4928STDY7071423	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	4928STDY7071358	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	4928STDY7071318	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	4928STDY7071647	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	4928STDY7071469	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	4928STDY7071427	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	4928STDY7071462	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	4928STDY7071385	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	FDAARGOS_396	Feces ( <i>Homo sapiens</i> )		
<i>Enterococcus durans</i>	NBRC 100479	Unkwnown		

**Figure S3.** Core genome SNP tree of 31 *E. durans*. The phylogenomic reconstruction was built using Parsnp and Fast tree 2. Strains related with 18S *E. durans* were represented in blue.



## Discussion

Complete knowledge of genome sequences may allow a precise genetic analysis of probiotic bacteria. This includes the genetic features that can be associated with beneficial effects and those potentially associated with undesirable characteristics. The genus *Enterococcus* contains strains associated with severe infections, while other strains form part of the commensal human microbiome of the mouth, skin, and intestine. Some strains have probiotic properties, including *E. durans* (Liaskovs'kyi et al. 2008; Li et al. 2018). Interestingly, virulence is very different among enterococci derived from community or hospital environments, which appears to be associated to some strain-specific genetic



features (Douillard and de Vos 2014). Thus, the study of whole genomes is relevant to acquire information on the potential benefits and drawbacks. In this work, the genome of *E. durans* LAB18S isolated from Minas Frescal cheese showed some desirable characteristics for a probiotic strain.

In the last decades, the survival of probiotic bacteria in the gastrointestinal tract has been extensively studied. Probiotics, after ingestion, find the acidic conditions and the activity of digestive enzymes of the stomach. *E. durans* LAB18S is equipped with a gene coding for Na<sup>+</sup>/H<sup>+</sup> antiporter, contributing to regulate intracellular pH (Guo et al. 2015). *E. durans* LAB18S demonstrated high ability to survive in the presence of simulated gastric juice containing pepsin and simulated intestinal juice containing pancreatin and bile salts (Pieniz et al. 2014).

The reduction of bacterial survival in the small intestine may be due to secretion of bile that breaks the microbial cell membrane. As well, tolerance to bile salt concentrations between 0.15 and 0.5% has been recommended for probiotics (Lavermicocca et al. 2008). Bile salt hydrolase (BSH) is an enzyme that hydrolyzes bile salts, decreasing its toxicity. The gene encoding cyclopropane-fatty-acyl-phospholipid synthase (HUO 05315), present in the genome of *E. durans* LAB18S, can be associated with bile salt tolerance. Comparative proteomic studies on *Lactobacillus plantarum* identified cyclopropane-fatty-acyl-phospholipid synthase as a key protein in bile tolerance (Hamon et al. 2011). Bile salt deconjugation by BSH has been also associated with reduced serum cholesterol level. The BSH identified in genome the *E. durans* KLDS6.0933, showing cholesterol removal ability, is different from that of other *Enterococcus* strains (Li et al. 2018).

Adhesive properties can prolong the contact between bacteria and the host and therefore enhance the desired probiotic effect (Wang et al. 2011). Auto-aggregation capacity

of LAB is correlated to their capacity to adhere to different kind of host cells, and it is considered as a desirable characteristic for preliminary probiotic screening (Botta et al. 2014). *E. durans* LAB18S genome does not show mucus-binding proteins and adhesion genes. However it presents an S-layer protein (LIURS 11695), and fibronectin-binding proteins (LIURS 07910 and LIURS 10480), which may contribute to adherence. In addition, a gene encoded aggregation-promoting factor (LIURS 03835) was also identified, suggesting that this strain can bind to receptors in the gut environment (Senan et al. 2015). Some EPS produced by probiotics can improve its adhesion properties and its persistence in the gut (Ruas-Madiedo et al. 2006), and the LAB18S genome carries an EPS cluster. All these genetic elements corroborate to the potential adhesive characteristics of *E. durans* LAB18S.

The benefit of bacteriocin-producing probiotic isolates against pathogenic bacteria in the gastrointestinal tract has been recognized (Gillor et al. 2008). Analysis for secondary metabolite clusters of LAB18S genome revealed the presence of genes associated with the synthesis of microcin J25, colicin V and enterocin A, which may endow competitive advantages to combat pathogenic bacteria. Colicin V is produced by many strains of *Escherichia coli* and its precursor peptide is similar to some bacteriocins of the *Enterobacteriaceae* family, which fits the definition of class II bacteriocins from Gram-positive bacteria (Håvarstein et al. 1994). Typical bacteriocins produced by LAB are generally not active against Gram-negative bacteria due to the presence and composition of the outer membrane. The transfer of genes encoding bacteriocins from Gram-negative bacteria, such as colicin V, to food-grade LAB host has been described (Langa et al. 2017).

The LAB18S genome contains genes of toxin-antitoxin systems, which have been associated with survival under stress conditions. Zeta-toxin is bactericidal for *Bacillus*

*subtilis* and bacteriostatic for *Escherichia coli*, while the toxin RelE degrades mRNA at specific sequences when it is bound to the ribosomal A site (Pedersen et al. 2003). The ability of several toxins to cleave mRNA stimulate the hypothesis that toxin-antitoxin systems are implicated in the quality control of prokaryotic gene expression. As a concern, the presence of omega/epsilon/zeta toxin-antitoxin system seems to stabilize plasmids carrying *vanA* in *E. faecium* and *E. faecalis* resistant to vancomycin (Fernández-Gracia et al. 2016).

Genes related with the metabolism of prebiotic molecules were also identified. The strain LAB18S presented genes related to the use of fructooligosaccharides (FOS), a non-digestible dietary component that undergo selective colonic fermentation. FOS cause significant changes in the composition of the gut microbiota, increasing the numbers of potentially health-promoting bacteria and reducing potentially harmful species, respectively (Slavin 2013). BGL gene was also detected in the genome. This enzyme is produced by several lactobacilli with both hydrolase and transglycosylase activities, beneficial from technological and health point of views for applications as probiotic cultures in dairy industry or synthesis of prebiotic GOS (Meira et al. 2012). Because they are not digested by humans, GOS represents a rich source of substrate for probiotic organisms, including *Enterococcus* (Park and Oh 2010). They also regulate the pH of the digestive tract and facilitate digestion. In addition to the prebiotic effects of GOS, transglycosylation also decreases the sugar concentration in foods and increases fiber content (Husain 2010).

In a previous study, it was observed that *E. durans* LAB18S bioaccumulates selenium when grown in medium containing  $\text{Na}_2\text{SeO}_3$  (Pieniz et al. 2017). Selenium was mainly found as selenoproteins, reaching 2.6 mg/g biomass. Selenium is an essential metalloid required for the expression of selenoproteins. Its antioxidant properties stimulates the activity of glutathione peroxidase, an antioxidant enzyme (Lin et al. 2015). Some enzymes

(selenoenzymes), including glutathione peroxidase, iodothyronine deiodinase, and thioredoxin reductase contain selenocysteine. To date, one biological form of Se has been identified as selenocysteine (Sec) (Hatfield and Gladyshev 2002). Selenium could form selenomethionine (SeMet) with the replacement of sulfur by methionine and thus could be incorporated into proteins instead of methionine (Schrauzer 2000). Although some microorganisms are capable of transforming high concentrations of selenium into selenate and selenite, only few studies on selenite uptake and biotransformation have been conducted with probiotic microorganisms (Zhang et al. 2009; Pieniz et al. 2017).

Selenoprotein genes, to insert SEC into UGA codons, have developed a stem-loop shaped RNA structure, called SECIS. These SECIS elements are located downstream of the Sec UGA codons in bacteria. Through a computer program we were able to identify conserved structural characteristics of these structures. Bacterial SECISearch recognize a bacterial consensus SECIS element in sequence databases and the results indicate the ability of the *E. durans* LAB18S to produce selenoproteins.

Enterococci may have resistance to various antibiotics, due to their innate resistance to widely used antibiotics such as penicillin or to their ability to easily acquire antimicrobial resistance, especially by horizontal gene transfer. Horizontal transfer of antimicrobial resistance in enterococci has been associated with mobile genetic elements, such as plasmids and transposons (Palmer et al. 2012; Beukers et al. 2015). Resistance to tetracycline in *Enterococcus* spp. is frequently associated with the resistance genes *tet(M)* and *tet(O)* (Roberts 2005; Anderson et al. 2016). Recently, a PCR-based plasmid classification system has been established by targeting specific replicon initiation genes (*rep*) of plasmid DNA. Rep-family, already found in the genus *Enterococcus*, may confer multiple antibiotic resistance as well as the mechanism of stabilization of toxin-antitoxin plasmids (Zankari et

al. 2012, Bonacina et al. 2017). The absence of such genetic elements in *E. durans* LAB18S reinforce its promising as probiotic strain. Another recent study concludes that a cheese isolate *E. faecalis* does not represent a substantial reservoir of antimicrobial resistance and virulence when compared to clinical strains (Silveti et al. 2019).

In summary, the genome of *E. durans* LAB18S presents a variety of genes that can be associated with probiotic properties, such as adhesion properties, viability at lower pH, bile salt tolerance, production of bacteriocins, and utilization of prebiotic molecules. Besides, this strain presents genes encoding for known selenoproteins, which should contribute to the antioxidant properties. In comparison with other *E. durans* genomes, LAB18S was the only food isolate with absence of plasmids, virulence and antimicrobial resistance genes. *E. durans* LAB18S exhibited a probiotic potential and its potential health benefit and application as probiotic strain in the feed industry merits future investigation. This work significantly improved the knowledge on the genetic characteristics of this promising strain.

### **Acknowledgments**

This work received financial support from CAPES, CNPq (grant 307455/2013-0) and FINEP Mais Água (grant 0112.0113).

### **Conflicts of interest**

The authors declare no conflicts of interest regarding this manuscript.

## References

- Anderson AC, Jonas D, Huber I, Karygianni L, Wölber J, Hellwig E, Arweiler N, Vach K, Wittmer A, Al-Ahmad A (2016) *Enterococcus faecalis* from food, clinical specimens, and oral sites: prevalence of virulence factors in association with biofilm formation. *Front Microbiol* 6:1534
- Beukers AG, Zaheer R, Cook SR, Stanford K, Chaves AV, Ward MP, McAllister TA (2015) Effect of in-feed administration and withdrawal of tylosin phosphate on antibiotic resistance in enterococci isolated from feedlot steers. *Front Microbiol* 6:483
- Bonacina J, Suárez N, Hormigo R, Fadda S, Lechner M, Saavedra L (2017) A genomic view of food-related and probiotic *Enterococcus* strains. *DNA Res* 24:1-14
- Botta C, Langerholc T, Cencic A, Cocolin L (2014) *In vitro* selection and characterization of new probiotic candidates from table olive microbiota. *PLoS ONE* 9:e94457
- Byappanahalli MN, Nevers MB, Korajkic A, Stanley ZR, Harwood VJ (2012) Enterococci in the environment. *Microbiol Mol Biol Rev* 76:685-706
- Carattoli A, Zankari E, García-Fernández A, Voldby LM, Lund O, Villa L, Aarestrup FM, Hasman H (2014) *In Silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 58:3895-3903
- Clemente JC, Ursell LK, Partry LW, Knight R (2012) The impact of the gut microbiota on human health: an integrative view. *Cell* 148:1258-1270
- Douillard FP, de Vos WM (2014) Functional genomics of lactic acid bacteria: from food to health. *Microb Cell Fact* 13:S1-S8
- Fernández-García L, Blasco L, Lopez M, Bou G, García-Contreras R, Wood T, Tomas M (2016) Toxin-Antitoxin systems in clinical pathogens. *Toxins* 8:227
- Franz CM, Huch M, Abriouel H, Holzapfel W, Gálvez A (2011) Enterococci as probiotics and their implications in food safety. *Int J Food Microbiol* 151:125-140
- Galano E, Mangiapane E, Bianga J, Palmese A, Pessione E, Szpunar J, Lobinski R, Amoresano A (2013) Privileged incorporation of selenium as selenocysteine in *Lactobacillus reuteri* proteins demonstrated by selenium-specific images and proteomics. *Mol Cell Proteom* 12:2196-2204
- Gillor O, Etzion A, Riley MA (2008) The dual role of bacteriocins as anti- and probiotics. *Appl Microbiol Biotechnol* 81:591-606
- Guo L, Li T, Tang Y, Yang L, Huo G (2015) Probiotic properties of *Enterococcus* strains isolated from traditional naturally fermented cream in China. *Microb Biotechnol* 9:737-745
- Hamon E, Horvatovich P, Izquierdo E, Bringel F, Marchioni E, Aoudé-Werner D, Ennahar S (2011) Comparative proteomic analysis of *Lactobacillus plantarum* for the identification of key proteins in bile tolerance. *BMC Microbiol* 11:63
- Hatfield DL, Gladyshev VN (2002) How selenium has altered our understanding of the genetic code. *Mol Cell Biol* 22:3565-3576
- Hatfield DL, Berry MJ, Gladyshev VN (2006) Selenium - Its molecular biology and role in human health. Springer, New York
- Havarstein LS, Holo H, Nes IF (1994) The leader peptide of colicin V shares consensus sequences with leader peptides that are common among peptide bacteriocins produced by Gram positive bacteria. *Microbiology* 140:2383-2389

- Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME (2014) Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 11:506-514
- Husain Q (2010) Beta galactosidases and their potential applications: a review. *Crit Rev Biotechnol* 30:41-62
- Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM (2014) Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol* 52:1501-1510
- Ladero V, Linares DM, del Rio B, Fernandez M, Martin MC, Alvarez MA (2013) Draft genome sequence of the tyramine producer *Enterococcus durans* strain IPLA 655. *Genome Announc* 1:265-213
- Langa S, Arqués J, Medina M, Landete J (2017) Coproduction of colicin V and lactic acid bacteria bacteriocins in lactococci and enterococci strains of biotechnological interest. *J Appl Microbiol* 122:1159-1167
- Lavermicocca P, Valerio F, Lonigro SL, Di Leo A, Visconti A (2008) Antagonistic activity of potential probiotic lactobacilli against the ureolytic pathogen *Yersinia enterocolitica*. *Curr Microbiol* 56:175-181
- Letunic I, Bork P (2019) Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 47:W256-W259
- Li B, Evvie SE, Jin D, Meng Y, Li N, Yan F, Huo G, Liu F (2018) Complete genome sequence of *Enterococcus durans* KLDS6.0933, a potential probiotic strain with high cholesterol removal ability. *Gut Pathog* 10:32
- Liaskovs'kyi TM, Pidhors'kyi VS, Kovalenko NK, Harmasheva IL, Muchnyk FV (2008) Identification of probiotic lactic acid bacteria strains. *Mikrobiol Z* 70:3-9
- Lin HC, Ho SC, Chen YY, Khoo KH, Hsu PH, Yen HCS (2015) CRL2 aids elimination of truncated selenoproteins produced by failed UGA/Sec decoding. *Science* 349:91-95
- Meira SMM, Helfer VE, Velho RV, Lopes FC, Brandelli A (2012) Probiotic potential of *Lactobacillus* spp. isolated from Brazilian regional ovine cheeses. *J Dairy Res* 79:119-127
- Palmer KL, Godfrey P, Griggs A, Kos VN, Zucker J, Desjardins C, Cerqueira G, Gevers D, Walker S, Wortman J, Feldgarden M, Haas B, Birren B, Gilmore MS (2012) Comparative genomics of enterococci: variation in *Enterococcus faecalis*, clade structure in *E. faecium*, and defining characteristics of *E. gallinarum* and *E. casseliflavus*. *MBio* 3:e00318-11
- Park AR, Oh DK (2010) Galacto-oligosaccharide production using microbial beta-galactosidase: current state and perspectives. *Appl Microbiol Biotechnol* 85:1279-1286
- Pedersen K, Zavialov AV, Pavlov M, Elf J, Gerdes K, Ehrenberg M (2003) The bacterial toxin RelE displays codon-specific cleavage of mRNAs in the ribosomal A site. *Cell* 112:131-140
- Pieniz S, Andreatza R, Anghinoni T, Camargo FAO, Brandelli A (2014) Probiotic potential, antimicrobial and antioxidant activities of *Enterococcus durans* strain LAB18s. *Food Control* 37:251-256
- Pieniz S, Andreatza R, Mann MB, Camargo FAO, Brandelli A (2017) Bioaccumulation and distribution of selenium in *Enterococcus durans*. *J Trace Elem Med Biol* 40:37-45
- Price MN, Dehal PS, Arkin AP (2010) FastTree 2 - Approximately maximum-likelihood trees for large alignments. *PLoS ONE* 5:e94-90

- Roberts MC (2005) Update on acquired tetracycline resistance genes. *FEMS Microbiol Lett* 245:195-203
- Ruas-Madiedo P, Gueimonde M, Margolles A, de los Reyes-Gavilán CG, Salminen S (2006) Exopolysaccharides produced by probiotic strains modify the adhesion of probiotics and enteropathogens to human intestinal mucus. *J Food Protec* 69:2011-2015
- Schrauzer GN (2000) Selenomethionine: a review of its nutritional significance, metabolism and toxicity. *J Nutr* 130:1653-1656
- Senan S, Prajapati JB, Joshi CG (2015) Whole-genome based validation of the adaptive properties of Indian origin probiotic *Lactobacillus helveticus* MTCC5463. *J Sci Food Agric* 95:321-328
- Silvetti T, Morandi S, Brasca M (2019) Does *Enterococcus faecalis* from traditional raw milk cheeses serve as a reservoir of antibiotic resistance and pathogenic traits? *Foodborne Pathog Dis* 16:359-367
- Slavin J (2013) Fiber and prebiotics: mechanisms and health benefits. *Nutrients* 5:1417-1435
- Treangen TJ, Ondov BD, Koren S, Phillippy AM (2014) The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. *Genome Biol* 15:2-15
- Wang Y, Wang J, Dai W (2011) Use of GFP to trace the colonization of *Lactococcus lactis* WH-C1 in the gastrointestinal tract of mice. *J Microbiol Meth* 86:390-392
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV (2012) Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 67:2640-2644
- Zhang Y, Gladyshev VN (2005) An algorithm for identification of bacterial selenocysteine insertion sequence elements and selenoprotein genes. *Bioinformatics* 21:2580-2589
- Zhang B, Zhou K, Zhang J, Chen Q, Liu G, Shang N, Qin W, Li P, Lin F (2009) Accumulation and species distribution of selenium in Se-enriched bacterial cells of the *Bifidobacterium animalis* 01. *Food Chem* 115:727-734
- Zhong Z, Zhang W, Song Y, Liu W, Xu H, Xi X, Menghe B, Zhang H, Sun Z (2017) Comparative genomic analysis of the genus *Enterococcus*. *Microbiol Res* 196:95-105



## ARTIGO CIENTÍFICO 2

---

### **Proteomic study of *Enterococcus durans* LAB18S growing on prebiotic oligosaccharides.**

Aceito para publicação no periódico Food Microbiology em 13 de Janeiro de 2020, fator de impacto 4.089.

**Proteomic study of *Enterococcus durans* LAB18S growing on prebiotic oligosaccharides.**

Carolina Baldisserotto Comerlato,

Ana Carolina Ritter,

Kendi Nishino Miyamoto,

Adriano Brandelli\*

Laboratório de Bioquímica e Microbiologia Aplicada, Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, 91501-970 Porto Alegre, Brazil

\* **Corresponding author:** A. Brandelli, ICTA-UFRGS, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brazil; E-mail: [abrand@ufrgs.br](mailto:abrand@ufrgs.br)

## Abstract

The study evaluates the ability of prebiotic carbohydrates, namely fructooligosaccharides (FOS) and galactooligosaccharides (GOS), to modulate the protein expression of *Enterococcus durans* LAB18S. This isolate has probiotic properties and grew in FOS, GOS and Glucose (used as control) at a concentration of 10 g L<sup>-1</sup>. Thus, proteins from these cultures were extracted for mass spectrometer analysis. A total of 771 proteins were identified and 135 *E. durans* proteins were validated by the Scaffold algorithm. The proteins were functionally categorized according to Gene Ontology terms. Both FOS and GOS were used as carbon source by *E. durans* LAB18 that may be associated with a positive regulation of protein production related to intestinal mucosa adhesion, carbohydrate and nitrogen metabolism and stress response. Cells grown with GOS showed an increased expression of the cell division protein divIVA, EF-Tu, GAPDH that can be associated with human epithelial cell adhesion. In addition to stimulating amino acid metabolism and energy conversion, the use of FOS stimulated the production of ClpX protein, which plays an important role in protein turnover. The results of this study suggest that FOS and GOS can be metabolized by *E. durans* and stimulate the microorganism to produce proteins related to some desirable characteristics for a probiotic. This approach provides information on implications of the use of different oligosaccharides on the physiology of probiotic bacteria.

**Keywords:** *Enterococcus*; proteome; fructooligosaccharides; galactooligosaccharides; probiotics; prebiotics.

## 1. Introduction

The genus *Enterococcus* comprise Gram-positive, catalase-negative, non-spore-forming, facultative anaerobic lactic acid bacteria, which are normal inhabitants of the gut microbiota of humans and other animals. Although some species/strains can be pathogenic, enterococci are traditionally recognized as a commensal bacterium (Fisher and Phillips, 2009). In addition, some *Enterococcus* strains have positive effects on gastrointestinal system by enhancement of the epithelium barrier, production of antimicrobial substances, competitive exclusion of pathogenic microorganism and modulation of the immune system (Gupta and Tiwari, 2015). These strains are usually referred as probiotics.

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (Hill et al., 2014). Probiotic microorganisms besides being able to survive in the gastrointestinal tract should also be able to multiply in the gut. In addition, some specific probiotic characteristics are required to improve health safety, namely mucus and epithelial host cell adherence, antimicrobial activity against pathogens, ability to hydrolyze bile salts, and absence of hemolytic activity (Gupta and Tiwari, 2015). Thus, the ability of probiotics to impart health benefits has prompted an increasing scientific interest. Some potential benefits of probiotic enterococci include reducing gut inflammation, improvement of immune system response and, in an indirect way, reducing the risk of colon cancer (Hew et al., 2007; Werner et al., 2013).

Currently, the most popular strategy to modulate the composition and/or metabolic activity of the human gut microbiome is the intake of probiotics, prebiotics or their combinations (Gibson et al., 2017). Prebiotics are substances not digestible by humans that modify the intestinal microbiota and play a selective role in stimulating the growth - or

activity - of beneficial bacterial species in the gut environment (Thomas, 2016; Moreno et al., 2017). Commercially available prebiotics, namely inulin, fructooligosaccharides (FOS), and galactooligosaccharides (GOS) are abundant in some foods and are mainly consumed by species of *Lactobacillus* and *Bifidobacterium* (Rastall and Gibson, 2015). Numerous studies evidence the prebiotic effects of FOS and GOS, which currently dominate this category of beneficial substances (Gibson et al., 2017). Advances in microbiological research led to formation of synbiotics, which are the combination of probiotics and prebiotics products (Tufarelli and Laudadio, 2016). The positive results of such combinations have attracted the interest of food and health companies, focusing in creating functional foods containing synbiotics. Research in this area is devoted on developing health-promoting foods by selecting new cultures with probiotic properties and ability to digest prebiotic substances (Hoseinifar et al., 2017).

*Enterococcus durans* strain LAB18S was isolated from a typical Brazilian soft cheese, and its probiotic and safety characteristics have been described (Pieniz et al., 2014). In addition, this strain is able to promote selenium bioaccumulation mostly as organic selenium in the form of selenoproteins (Pieniz, et al., 2017). In this study, the ability of prebiotic carbohydrates to support the growth of *E. durans* LAB18S was investigated, and the protein expression under different conditions was studied using a proteomic approach. The data from this study provided a significant amount of complementary and comparative information of the different proteins and metabolic pathways by the utilization of prebiotics by the probiotic strain.

## 2. Materials and methods

### 2.1. Bacterial strain and growth conditions

*Enterococcus durans* LAB18S was recovered from the collection of the Laboratory of Applied Microbiology and Biochemistry (Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil). The strain was maintained as frozen stock cultures in Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK) containing 20% (v/v) glycerol.

The carbohydrates tested as carbon source to analyze the growth and protein expression of *E. durans* LAB18S were FOS (Sigma-Aldrich, St. Louis, MO. USA), GOS (Yakult, Almere, Netherlands) and glucose (Synth, Diadema, Brazil), used as control. The carbohydrates were added to a semisynthetic medium (SM) to obtain a working concentration of 10 g L<sup>-1</sup> (Rossi et al., 2005). *E. durans* inoculum was obtained in MRS broth (Kasvi, São José dos Pinhais, Brazil) under anaerobic conditions (GasPak, BD Diagnostics, Sparks, USA). After 24 h this culture was inoculated (2%, v/v) into 10 mL of SM containing 10 g L<sup>-1</sup> of each carbon source (glucose, FOS or GOS). The cultures were incubated anaerobically at 37°C for 30 h. Growth was determined by measuring the final pH and the optical density at 600 nm (OD<sub>600</sub>) at 0, 4, 8, 12, 24 and 30 h post-inoculation. Each experiment was performed in triplicate.

## **2.2. Protein extraction and quantification**

For protein extraction, samples were removed at late exponential growth phase (8 h). The harvested cells were washed with PBS buffer and centrifuged three times at 10,000 g for 5 min. Samples were lyophilized and macerated with liquid nitrogen in order to disrupt the cells, and then suspended in 50 mM Tris-HCl pH 7.5 containing protease inhibitors (Halt Protease, Thermo Scientific, Waltham, MA, USA). The protein suspensions were vortexed for 5 min at intervals of 1 min on ice. This procedure was repeated five times, followed by centrifugation at 10,000 g for 20 min at 4°C. After centrifugation, each supernatant was pooled with the first supernatant and stored at -80°C. The protein concentration was determined by the Coomassie dye binding assay (Bradford, 1976) using known concentrations of bovine serum albumin as a standard.

## **2.3. Sample preparation for mass spectrometry**

The protocol for protein digestion was adapted from Villén and Gygi (2008). The extracted proteins were denatured in 8 M urea (1:1 v/v) for 30 min at room temperature, reduced and alkylated in 5 mM dithiothreitol (DTT, Sigma-Aldrich) and 14 mM iodoacetamide (IAA, Sigma-Aldrich), respectively. A further addition of 5 mM DTT for 15 min was performed to eliminate the unreacted IAA. Samples were diluted in 50 mM ammonium bicarbonate (1:5 v/v) and 1 mM CaCl<sub>2</sub> was added to serve as a trypsin co-factor. Proteins were digested using 20 µg of trypsin (Sequencing Grade Modified Trypsin V5111, Promega, Fitchburg, WI, USA) suspended in 50 mM ammonium bicarbonate (final

concentration of 20 ng/ $\mu$ L). The digestion was stopped with 0.4% (v/v) trifluoroacetic acid and then centrifuged at 25,000 g for 10 min. The peptide extracts were desalinated with a C18 Sep-Pak column (Waters, Milford, MA, USA), according to the manufacturer's instructions and further dried in a Speed-Vac apparatus (Eppendorf AG, Hamburg, Germany). Three biological replicates for each culture (glucose, FOS and GOS) were utilized for proteomic experiments. Each sample eluted from the C18 resin was run three times (LC-MS/MS technical replicates).

#### **2.4. LC-MS/MS sample analysis**

LC-MS/MS analysis was performed at the Brazilian Biosciences National Laboratory (LNBio-CNPEM, Campinas, Brazil). Each test and control samples were analyzed separately using an ESI-Q-ToF Premier API mass spectrometer (MicroMass/Waters), attached to a nanoACQUITY™ ultra performance liquid chromatography (UPLC) system (Waters). Ten micrograms of each peptide sample was injected in an analytic ACQUITY UPLC peptide BEH C18 nanoACQUITY column (130 Å, 1.7  $\mu$ m, 100  $\mu$ m  $\times$  100 mm) with a 2-90% acetonitrile gradient in 0.1% formic acid for 60 min, at a 200 nL/min flow rate.

The MS spectra were recorded (m/z range 100-2000 Da), with 1-second search time. The MS/MS spectra with a mass between m/z 50-2000 Da with the same search time mentioned for MS mode were obtained using the MassLynx software system (Waters). Samples were analyzed in a data dependent acquisition mode, which means that MS mode run was followed by three MS/MS runs of the three most intense multiple charged ions. The



energy of collision for peptide fragmentation were set by the +2, +3 and +4 ion charges data available by the MassLynx software. The raw MS/MS data were processed using Mascot distiller v. 2.3.02 (Matrix Science, London, UK) to generate the peak list files.

## **2.5. LC-MS/MS data analysis**

The protein search was based on the amino acid sequences (24018 entries) from *Enterococcus durans* KLDS60933 strain, available at UniProtKB website (<http://www.uniprot.org/proteomes/>, proteome ID 53345, last update: 26/06/2018). The raw MS/MS data were processed using the Mascot ion version 2.3.02, and the cysteine carbamidomethylation (monoisotopic mass 57.0215 Da) was used as a fixed modification, the oxidation of methionine (monoisotopic mass 15.9949 Da) as a variable modification, and a peptide and MS/MS fragment ion mass tolerance of 0.1 Da. Some other parameters were set to include missed cleavages, and Mascot's automated decoy database search was selected. The obtained \*.dat files of all fractions were processed by Scaffold (version Scaffold\_4.8.7, Proteome Software Inc., Portland, OR, USA). In order to improve the reliability of protein identification some parameters were used such as: fragment ion mass tolerance of 0.100 Da, parent ion and parent ion tolerance of 0.100 Da and the charge state deconvolution and deisotoping were not performed. Protein identifications were accepted if they could be established at greater than 57% probability (using Peptide Prophet algorithm) and peptide identifications were accepted if greater than 73% (using Scaffold Local FDR algorithm) contained at least 1 identified peptide and obtaining a FDR <1%. The normalized spectral abundance factor (NSAF) was calculated for each protein, and the quantitative

differences were analyzed by ANOVA through Scaffold. Values of  $p < 0.05$  were considered statistically significant. Hypothetical proteins were identified from blastx and blastp searches of the GenBank database and all identified proteins were categorized according to the terms of the gene ontology using Blast2GO software version 3.0.7 (BioBam, Valencia, Spain).

### 3. Results

*E. durans* LAB18S reached a maximum OD600 of 1.405 during growth in SM medium supplemented with glucose (Fig 1). Distinct growth amounts were observed for the strain when the oligosaccharides FOS and GOS were used as source of carbon/energy. Growth with GOS had a maximum OD600 of 0.867, whereas the growth using FOS resulted a maximum OD600 of 0.748. Comparing the three different carbon sources tested, the glucose-containing medium resulted in greater biomass accumulation. However, the growth curves showed a similar profile, with the stationary phase starting at about 8 h post inoculation and maintained until the end of incubation at 30 h.

The proteins extracted from *E. durans* LAB18S growing on different carbon sources were studied by mass spectrometry. The analysis of MS/MS data resulted in the identification of 771 proteins (Supplementary Table S1). Thereafter, the parameters of Scaffold (see methods section) were adjusted to assure the precise identification of peptides and proteins. These settings allowed the identification of 135 *E. durans* proteins (Supplementary Table S2) and these were considered proper for statistical analysis.

Reproducibility of the biological replicates were evaluated and approximately 95% of coincidence among them were shown for each type of sample (GLU, FOS and GOS).

The 135 Scaffold validated *E. durans* proteins were functionally categorized according to GO terms, being associated to different biological processes (150 terms), molecular functions (229 terms), and cellular component (78 terms). The GO profile is depicted in Fig. 2. Regarding the biological processes, the proteins identified were mostly associated with metabolic processes (Fig. 2A), including organic substance, primary, cellular, biosynthetic, and small molecule metabolic processes. Interestingly, proteins associated with regulation of metabolic process, protein folding, stress response and cell homeostasis were lesser expressed. GO analysis in relation to molecular functions showed that proteins related with binding to organic, heterocyclic and small molecules were among the most relevant (Fig. 2B). A diversity of molecular functions was observed among proteins expressed, including oxidoreductase, isomerase and lyase activities. Considering the cellular components, the proteins were essentially categorized as intracellular part and ribonucleoprotein complex (Fig. 2C). Some proteins associated with cell periphery/plasma membrane and transporter complex were also assigned as differentially regulated in response to prebiotics.

The detailed assignment of 135 Scaffold validated proteins is presented in Supplementary Table S3. The results of GO analysis provide a global view of the proteins expressed after growth on different carbohydrates. The assortment of molecular functions and cellular component assignments could be related to the diversity of proteins that were uniquely identified or upregulated in response to different carbohydrates.

The majority of identified proteins were present in all conditions tested. The number of proteins commonly expressed in *E. durans* grown on FOS, GOS and glucose was 81 (Fig. 3). Seven proteins were only observed in GOS culture, related with serine metabolism, ribosomal proteins and stress response proteins. In the FOS culture, 11 exclusive proteins were detected, including enzymes related with carbohydrate metabolism, a mannose transporter, and proteins implicated in ribosome function. Six proteins were only found in the glucose-cultured cells, such as enzymes associated with nitrogen metabolism, pyruvate oxidase, and a Clp protease associated with stress response.

Proteins showing significant differential expression when comparing the treatments glucose (GLU), FOS and GOS are listed in Table 1. GO classification showed that these proteins are mostly related with metabolic processes, molecular binding and stress response mechanisms. Among these proteins, five were overexpressed in the group treated with FOS. CplX protease is related with bacterial stress. In addition, a hypothetical protein LIANG\_08515 was overexpressed in FOS-treated group and it was identified as similar to the Y1bF family regulator. Other proteins overexpressed in cells treated with FOS were ornithine carbamoyl transferase and 2-oxoisovalerate dehydrogenase, involved in amino acids metabolism. In FOS cultures, specific proteins related with ribosome structure were identified. GOS group showed overexpressed proteins associated with hexose metabolism such as tagatose-bisphosphate aldolase and glucose-6-phosphate isomerase. EF-Tu factor and GAPDH (glyceraldehyde 3-phosphate dehydrogenase), which have been implied with cell adherence, were overexpressed in GOS and glucose cultures (Table 1). The proteins overexpressed in FOS and glucose culture were a hypothetical protein identified as periplasmic binding domain of basic membrane lipoprotein (PnrA) and a SDR family

oxidoreductase. About the cells treated with glucose, overexpressed proteins were related with hexose-monophosphate pathway and amino acids metabolism.

#### **4. Discussion**

In this study, a comparative proteomic analysis was performed to investigate the influence of the prebiotics FOS and GOS on the protein expression of *E. durans* LAB18S, a strain showing probiotic properties and selenium bioaccumulation capability (Pieniz et al., 2017). Proteomics of lactic acid bacteria can be useful to study features and adaptation of probiotics in the gastrointestinal tract conditions such as the effect of nutrient sources and various stress conditions (Aires and Butel, 2011).

Glucose served as a positive control as it often supports good growth for lactic acid bacteria and is a preferred carbon source due to the greater facility of metabolizing this compound (Watson et al., 2013). In terms of growth support for the *E. durans*, it has been observed a similar performance for both prebiotic oligosaccharides GOS and FOS. In addition, most Scaffold validated proteins were present in all conditions, suggesting that the strain LAB18S can develop on prebiotic carbohydrates like FOS and GOS as sole carbon source without extensive metabolic adaptation.

FOS and GOS are currently the main prebiotics, as evidenced by numerous studies about their effects on probiotic bacteria. An overview on microbiota interactions and comparative studies on prebiotics suggest that particularly FOS and GOS seem to promote increased abundance of bifidobacteria within the gut microbiota (Simpson and Campbell, 2015). The change of carbon source can promote in the probiotic microorganism a

modification of metabolic pathways (Skalkam et al., 2016). In this study, an increase in the expression of tagatose-bisphosphate aldolase was observed upon cultivation in GOS. This enzyme is typically related to galactose catabolism (Van der Heiden et al., 2015), indicating a direction for metabolic pathways involved with galactose in the cells whose carbon source was GOS.

Cells grown with GOS showed an increased expression of the cell division protein *divIVA*. In Gram-positive bacteria, *divIVA* has been associated to selection of cell division site, peptidoglycan biosynthesis and sporulation (Oliva et al., 2010). A proteomic study on *Listeria monocytogenes* biofilms showed that the induction of the *divIVA* gene expression is directly related with biofilm formation, swarming motility, invasiveness and cell-to-cell spread (Halbedel et al., 2012). *divIVA* is a multifunctional protein in *Enterococcus* spp. related with cell division, viability, polar growth, complete septum closure, morphogenesis and chromosome segregation (Bohle et al., 2010). *E. durans* LAB18S exhibited adhesion properties and a strong capacity of biofilm formation, but this capacity was not associated with virulence-associated genes (Pieniz et al., 2015). Thus, the prebiotic GOS stimulus to the overexpression of *divIVA* protein is probably associated with spreading and adhesion properties, a desirable characteristic of probiotic strains.

EF-Tu was highly expressed in *E. durans* LAB18S growing on GOS and glucose. This protein functions as an essential and conserved GTPase that ensures translational accuracy by catalyzing the reaction adding the correct amino acid to a growing nascent polypeptide chain. However, proteins formerly thought to be limited to the bacterial cytoplasm like EF-Tu and GroEL, have been identified on the surface of *L. johnsonii* La1 (Bergonzelli et al., 2006), suggesting that EF-Tu can be found associated with the cell envelope of lactic acid bacteria. Indeed, these proteins were further described as cell wall-

associated proteins that can be released from lactobacilli by osmotic shock and have been associated with adhesion to mucin and human epithelial cells (Gilad et al., 2011). Besides, the glycolytic enzymes GAPDH and glucose-6-phosphate isomerase were associated to the cell wall of lactic acid bacteria and GAPDH has been enrolled in the adhesion of *Enterococcus* to the intestinal epithelium (Kinoshita et al., 2012). These proteins were also overexpressed in GOS and glucose cultures of *E. durans* LAB18S. The ability to adhere to intestinal epithelial cells is considered an important feature of probiotic strains. These results suggest that GOS and glucose were able to induce proteins related with adhesion in gut epithelium as compared to FOS culture.

Two enzymes involved in amino acid metabolism and energy conversion were among the proteins up-regulated during *E. durans* growth in FOS. Ornithine carbamoyl transferase not only assists the biosynthesis of the amino acid arginine in prokaryotes, but is also involved in the arginine deiminase (ADI) pathway. Lactic acid bacteria can use the ADI pathway to convert arginine into ornithine via citrulline while producing ATP and ammonia, thus gaining an energetic advantage and handling with acid stress (Vrancken et al., 2009). The overexpression of 2-oxoisovalerate dehydrogenase, implicated in Leu, Ile, Val, Phe, Tyr, Trp, Asp and Asn catabolism, was observed in FOS. This enzyme is a functional component of the branched-chain alpha-keto dehydrogenase complex, which catalyzes the general conversion of alpha-keto acids to acyl-CoA and CO<sub>2</sub>. This enzyme was also overexpressed when *Enterococcus mundtii* CRL35 was co-cultivated for 6 h with *E. coli* (Orihuel et al., 2018), suggesting a role in gathering nitrogen metabolism and energy production during the first hours of growth.

Another protein up-regulated in FOS-treated cells was ClpX, a member of Clp proteases that are conserved in most bacterial species and have an important role in protein

turnover. Besides protein homeostasis and degradation of misfolded proteins, Clp have a key role in several regulatory processes by targeting transcriptional regulators associated with cell division, morphological differentiation, stress tolerance and antibiotic resistance (Frees et al., 2007; Konovalova et al., 2014). The amounts of major growth-phase-regulated proteins in *E. coli* are regulated at some point by the activity of at least one of the Clp proteins. Wild type cultures showed increased viability during extended stationary phase as compared with strains lacking functional ClpP or ClpX (Weichart et al., 2003). A proteomic study in *E. coli* suggested that ClpXP has great capacity to process damage-response proteins, controlling the levels of many stress response proteins, and contributing to cell survival (Neher et al., 2006).

In a recent study, the dairy-isolated *E. faecalis* D27 was compared to a commercial probiotic *E. faecalis* Symbioflor1 and to a clinical isolate *E. faecalis* UW3114 (Cirrincione et al., 2019). The Symbioflor1 proteome revealed several proteins that support its probiotic role, specifically involved in stress response, which are essential for bacterial survival in the gastrointestinal tract. In addition, the strain D27 showed some proteins possibly involved in pathogenicity, such as hemolysin and penicillin-binding protein 1B, which were absent in the probiotic.

Two hypothetical proteins were observed among the differentially expressed proteins and identified on blastx and blastp searches with sequence similarity with the *E. durans* species. One of them is LIANG\_08515, which belongs to YlbF protein superfamily and was overexpressed in FOS treatment. This protein is part of the YmcA-YlbF-YaaT complex that regulates sporulation, biofilm formation and transformation capacity (Dubnau et al., 2016). In this regard, *E. durans* has been recognized as a bacterium that can develop biofilms and become competent to acquire extracellular DNA from the environment. In *B. subtilis*, the



YlbF-YmcA-YaaT complex interacts with Rny ribonuclease, and this interaction stimulates the disruption of the *sinR* transcript (DeLoughery et al., 2016). This condition avoids the accumulation of SinR, a repressor of biofilm formation.

The second differentially expressed hypothetical protein, LIANG\_06205, presents a periplasmic binding domain of basic membrane lipoprotein PnrA (purine nucleoside receptor A). *Treponema pallidum* PnrA was described as a new bacterial transporter of nucleosides, the first one reported in spirochetes (Deka et al., 2006). It functions as an ATP-binding cassette (ABC)-type substrate-binding protein, which is a class of proteins associated with molecular trafficking in bacteria (Maqbool et al., 2015). Access to nucleosides appears vital for the survival of *E. durans* in the host and recent studies show that purines and pyrimidines can stimulate the growth of some *Enterococcus* strains (Khan et al., 2013). Thus, the ability to uptake purines from the environment could be an important determinant of *E. durans* LAB18S aptness.

The current knowledge on the complexity and usability of prebiotic oligosaccharides has increased because of the development of various 'omic' tools (Moreno et al., 2017). The data presented here suggest that FOS and GOS can be used as a carbon source by probiotic *E. durans* as well as stimulated the microorganism to produce different proteins and/or induced different levels of protein expression, including intestinal mucosa adhesion proteins, metabolic enzymes, and stress response proteins. The upregulation of enzymes implicated in carbohydrate and nitrogen metabolism, energy production and ribosomal proteins can be related with the metabolism and the physiological state during the first hours of growth. Although the pure culture models used in this work do not reflect the environmental experiences that bacteria face in the host, this approach provides relevant information on the

physiological implications of using different oligosaccharides, contributing to the understanding of probiotic bacteria functionalities.

## **Acknowledgments**

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) [grant 306936/2017-8]. Authors thank the National Laboratory of Biosciences (LNBio, Brazil, Project MAS-21023) for support in mass spectrometry analysis.

## **Conflicts of interest**

Authors declare no conflicts of interest regarding this manuscript.

## **References**

Aires, J., Butel M.J., 2011. Proteomics, human gut microbiota and probiotics. *Expert Rev. Proteomics* 8, 279-288.

Bergonzelli, G.E., Granato, D., Pridmore, R.D., Marvin-Guy, L.F., Donnicola, D., Cortesy-Theulaz, I.E., 2006. GroEl of *Lactobacillus johnsonii* La1 (NCC533) is cell surface associated: potential role in interactions with the host and the gastric pathogen *Helicobacter pylori*. *Infect. Immun.* 74, 425-434.

- Bohle, L.A., Færgestad, E.M., Veiseth-Kent, E., Steinmoen, H., Nes, I.F., Eijsink, V.G., Mathiesen, G., 2010. Identification of proteins related to the stress response in *Enterococcus faecalis* V583 caused by bovine bile. *Proteome Sci.* 8, 37.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248-254.
- Cirrinzione, S., Neumann, B., Zühlke, D., Riedel, K., Pessione, E., 2019. Detailed soluble proteome analyses of a dairy-isolated *Enterococcus faecalis*: a possible approach to assess food safety and potential probiotic value. *Front. Nutr.* 6, 71.
- Deka, R.K., Brautigam, C.A., Yang, X.F., Blevins, J.S., Machius, M., Tomchick, D.R., Norgard, M.V., 2006. The PnrA (Tp0319; TmpC) lipoprotein represents a new family of bacterial purine nucleoside receptor encoded within an ATP-binding cassette (ABC)-like operon in *Treponema pallidum*. *J. Biol. Chem.* 281, 8072-8081.
- DeLoughery, A., Dengler, V., Chai, Y., Losick, R., 2016. Biofilm formation by *Bacillus subtilis* requires an endoribonuclease-containing multisubunit complex that controls mRNA levels for the matrix gene repressor SinR. *Mol. Microbiol.* 99, 425-437.
- Dubnau, E.J., Carabetta, V.J., Tanner, A.W., Miras, M., Diethmaier, C., Dubnau, D., 2016. A protein complex supports the production of Spo0A-P and plays additional roles for biofilms and the K-state in *Bacillus subtilis*. *Mol. Microbiol.* 101, 606-624.
- Fisher, K., Phillips, C., 2009. The ecology, epidemiology and virulence of *Enterococcus*. *Microbiology* 155, 1749-1757.
- Frees, D., Savijoki, K., Varmanen, P., Ingmer, H., 2007. Clp ATPases and ClpP proteolytic complexes regulate vital biological processes in low GC, gram-positive bacteria. *Mol. Microbiol.* 63, 1285-1295.
- Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J., Scott, K., Stanton, C., Swanson, K.S., Cani, P.D., Verbeke, K., Reid, G., 2017. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* 14, 491-502.
- Gilad, O., Svensson, B., Viborg, A.H., Stuer-Lauridsen, B., Jacobsen, S., 2011. The extracellular proteome of *Bifidobacterium animalis* subsp. *lactis* BB-12 reveals proteins with putative roles in probiotic effects. *Proteomics* 12, 2503-2514.
- Gupta A., Tiwari S.K., 2014. Probiotic potential of *Lactobacillus plantarum* LD1 isolated from batter of DOSA, a South Indian fermented food. *Probiotics Antimicrob. Proteins* 6, 73-81.
- Halbedel, S., Hahn, B., Daniel, R.A., Flieger, A., 2012. DivIVA affects secretion of virulence related autolysins in *Listeria monocytogenes*. *Mol. Microbiol.* 83, 821-839.

- Hew, C.M., Korakli, M., Vogel, R.F., 2007. Expression of virulence-related genes by *Enterococcus faecalis* in response to different environments. *Syst. Appl. Microbiol.* 30, 257-267.
- Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C., Sanders, M.E., 2014. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* 11, 506-514.
- Hoseinifar, S.H., Ahmadi, A., Raeisi, M., Hoseini, S.H., Khalili, M., Behnampour, N., 2017. Comparative study on immunomodulatory and growth enhancing effects of three prebiotics (galactooligosaccharide, fructooligosaccharide and inulin) in common carp (*Cyprinus carpio*). *Aquac. Res.* 48, 3298-3307.
- Khan, H., Flint, S.H., Yu, P.L., 2013. Development of a chemically defined medium for the production of enterolysin A from *Enterococcus faecalis* B9510. *J. Appl. Microbiol.* 114, 1092-1102.
- Kinoshita, H., Imoto, S., Suda, Y., Ishida, M., Watanabe, M., Kawai, Y., Saito, T., 2012. Proposal of screening method for intestinal mucus adhesive lactobacilli using the enzymatic activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). *Anim. Sci. J.* 84, 150-158.
- Konovalova, A., Sjøgaard-Andersen, L., Kroos, L., 2014. Regulated proteolysis in bacterial development. *FEMS Microbiol. Rev.* 38, 493-522.
- Maqbool, A., Horler, R.S.P., Muller, A., Wilkinson, A.J., Wilson, K.S., Thomas, G.H., 2015. The substrate-binding protein in bacterial ABC transporters: dissecting roles in the evolution of substrate specificity. *Biochem. Soc. Transact.* 43, 1011-1017.
- Moreno, F.J., Corzo, N., Montilla, A., Villamiel, M., Olano, A., 2017. Current state and latest advances in the concept, production and functionality of prebiotic oligosaccharides. *Curr. Opin. Food Sci.* 13, 50-55.
- Neher, S.B., Villén, J., Oakes, E.C., Bakalarski, C.E., Sauer, R.T., Gygi, S.P., Baker T.A., 2006. Proteomic profiling of ClpXP substrates after DNA damage reveals extensive instability within SOS regulon. *Mol. Cell* 22, 193-204.
- Oliva, M.A., Halbedel, S., Freund, S.M., Dutow, P., Leonard, T.A., Veprintsev, D.B., Hamoen, L.W., Löwe, J., 2010. Features critical for membrane binding revealed by DivIVA crystal structure. *EMBO J.* 29, 1988-2001.
- Orihuel, A., Terán, L., Renaut, J., Vignolo, G.M., Almeida, A.M., Saavedra, M.L., Fadda, S., 2018. Differential proteomic analysis of lactic acid bacteria-*Escherichia coli* O157:H7 interaction and its contribution to bioprotection strategies in meat. *Front. Microbiol.* 9, 1083.
- Pieniz, S., Andreazza, R., Anghinoni, T., Camargo, F.A.O., Brandelli, A., 2014. Probiotic potential, antimicrobial and antioxidant activities of *Enterococcus durans* strain LAB18s. *Food Control* 37, 251-256.

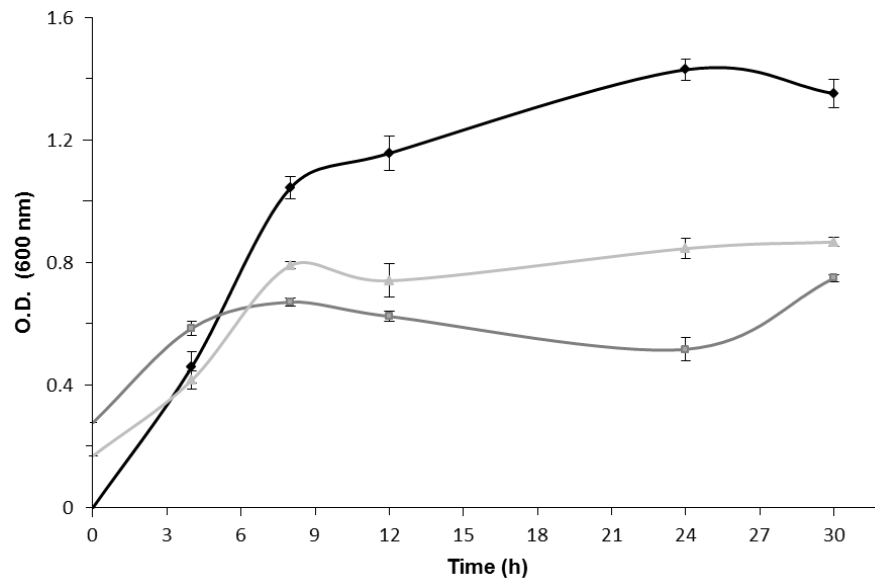
- Pieniz, S., Moura, T.M., Cassenego, A.P., Andrezza, R., Frazzon, A.P.G., Camargo, F.A.O., Brandelli, A., 2015. Evaluation of resistance genes and virulence factors in a food isolated *Enterococcus durans* with probiotic potential. *Food Control* 51, 49-54.
- Pieniz, S., Andrezza, R., Mann, M.B., Camargo, F.A.O., Brandelli, A., 2017. Bioaccumulation and distribution of selenium in *Enterococcus durans*. *J. Trace Elem. Med. Biol.* 40, 37-45.
- Rastall, R.A., Gibson, G.R., 2015. Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. *Curr. Opin. Biotechnol.* 32, 42-46.
- Rossi, M., Corradini, C., Amaretti, A., Nicolini, M., Pompei, A., Zanoni, S., Matteuzzi, D., 2005. Fermentation of fructooligosaccharides and inulin by bifidobacteria: A comparative study of pure and fecal cultures. *Appl. Environ. Microbiol.* 71, 6150-6158.
- Simpson, H.L., Campbell, B.J., 2015. Review article: dietary fibre-microbiota interactions. *Alim. Pharmacol. Ther.* 42, 158-179.
- Skalkam, M.L., Wiese, M., Nielsen, D.S., van Zanten, G., 2016. In vitro screening and evaluation of synbiotics. In: *Probiotics, Prebiotics, and Synbiotics*, Watson R.R., Preedy V.R. (eds.). Elsevier, London, pp. 477-486.
- Thomas, L.V., 2016. Probiotics - the journey continues. *Int. J. Dairy Technol.* 69, 1-12.
- Tufarelli, V., Laudadio, V., 2016. An overview on the functional food concept: perspectives and applied researches in probiotics, prebiotics and symbiotics. *J. Exp. Biol. Agric. Sci.* 4, 274-278.
- Van der Heiden, E., Delmarcelle, M., Simon, P., Counson, M., Galleni, M., Freedberg, D.I., Thompson, J., Joris, B., Battistel, M.D., 2015. Synthesis and physicochemical characterization of D-tagatose-1-phosphate: The substrate of the tagatose-1-phosphate kinase in the phosphotransferase system-mediated D-tagatose catabolic pathway of *Bacillus licheniformis*. *J. Mol. Microbiol. Biotechnol.* 25, 106-119.
- Villén, J., Gygi, S.P., 2008. The SCX/IMAC enrichment approach for global phosphorylation analysis by mass spectrometry. *Nat. Protoc.* 3, 1630-1638.
- Vrancken, G., Rimaux, T., Wouters, D., Leroy, F., De Vuyst, L., 2009. The arginine deaminase pathway of *Lactobacillus fermentum* IMDO 130101 responds to growth under stress conditions of both temperature and salt. *Food Microbiol.* 26, 720-727.
- Watson, D., O'Connell Motherway, M., Schoterman, M.H., van Neerven, R.J., Nauta, A., van Sinderen D., 2013. Selective carbohydrate utilization by lactobacilli and bifidobacteria. *J. Appl. Microbiol.* 114, 1132-1146.
- Weichart, D., Querfurth, N., Dreger, M., Hengge-Aronis, R., 2003. Global role for ClpP-containing proteases in stationary-phase adaptation of *Escherichia coli*. *J. Bacteriol.* 185, 115-125.
- Werner, G., Coque, T.M., Franz, C.M., Grohmann, E., Hegstad, K., Jensen, L., van Schaik, W., Weaver K., 2013. Antibiotic resistant enterococci-tales of a drug resistance gene trafficker. *Int. J. Med. Microbiol.* 303, 360-379.

## Figure legends

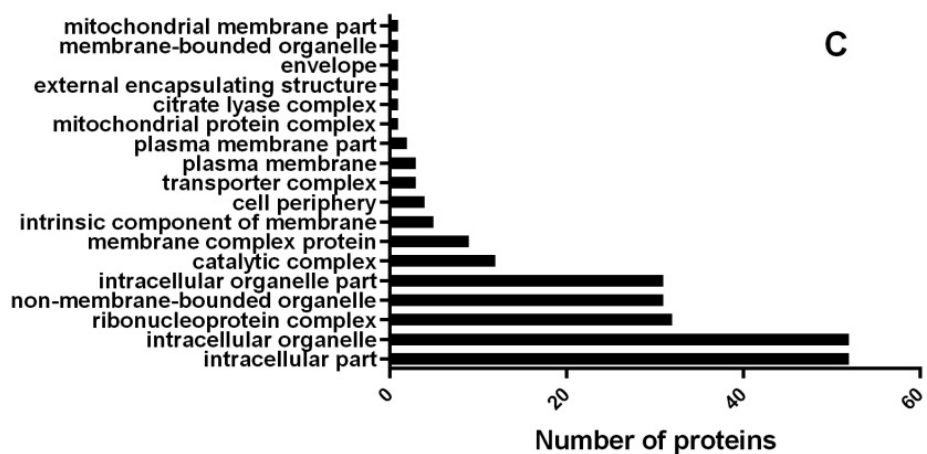
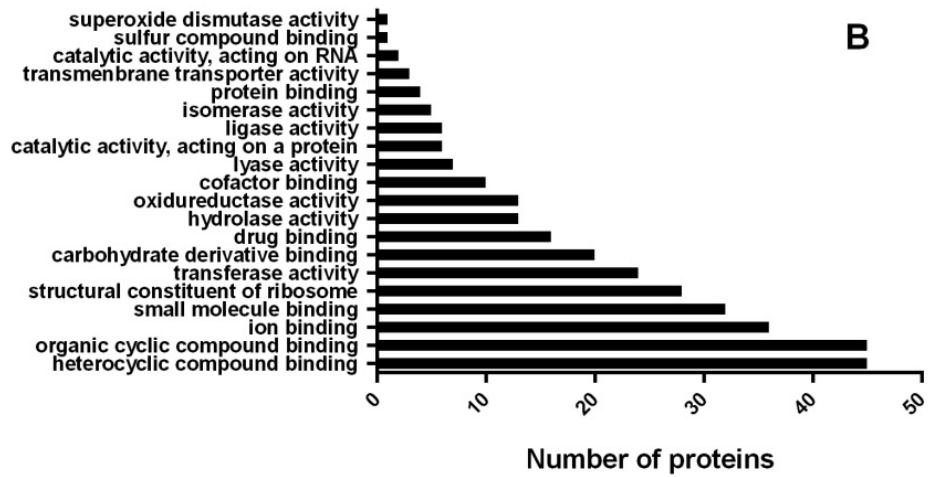
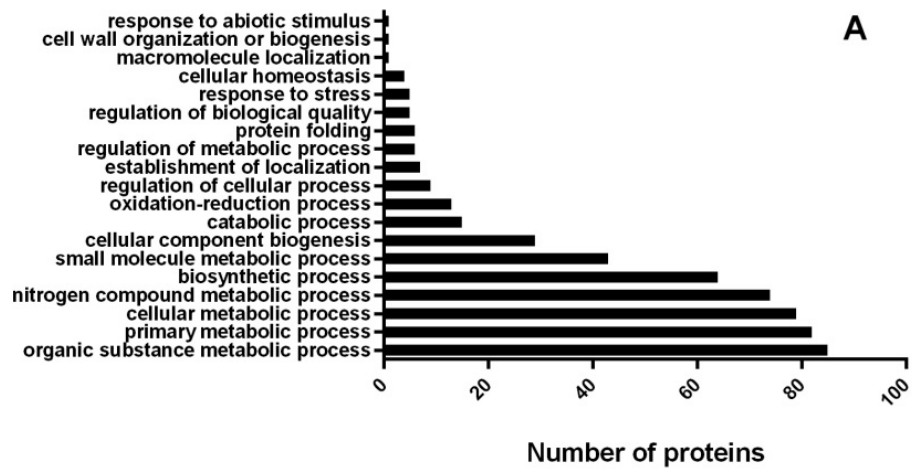
**Fig. 1.** Growth curves of *Enterococcus durans* LAB18S cultivated anaerobically at 37°C in media containing 10 g L<sup>-1</sup> glucose (◆), FOS (■) or GOS (▲) as carbon sources.

**Fig. 2.** Gene ontology profile of identified proteins from *Enterococcus durans* LAB18S growing on FOS, GOS and glucose. The bar charts represent the functional annotations of most relevant biological processes (A), molecular functions (B), and cellular components (C) at the third level of complexity.

**Fig. 3.** Venn diagrams of all identified and Scaffold-validated proteins under different growth conditions. In the center of the diagram, in yellow, the number of proteins common to the three treatments (FOS, GOS and GLU) is represented.

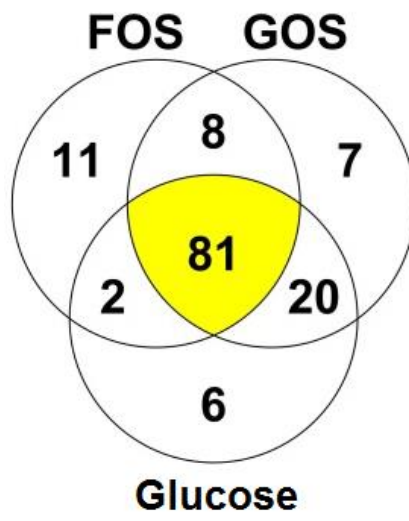


Comerlato et al., Fig. 1



Comerlato et al., Fig. 2





Comerlato et al., Fig. 3

**Table 1.** Differentially expressed proteins in *E. durans* LAB18S growing on FOS, GOS and glucose (GLU) media.

Protein description	Accession number	p-value	Biological process	Expression		
				FOS	GOS	GLU
Clp protease ClpX	AKX87246.1	0.038	Protein binding	High	Low	Low
Ornithine carbamoyl transferase	AKX84989.1	0.025	Amino acid binding	High	Low	Low
2-oxoisovalerate dehydrogenase	AKX86820.1	0.025	Catalytic activity	High	Low	Low
Uncharacterized protein LIANG_08515, similar to YlbF family regulator	AKX86201.1	0.0058	Biofilm formation and spore formation	High	Low	Low
30S ribosomal protein S19	AKX85806.1	0.0071	Structural constituent of ribosome	High	Low	Low
Tagatose-biphosphate aldolase	AKX86137.1	0.0021	Lyase activity	Low	High	Low
Cell division protein	AKX86522.1	0.037	Cellular component	Low	High	Low
Ribosome recycling factor	AKX84983.1	0.043	Structural constituent of ribosome	Low	Low	High
Transketolase	AKX86919.1	0.0042	Key enzyme of pentose phosphate pathway	Low	Low	High
3-deoxy-7-phosphoheptulonate synthase	AKX85527.1	0.015	Lyase activity	Low	Low	High
Aspartate carbamoyltransferase catalytic subunit	AKZ48309.1	0.0089	Catalytic activity	Low	Low	High
Glucose-6-phosphate isomerase	AKX85027.1	0.049	Isomerase activity	Low	High	High

Elongation factor Tu	AKX86680.1	0.018	Translation	Low	High	High
Oxidoreductase SDR family	AKX84788.1	0.035	Oxidoreductase activity	High	Low	High
Uncharacterized protein LIANG_06205, periplasmic binding domain of basic membrane lipoprotein PrnA	AKX85830.1	0.025	Transport of nucleosides	High	Low	High
30S ribosomal protein S10	AKX85811.1	0.017	Structural constituent of ribosome	High	Low	High
Glyceraldehyde-3-phosphate dehydrogenase	AKX86905.1	0.0082	Involved with glycolysis	Low	High	High

---

**Supplementary Table 1 - Overview protein/peptide identification reports of FOS, GOS and Glucose treated *E. durans* LAB18S sample.** Complete list of LC-MS/MS identified proteins prior to Scaffold validation.

	Protein name	Accession numbers	Molecular weight (Da)
1	general stress protein [Enterococcus durans]	gb AKX85160.1 ,gb AKZ48823.1 ,gb EMS74678.1 ,gb EOT31758.1 ,gb EOU18551.1 ,gb OQO81463.1	21.119,5
2	30S ribosomal protein S9 [Enterococcus durans]	gb AKX85907.1 ,gb AKZ47286.1 ,gb EMS74287.1 ,gb EOT28125.1 ,gb EOU16435.1 ,gb OQO79024.1	14.362,8
3	hypothetical protein LIANG_06205 [Enterococcus durans]	gb AKX85830.1 ,gb AKZ47209.1 ,gb EMS74571.1 ,gb EOT33799.1 ,gb EOU25430.1 ,gb OQO82120.1	37.834,8
4	30S ribosomal protein S12 [Enterococcus durans]	gb AKX85821.1 ,gb AKZ47199.1 ,gb EMS74561.1 ,gb EOT33809.1 ,gb EOU25440.1 ,gb OQO82130.1	15.215,9
5	hypothetical protein H318_07903 [Enterococcus durans IPLA 655]	gb EMS75618.1 ,gb EOT32232.1 ,gb EOU20033.1 ,gb OQO81395.1 ,ref WP_005878408.1	16.010,1
6	30S ribosomal protein S11 [Enterococcus durans]	gb AKX85785.1 ,gb AKZ47163.1 ,gb EMS74526.1 ,gb EOT33845.1 ,gb EOU25476.1 ,gb OQO82165.1	13.736,0
7	stress response regulator Gls24 [Enterococcus durans]	gb AKX84772.1 ,gb EMS76996.1 ,gb EOT35451.1 ,gb EOU19160.1 ,gb OQO82729.1 ,ref WP_005875244.1	20.508,5
8	glutamine synthetase [Enterococcus durans]	gb AKX85323.1 ,gb AKZ48982.1 ,gb EMS76744.1 ,gb EOT32128.1 ,gb EOU19929.1 ,gb OQO78738.1	50.675,0
9	isomerase [Enterococcus durans]	gb AKX86085.1 ,gb AKZ47458.1 ,gb EMS75798.1 ,gb EOT26126.1 ,gb EOU22385.1 ,gb OQO81567.1	64.557,2
10	50S ribosomal protein L17 [Enterococcus durans]	gb AKX85783.1 ,gb AKZ47161.1 ,gb EMS74524.1 ,gb EOT33847.1 ,gb EOU25478.1 ,gb OQO82167.1	14.344,9
11	ATP FOF1 synthase subunit alpha [Enterococcus durans]	gb AKX85198.1 ,gb AKZ48860.1 ,gb EMS75647.1 ,gb EOT32261.1 ,gb EOU20062.1 ,gb OQO81424.1	56.397,4
12	ferritin [Enterococcus durans]	gb AKX85904.1 ,gb AKZ47283.1 ,gb EMS74283.1 ,gb EOT28129.1 ,gb EOU16439.1 ,gb OQO79021.1	17.950,9
13	pyruvate kinase [Enterococcus durans]	gb AKX86645.1 ,gb AKZ47998.1 ,gb EMS77112.1 ,gb EOT34121.1 ,gb EOU26238.1 ,gb OQO80009.1	63.662,1
14	hypothetical protein LIANG_08385 [Enterococcus durans]	gb AKX86179.1 ,gb AKZ47551.1 ,gb EMS75204.1 ,ref WP_005879148.1	16.865,4
15	S-adenosylmethionine synthetase [Enterococcus durans]	gb AKX86039.1 ,gb AKZ47417.1 ,gb EMS75771.1 ,gb EOT26478.1 ,gb EOU22338.1 ,gb OQO81524.1	43.278,6
16	elongation factor G [Enterococcus durans]	gb AKX85819.1 ,gb AKZ47197.1 ,gb EMS74559.1 ,gb EOT33811.1 ,gb EOU25442.1 ,gb OQO82132.1	76.742,1
17	hypothetical protein LIANG_03665 [Enterococcus durans]	gb AKX85374.1 ,gb AKZ49033.1 ,gb EMS76367.1 ,gb EOT29603.1 ,gb EOU22725.1 ,gb OQO78273.1	12.186,8
18	6-phosphofructokinase [Enterococcus durans]	gb AKX86644.1 ,gb AKZ47997.1 ,gb EMS77111.1 ,gb EOT34120.1 ,gb EOU26237.1 ,gb OQO80010.1	34.187,9
19	cell division protein FtsZ [Enterococcus durans]	gb AKX86518.1 ,gb EMS74412.1 ,gb EOT33976.1 ,gb EOU26093.1 ,gb OQO81852.1 ,ref WP_005881040.1	44.328,3
20	NAD(FAD)-dependent dehydrogenase [Enterococcus durans]	gb AKX85538.1 ,gb AKZ49189.1 ,gb EMS75354.1 ,gb EOT25774.1 ,gb EOU22483.1 ,ref WP_005879034.1	50.332,3
21	molecular chaperone DnaK [Enterococcus durans]	gb AKX84948.1 ,gb AKZ48610.1 ,gb EMS74968.1 ,gb OQO78467.1 ,ref WP_005880047.1	65.692,7
22	glucose-6-phosphate isomerase [Enterococcus durans]	gb AKX85027.1 ,gb AKZ48688.1 ,gb EMS76330.1 ,gb EOT36447.1 ,gb EOU18787.1 ,gb OQO77954.1	49.752,2
23	glucokinase [Enterococcus durans]	gb AKX85004.1 ,gb AKZ48666.1 ,gb EMS75062.1 ,gb EOT36277.1 ,gb EOU18865.1 ,gb OQO79324.1	33.859,2
24	cell division protein DivIVA [Enterococcus durans]	gb AKX86522.1 ,gb AKZ47882.1 ,gb EMS74407.1 ,gb EOT33981.1 ,gb EOU26098.1 ,gb OQO81847.1	26.724,9
25	cold-shock protein [Enterococcus durans]	gb AKX85335.1 ,gb AKZ48994.1 ,gb EMS76731.1 ,gb EOT32114.1 ,gb EOU19915.1 ,gb OQO78750.1	7.258,7

26	phosphoenolpyruvate-protein phosphotransferase	gb AKX86601.1 ,gb AKZ47958.1 ,ref WP_053108894.1	63.353,5
27	peptidase M29 [Enterococcus durans]	gb AKX86541.1 ,gb AKZ47900.1 ,ref WP_053108834.1	45.096,0
28	Clp protease ClpX (plasmid) [Enterococcus durans]	gb AKX87246.1 ,gb EMS76214.1 ,gb EOT30301.1 ,gb EOU15544.1 ,ref WP_005876977.1	77.848,4
29	osmotically inducible protein C [Enterococcus durans]	gb AKX86277.1 ,gb AKZ47647.1 ,gb EMS75167.1 ,gb EOT34377.1 ,gb EOU25801.1 ,gb OQO78110.1	14.438,9
30	30S ribosomal protein S7 [Enterococcus durans]	gb AKX85820.1 ,gb AKZ47198.1 ,gb EMS74560.1 ,gb EOT33810.1 ,gb EOU25441.1 ,gb OQO82131.1	17.843,7
31	ATP synthase beta-subunit, partial [Enterococcus durans]	gb ADO14905.1 ,gb AKX85196.1 ,gb AKZ48858.1 ,gb EMS75649.1 ,gb EOT32263.1 ,gb EOU20064.1	51.167,8
32	arginine deiminase [Enterococcus durans]	gb AKX84990.1 ,gb AKZ48652.1 ,gb EMS76655.1 ,gb EOT36297.1 ,gb EOU18885.1 ,gb OQO79339.1	46.057,9
33	50S ribosomal protein L7/L12 [Enterococcus durans]	gb AKX85495.1 ,gb AKZ49148.1 ,gb EMS75307.1 ,gb EOT25821.1 ,gb EOU22530.1 ,gb OQO81709.1	12.497,9
34	NAD synthetase [Enterococcus durans]	gb AKX85456.1 ,gb AKZ49113.1 ,gb EMS74740.1 ,gb EOT29798.1 ,gb EOU22632.1 ,gb OQO81755.1	30.855,6
35	branched-chain amino acid aminotransferase	gb AKX86897.1 ,gb AKZ48249.1 ,gb EMS76178.1 ,gb EOT35275.1 ,gb EOU19381.1 ,gb OQO82535.1	37.297,0
36	adenylosuccinate synthetase [Enterococcus durans]	gb AKX85849.1 ,gb AKZ47228.1 ,gb EMS75973.1 ,gb EOT33753.1 ,gb EOU25384.1 ,gb OQO82072.1	47.817,8
37	ornithine carbamoyltransferase [Enterococcus durans]	gb AKX84989.1 ,gb AKZ48651.1 ,gb EMS76654.1 ,gb EOT36298.1 ,gb EOU18886.1 ,gb OQO79340.1	38.341,7
38	dihydrolipoamide dehydrogenase [Enterococcus durans]	gb AKX86822.1 ,gb AKZ48179.1 ,gb EMS76473.1 ,gb EOT34966.1 ,gb EOU19458.1 ,gb OQO82466.1	49.261,4
39	Clp protease ClpB [Enterococcus durans]	gb AKX84844.1 ,gb AKX86599.1 ,gb AKZ47956.1 ,gb AKZ48506.1 ,gb EMS74863.1 ,gb EMS76503.1	98.085,9
40	aspartate carbamoyltransferase catalytic subunit	gb AKZ48309.1 ,gb EMS74996.1 ,gb EOT35362.1 ,gb EOU19315.1 ,gb OQO82592.1 ,ref WP_005876975.1	34.904,9
41	general stress protein [Enterococcus durans]	gb AKX84785.1 ,gb AKZ48444.1 ,gb EMS75708.1 ,gb EOT35438.1 ,gb EOU19146.1 ,gb OQO78700.1	30.065,8
42	oxidoreductase [Enterococcus durans]	gb AKX84788.1 ,gb AKZ48447.1 ,gb EMS75711.1 ,gb EOT35435.1 ,gb EOU19143.1 ,gb OQO78703.1	31.934,8
43	carbamate kinase [Enterococcus durans]	gb AKX84988.1 ,gb AKZ48650.1 ,gb EMS76653.1 ,gb EOT36299.1 ,gb EOU18887.1 ,gb OQO79341.1	33.886,7
44	dihydrolipoamide acetyltransferase [Enterococcus durans]	gb AKX86821.1 ,gb AKZ48178.1 ,gb EMS76472.1 ,gb EOT34967.1 ,gb EOU19459.1 ,gb OQO82465.1	57.943,6
45	glutamyl-tRNA synthetase [Enterococcus durans]	gb AKZ47353.1 ,gb EMS76247.1 ,gb EOT28433.1 ,gb EOU16409.1 ,gb OQO78070.1 ,ref WP_005876899.1	55.356,2
46	phosphocarrier protein HPr [Enterococcus durans]	gb AKX86600.1 ,gb AKZ47957.1 ,gb EMS76502.1 ,gb EOT34074.1 ,gb EOU26191.1 ,gb OQO80053.1	9.312,5
47	2-oxoisovalerate dehydrogenase [Enterococcus durans]	gb AKX86820.1 ,gb AKZ48177.1 ,gb EMS76471.1 ,gb EOT34968.1 ,gb EOU19460.1 ,gb OQO82464.1	35.384,6
48	N-acetylglucosamine-6-phosphate deacetylase	gb AKX86783.1 ,gb AKZ48142.1 ,gb EMS76427.1 ,gb EOT35012.1 ,gb EOU19504.1 ,gb OQO82426.1	41.332,0
49	30S ribosomal protein S6 [Enterococcus durans]	gb AKX85856.1 ,gb AKZ47235.1 ,gb EMS76722.1 ,gb EOT33747.1 ,gb EOU25378.1 ,gb OQO82066.1	11.578,8
50	30S ribosomal protein S10 [Enterococcus durans]	gb AKX85811.1 ,gb AKZ47189.1 ,gb EMS74552.1 ,gb EOT33819.1 ,gb EOU25450.1 ,gb OQO82140.1	18.006,8
51	GroES, partial [Enterococcus durans]	gb AAN32674.1 AF417585_1,gb AKX85467.1 ,gb AKZ49124.1 ,gb EMS74729.1 ,gb EOT29787.1	10.031,4
52	50S ribosomal protein L2 [Enterococcus durans]	gb AKX85807.1 ,gb AKZ47185.1 ,gb EMS74548.1 ,gb EOT33823.1 ,gb EOU25454.1 ,gb OQO82144.1	30.326,6
53	lactate dehydrogenase [Enterococcus durans]	gb AKX86027.1 ,gb AKZ47406.1 ,gb EMS77070.1 ,gb EOT29104.1 ,gb EOU16350.1 ,gb OQO79291.1	35.131,4
54	uridylate kinase [Enterococcus durans]	gb AKX87068.1 ,gb AKZ49357.1 ,gb EMS76648.1 ,gb EOT36304.1 ,gb EOU18892.1 ,gb OQO79362.1	26.031,2

55	ribosome-recycling factor [Enterococcus durans]	gb AKX84983.1 ,gb AKZ48645.1 ,gb EMS76647.1 ,gb EOT36305.1 ,gb EOU18893.1 ,gb OQO79346.1	20.881,9
56	carbamoyl phosphate synthase large subunit	gb AKX86955.1 ,gb AKZ48312.1 ,gb EMS74993.1 ,gb EOT35359.1 ,gb EOU19312.1 ,gb OQO82595.1	117.433,8
57	trigger factor [Enterococcus durans]	gb AKX84928.1 ,gb AKZ48590.1 ,gb EMS74945.1 ,gb EOT36106.1 ,gb EOU18964.1 ,gb OQO78488.1	47.671,9
58	30S ribosomal protein S2 [Enterococcus durans]	gb AKX84985.1 ,gb AKZ48647.1 ,gb EMS76650.1 ,gb EOT36302.1 ,gb EOU18890.1 ,gb OQO79344.1	29.298,5
59	30S ribosomal protein S13 [Enterococcus durans]	gb AKX85786.1 ,gb AKZ47164.1 ,gb EMS74527.1 ,gb EOT33844.1 ,gb EOU25475.1 ,gb OQO82164.1	13.534,1
60	transketolase [Enterococcus durans]	gb AKX86919.1 ,gb AKZ48271.1 ,gb EMS76686.1 ,gb EOT35404.1 ,gb EOU19357.1 ,ref WP_005875855.1	72.147,2
61	DNA-binding protein [Enterococcus durans]	gb AKX86714.1 ,gb AKZ48069.1 ,gb EMS75473.1 ,gb EOT34733.1 ,gb EOU19591.1 ,gb OQO81189.1	9.682,3
62	50S ribosomal protein L4 [Enterococcus durans]	gb AKX85809.1 ,gb AKZ47187.1 ,gb EMS74550.1 ,gb EOT33821.1 ,gb EOU25452.1 ,gb OQO82142.1	22.462,4
63	decarboxylase [Enterococcus durans]	gb AKX85643.1 ,gb AKZ47021.1 ,gb EMS76937.1 ,gb EOT32744.1 ,gb EOU25647.1 ,gb OQO82311.1	70.192,3
64	50S ribosomal protein L22 [Enterococcus durans]	gb AKX85805.1 ,gb AKZ47183.1 ,gb EMS74546.1 ,gb EOT33825.1 ,gb EOU25456.1 ,gb OQO82146.1	12.450,7
65	phosphoglycerate kinase [Enterococcus durans]	gb AKX85214.1 ,gb AKZ48876.1 ,gb EMS75629.1 ,gb EOT32243.1 ,gb EOU20044.1 ,gb OQO81407.1	41.855,5
66	pyrrolidone-carboxylate peptidase [Enterococcus durans]	gb AKX86427.1 ,gb AKZ47791.1 ,gb EMS76129.1 ,gb EOT34212.1 ,gb EOU25995.1 ,gb OQO81945.1	22.969,3
67	3-deoxy-7-phosphoheptulonate synthase [Enterococcus durans]	gb AKX85527.1 ,gb AKZ49178.1 ,gb EMS75341.1 ,gb EOT25786.1 ,gb EOU22495.1 ,gb OQO81675.1	37.495,2
68	molecular chaperone GroEL [Enterococcus durans]	gb AKX85466.1 ,gb AKZ49123.1 ,gb EMS74730.1 ,gb OQO81745.1 ,ref WP_005880136.1	57.148,7
69	tyrosine--tRNA ligase [Enterococcus durans]	gb AKX85644.1 ,gb AKZ47022.1 ,gb EMS76936.1 ,gb EOT32743.1 ,gb EOU25646.1 ,gb OQO82310.1	47.335,7
70	osmotically inducible protein C [Enterococcus durans]	gb AKX86185.1 ,gb AKZ47557.1 ,gb EMS74585.1 ,gb OQO80135.1 ,ref WP_002292291.1	14.674,3
71	glyceraldehyde-3-phosphate dehydrogenase	gb AKX86905.1 ,gb AKZ48257.1 ,gb EMS75963.1 ,gb EOT35299.1 ,gb EOU19372.1 ,gb OQO82543.1	36.155,7
72	cysteine synthase [Enterococcus durans]	gb AKX86921.1 ,gb AKZ48273.1 ,gb EMS76684.1 ,gb EOT35402.1 ,gb EOU19355.1 ,gb OQO82556.1	32.481,4
73	enolase [Enterococcus durans]	gb AKX85212.1 ,gb AKZ48874.1 ,gb EMS75631.1 ,gb EOT32245.1 ,gb EOU20046.1 ,gb OQO81409.1	46.481,0
74	hypothetical protein LIANG_10220 [Enterococcus durans]	gb AKX86497.1 ,gb AKZ47857.1 ,gb EMS74433.1 ,gb EOT33955.1 ,gb EOU26072.1 ,gb OQO81873.1	15.537,3
75	triosephosphate isomerase [Enterococcus durans]	gb AKX85213.1 ,gb AKZ48875.1 ,gb EMS75630.1 ,gb OQO81408.1 ,ref WP_005878431.1	26.877,8
76	dihydroxyacetone kinase [Enterococcus durans]	gb AKX85646.1 ,gb AKZ47024.1 ,gb EMS76934.1 ,gb EOT32741.1 ,gb EOU15552.1 ,gb EOU25644.1	21.837,3
77	50S ribosomal protein L29 [Enterococcus durans]	gb AKX85802.1 ,gb AKZ47180.1 ,gb EMS74543.1 ,gb EOT33828.1 ,gb EOU25459.1 ,gb OQO82149.1	7.344,0
78	50S ribosomal protein L31 type B [Enterococcus durans]	gb AKX85318.1 ,gb AKZ48977.1 ,gb EMS76749.1 ,gb EOT32133.1 ,gb EOU19934.1 ,gb OQO78733.1	9.936,9
79	NADPH:quinone reductase [Enterococcus durans]	gb AKX85133.1 ,gb AKZ48795.1 ,gb EMS74649.1 ,gb EOT31576.1 ,gb EOU18586.1 ,gb OQO81494.1	33.960,6
80	GMP synthase [Enterococcus durans]	gb AKX85836.1 ,gb AKZ47215.1 ,gb EMS74578.1 ,gb EOT33792.1 ,gb EOU25423.1 ,gb OQO82113.1	57.759,7
81	tagatose-bisphosphate aldolase [Enterococcus durans]	gb AKX86137.1 ,gb AKZ47509.1 ,gb EMS75410.1 ,ref WP_002290551.1 ,ref WP_005878819.1	36.445,1
82	2,5-diketo-D-gluconic acid reductase [Enterococcus durans]	gb AKX86672.1 ,gb AKZ48028.1 ,gb EMS75514.1 ,gb OQO81227.1 ,ref WP_005878645.1	31.865,6
83	30S ribosomal protein S15 [Enterococcus durans]	gb AKX85715.1 ,gb EMS76858.1 ,gb EOT33619.1 ,gb EOU25559.1 ,gb OQO82242.1	10.595,5

84	uracil phosphoribosyltransferase [Enterococcus durans]	gb AKX86332.1 ,gb AKZ47698.1 ,gb EMS75108.1 ,gb EOT34441.1 ,gb EOU25865.1 ,gb OQO80536.1	22.888,5
85	phosphoglyceromutase [Enterococcus durans]	gb OQO82127.1 ,ref WP_081133709.1	25.914,4
86	30S ribosomal protein S8 [Enterococcus durans]	gb AKX85796.1 ,gb AKZ47174.1 ,gb EMS74537.1 ,gb EOT33834.1 ,gb EOU25465.1 ,gb OQO82155.1	14.840,7
87	50S ribosomal protein L6 [Enterococcus durans]	gb AKX85795.1 ,gb AKZ47173.1 ,gb EMS74536.1 ,gb EOT33835.1 ,gb EOU25466.1 ,gb OQO82156.1	19.209,3
88	glyceraldehyde-3-phosphate dehydrogenase [Enterococcus durans]	gb AKX85215.1 ,gb AKZ48877.1 ,gb EMS75628.1 ,gb EOT32242.1 ,gb EOU20043.1 ,gb OQO81406.1	35.774,4
89	50S ribosomal protein L13 [Enterococcus durans]	gb AKX87109.1 ,gb AKZ49295.1 ,gb EMS74286.1 ,gb EOT28126.1 ,gb EOU16436.1 ,gb OQO79028.1	15.652,5
90	universal stress protein UspA [Enterococcus durans]	gb AKX86231.1 ,gb AKZ47601.1 ,gb EMS76048.1 ,gb EOT34514.1 ,gb EOU25751.1 ,gb OQO79639.1	17.246,0
91	hypothetical protein LIANG_08515 [Enterococcus durans]	gb AKX86201.1 ,gb AKZ47572.1 ,gb EMS76564.1 ,gb EOT32805.1 ,gb EOU25708.1 ,gb OQO80094.1 ,	13.151,2
92	50S ribosomal protein L21 [Enterococcus durans]	gb AKX86487.1 ,gb AKZ47845.1 ,gb EMS74445.1 ,gb EOT33943.1 ,gb EOU26060.1 ,gb OQO81885.1	11.174,6
93	fructose-bisphosphate aldolase [Enterococcus durans]	gb AKX85321.1 ,gb AKZ48980.1 ,gb EMS76746.1 ,gb EOT32130.1 ,gb EOU19931.1 ,gb OQO78736.1	30.809,7
94	acyl carrier protein [Enterococcus durans]	gb AKX86608.1 ,gb AKZ47964.1 ,gb EMS76493.1 ,gb EOT34083.1 ,gb EOU26200.1 ,gb OQO80044.1	8.562,8
95	30S ribosomal protein S5 [Enterococcus durans]	gb AKX85793.1 ,gb AKZ47171.1 ,gb EMS74534.1 ,gb EOT33837.1 ,gb EOU25468.1 ,gb OQO82157.1	17.476,9
96	hypothetical protein LIANG_02015 [Enterococcus durans]	gb AKX85093.1 ,gb AKZ48753.1 ,gb EMS76784.1 ,gb EOT31621.1 ,gb EOU18631.1 ,gb OQO81358.1	41.652,5
97	elongation factor Tu [Enterococcus durans]	gb AKX85818.1 ,gb AKZ47196.1 ,gb EMS74558.1 ,gb EOT33812.1 ,gb EOU25443.1 ,gb OQO82133.1	43.162,2
98	cold-shock protein [Enterococcus durans]	gb AKX86580.1 ,gb AKZ47937.1 ,gb EMS76523.1 ,gb EOT34050.1 ,gb EOU26167.1 ,gb OQO77812.1	7.204,7
99	elongation factor Tu [Enterococcus durans]	gb AKX86680.1 ,gb AKZ48035.1 ,gb EMS75506.1 ,gb EOT34767.1 ,gb EOU19625.1 ,gb OQO81219.1	43.213,0
100	50S ribosomal protein L30 [Enterococcus durans]	gb AKX85792.1 ,gb AKZ47170.1 ,gb EMS74533.1 ,gb EOT33838.1 ,gb EOU25469.1 ,gb OQO82158.1	6.427,5
101	30S ribosomal protein S1 [Enterococcus durans]	gb AKX86712.1 ,gb AKZ48067.1 ,gb EMS75475.1 ,gb EOT34735.1 ,gb EOU19593.1 ,gb OQO81191.1	44.559,6
102	hypothetical protein LIANG_11460 [Enterococcus durans]	gb AKX86705.1 ,gb AKZ48060.1 ,gb EMS75482.1 ,gb EOT34742.1 ,gb EOU19600.1 ,gb OQO81198.1	49.365,7
103	50S ribosomal protein L15 [Enterococcus durans]	gb AKX85791.1 ,gb AKZ47169.1 ,gb EMS74532.1 ,gb EOT33839.1 ,gb EOU25470.1 ,gb OQO82159.1	15.424,8
104	30S ribosomal protein S3 [Enterococcus durans]	gb AKX85804.1 ,gb AKZ47182.1 ,gb EMS74545.1 ,gb EOT33826.1 ,gb EOU25457.1 ,gb OQO82147.1	24.406,0
105	30S ribosomal protein S5 [Enterococcus durans]	gb AKX85793.1 ,gb AKZ47171.1 ,gb EMS74534.1 ,gb EOT33837.1 ,gb EOU25468.1 ,gb OQO82157.1	17.476,9
106	elongation factor G [Enterococcus durans]	gb AKX85819.1 ,gb AKZ47197.1 ,gb EMS74559.1 ,gb EOT33811.1 ,gb EOU25442.1 ,gb OQO82132.1	76.742,1
107	50S ribosomal protein L4 [Enterococcus durans]	gb AKX85809.1 ,gb AKZ47187.1 ,gb EMS74550.1 ,gb EOT33821.1 ,gb EOU25452.1 ,gb OQO82142.1	22.462,4
108	glyceraldehyde-3-phosphate dehydrogenase	gb AKX86905.1 ,gb AKZ48257.1 ,gb EMS75963.1 ,gb EOT35299.1 ,gb EOU19372.1 ,gb OQO82543.1	36.155,7
109	50S ribosomal protein L22 [Enterococcus durans]	gb AKX85805.1 ,gb AKZ47183.1 ,gb EMS74546.1 ,gb EOT33825.1 ,gb EOU25456.1 ,gb OQO82146.1	12.450,7
110	carbamate kinase [Enterococcus durans]	gb AKX84988.1 ,gb AKZ48650.1 ,gb EMS76653.1 ,gb EOT36299.1 ,gb EOU18887.1 ,gb OQO79341.1	33.886,7
111	phosphoglyceromutase [Enterococcus durans]	gb OQO82127.1 ,ref WP_081133709.1	25.914,4
112	Clp protease ClpX (plasmid) [Enterococcus durans]	gb AKX87246.1 ,gb EMS76214.1 ,gb EOT30301.1 ,gb EOU15544.1 ,ref WP_005876977.1	77.848,4

113	decarboxylase [Enterococcus durans]	gb AKX85643.1 ,gb AKZ47021.1 ,gb EMS76937.1 ,gb EOT32744.1 ,gb EOU25647.1 ,gb OQO82311.1	70.192,3
114	30S ribosomal protein S7 [Enterococcus durans]	gb AKX85820.1 ,gb AKZ47198.1 ,gb EMS74560.1 ,gb EOT33810.1 ,gb EOU25441.1 ,gb OQO82131.1	17.843,7
115	lactate dehydrogenase [Enterococcus durans]	gb AKX86027.1 ,gb AKZ47406.1 ,gb EMS77070.1 ,gb EOT29104.1 ,gb EOU16350.1 ,gb OQO79291.1	35.131,4
116	adenylosuccinate synthetase [Enterococcus durans]	gb AKX85849.1 ,gb AKZ47228.1 ,gb EMS75973.1 ,gb EOT33753.1 ,gb EOU25384.1 ,gb OQO82072.1	47.817,8
117	osmotically inducible protein C [Enterococcus durans]	gb AKX86185.1 ,gb AKZ47557.1 ,gb EMS74585.1 ,gb OQO80135.1 ,ref WP_002292291.1	14.674,3
118	phosphoenolpyruvate-protein phosphotransferase	gb AKX86601.1 ,gb AKZ47958.1 ,ref WP_053108894.1	63.353,5
119	3-deoxy-7-phosphoheptulonate synthase [Enterococcus durans]	gb AKX85527.1 ,gb AKZ49178.1 ,gb EMS75341.1 ,gb EOT25786.1 ,gb EOU22495.1 ,gb OQO81675.1	37.495,2
120	enolase [Enterococcus durans]	gb AKX85212.1 ,gb AKZ48874.1 ,gb EMS75631.1 ,gb EOT32245.1 ,gb EOU20046.1 ,gb OQO81409.1	46.481,0
121	50S ribosomal protein L30 [Enterococcus durans]	gb AKX85792.1 ,gb AKZ47170.1 ,gb EMS74533.1 ,gb EOT33838.1 ,gb EOU25469.1 ,gb OQO82158.1	6.427,5
122	30S ribosomal protein S13 [Enterococcus durans]	gb AKX85786.1 ,gb AKZ47164.1 ,gb EMS74527.1 ,gb EOT33844.1 ,gb EOU25475.1 ,gb OQO82164.1	13.534,1
123	elongation factor Tu [Enterococcus durans]	gb AKX85818.1 ,gb AKZ47196.1 ,gb EMS74558.1 ,gb EOT33812.1 ,gb EOU25443.1 ,gb OQO82133.1	43.162,2
124	ATP synthase beta-subunit, partial [Enterococcus durans]	gb ADO14905.1 ,gb AKX85196.1 ,gb AKZ48858.1 ,gb EMS75649.1 ,gb EOT32263.1 ,gb EOU20064.1	51.167,8
125	osmotically inducible protein C [Enterococcus durans]	gb AKX86277.1 ,gb AKZ47647.1 ,gb EMS75167.1 ,gb EOT34377.1 ,gb EOU25801.1 ,gb OQO78110.1	14.438,9
126	trigger factor [Enterococcus durans]	gb AKX84928.1 ,gb AKZ48590.1 ,gb EMS74945.1 ,gb EOT36106.1 ,gb EOU18964.1 ,gb OQO78488.1	47.671,9
127	ferritin [Enterococcus durans]	gb AKX85904.1 ,gb AKZ47283.1 ,gb EMS74283.1 ,gb EOT28129.1 ,gb EOU16439.1 ,gb OQO79021.1 ,	17.950,9
128	tyrosine--tRNA ligase [Enterococcus durans]	gb AKX85644.1 ,gb AKZ47022.1 ,gb EMS76936.1 ,gb EOT32743.1 ,gb EOU25646.1 ,gb OQO82310.1	47.335,7
129	30S ribosomal protein S8 [Enterococcus durans]	gb AKX85796.1 ,gb AKZ47174.1 ,gb EMS74537.1 ,gb EOT33834.1 ,gb EOU25465.1 ,gb OQO82155.1	14.840,7
130	phosphoglycerate kinase [Enterococcus durans]	gb AKX85214.1 ,gb AKZ48876.1 ,gb EMS75629.1 ,gb EOT32243.1 ,gb EOU20044.1 ,gb OQO81407.1	41.855,5
131	ribosome-recycling factor [Enterococcus durans]	gb AKX84983.1 ,gb AKZ48645.1 ,gb EMS76647.1 ,gb EOT36305.1 ,gb EOU18893.1 ,gb OQO79346.1	20.881,9
132	30S ribosomal protein S9 [Enterococcus durans]	gb AKX85907.1 ,gb AKZ47286.1 ,gb EMS74287.1 ,gb EOT28125.1 ,gb EOU16435.1 ,gb OQO79024.1	14.362,8
133	oxidoreductase [Enterococcus durans]	gb AKX84788.1 ,gb AKZ48447.1 ,gb EMS75711.1 ,gb EOT35435.1 ,gb EOU19143.1 ,gb OQO78703.	31.934,8
134	triosephosphate isomerase [Enterococcus durans]	gb AKX85213.1 ,gb AKZ48875.1 ,gb EMS75630.1 ,gb OQO81408.1 ,ref WP_005878431.1	26.877,8
135	30S ribosomal protein S6 [Enterococcus durans]	gb AKX85856.1 ,gb AKZ47235.1 ,gb EMS76722.1 ,gb EOT33747.1 ,gb EOU25378.1 ,gb OQO82066.1	11.578,8
136	50S ribosomal protein L15 [Enterococcus durans]	gb AKX85791.1 ,gb AKZ47169.1 ,gb EMS74532.1 ,gb EOT33839.1 ,gb EOU25470.1 ,gb OQO82159.1	15.424,8
137	aspartate carbamoyltransferase catalytic subunit	gb AKZ48309.1 ,gb EMS74996.1 ,gb EOT35362.1 ,gb EOU19315.1 ,gb OQO82592.1 ,ref WP_005879675.1	34.904,9
138	GMP synthase [Enterococcus durans]	gb AKX85836.1 ,gb AKZ47215.1 ,gb EMS74578.1 ,gb EOT33792.1 ,gb EOU25423.1 ,gb OQO82113.1	57.759,7
139	30S ribosomal protein S11 [Enterococcus durans]	gb AKX85785.1 ,gb AKZ47163.1 ,gb EMS74526.1 ,gb EOT33845.1 ,gb EOU25476.1 ,gb OQO82165.1	13.736,0
140	cold-shock protein [Enterococcus durans]	gb AKX86580.1 ,gb AKZ47937.1 ,gb EMS76523.1 ,gb EOT34050.1 ,gb EOU26167.1 ,gb OQO77812.1	7.204,7
141	elongation factor Tu [Enterococcus durans]	gb AKX86680.1 ,gb AKZ48035.1 ,gb EMS75506.1 ,gb EOT34767.1 ,gb EOU19625.1 ,gb OQO81219.1	43.213,0



142	glucose-6-phosphate isomerase [Enterococcus durans]	gb AKX85027.1 ,gb AKZ48688.1 ,gb EMS76330.1 ,gb EOT36447.1 ,gb EOU18787.1 ,gb OQO77954.1	49.752,2
143	GroES, partial [Enterococcus durans]	gb AAN32674.1 AF417585_1,gb AKX85467.1 ,gb AKZ49124.1 ,gb EMS74729.1 ,gb EOT29787.1	10.031,4
144	molecular chaperone GrpE [Enterococcus durans]	gb AKX84949.1 ,gb AKZ48611.1 ,gb EMS74969.1 ,gb EOT36158.1 ,gb EOU18940.1 ,gb OQO78466.1	21.149,6
145	glyceraldehyde-3-phosphate dehydrogenase	gb AKX85215.1 ,gb AKZ48877.1 ,gb EMS75628.1 ,gb EOT32242.1 ,gb EOU20043.1 ,gb OQO81406.1	35.774,4
146	pyruvate kinase [Enterococcus durans]	gb AKX86645.1 ,gb AKZ47998.1 ,gb EMS77112.1 ,gb EOT34121.1 ,gb EOU26238.1 ,gb OQO80009.1	63.662,1
147	30S ribosomal protein S10 [Enterococcus durans]	gb AKX85811.1 ,gb AKZ47189.1 ,gb EMS74552.1 ,gb EOT33819.1 ,gb EOU25450.1 ,gb OQO82140.1	18.006,8
148	50S ribosomal protein L29 [Enterococcus durans]	gb AKX85802.1 ,gb AKZ47180.1 ,gb EMS74543.1 ,gb EOT33828.1 ,gb EOU25459.1 ,gb OQO82149.1	7.344,0
149	50S ribosomal protein L13 [Enterococcus durans]	gb AKX87109.1 ,gb AKZ49295.1 ,gb EMS74286.1 ,gb EOT28126.1 ,gb EOU16436.1 ,gb OQO79028.1	15.652,5
150	arginine deiminase [Enterococcus durans]	gb AKX84990.1 ,gb AKZ48652.1 ,gb EMS76655.1 ,gb EOT36297.1 ,gb EOU18885.1 ,gb OQO79339.1	46.057,9
151	hypothetical protein LIANG_11460 [Enterococcus durans]	gb AKX86705.1 ,gb AKZ48060.1 ,gb EMS75482.1 ,gb EOT34742.1 ,gb EOU19600.1 ,gb OQO81198.1	49.365,7
152	transketolase [Enterococcus durans]	gb AKX86919.1 ,gb AKZ48271.1 ,gb EMS76686.1 ,gb EOT35404.1 ,gb EOU19357.1 ,ref WP_005875855.1 ,	72.147,2
153	30S ribosomal protein S15 [Enterococcus durans]	gb AKX85715.1 ,gb EMS76858.1 ,gb EOT33619.1 ,gb EOU25559.1 ,gb OQO82242.1 ,ref WP_005875316.1	10.595,5
154	fructose-bisphosphate aldolase [Enterococcus durans]	gb AKX85321.1 ,gb AKZ48980.1 ,gb EMS76746.1 ,gb EOT32130.1 ,gb EOU19931.1 ,gb OQO78736.1	30.809,7
155	pyrrolidone-carboxylate peptidase [Enterococcus durans]	gb AKX86427.1 ,gb AKZ47791.1 ,gb EMS76129.1 ,gb EOT34212.1 ,gb EOU25995.1 ,gb OQO81945.1	22.969,3
156	pyruvate oxidase [Enterococcus durans]	gb AKZ47224.1 ,gb EMS75969.1 ,gb EOT33757.1 ,gb EOU25388.1 ,gb OQO82076.1 ,ref WP_005877532.1	63.591,9
157	30S ribosomal protein S2 [Enterococcus durans]	gb AKX84985.1 ,gb AKZ48647.1 ,gb EMS76650.1 ,gb EOT36302.1 ,gb EOU18890.1 ,gb OQO79344.1	29.298,5
158	molecular chaperone GroEL [Enterococcus durans]	gb AKX85466.1 ,gb AKZ49123.1 ,gb EMS74730.1 ,gb OQO81745.1 ,ref WP_005880136.1	57.148,7
159	general stress protein [Enterococcus durans]	gb AKX85160.1 ,gb AKZ48823.1 ,gb EMS74678.1 ,gb EOT31758.1 ,gb EOU18551.1 ,gb OQO81463.1	21.119,5
160	molecular chaperone DnaK [Enterococcus durans]	gb AKX84948.1 ,gb AKZ48610.1 ,gb EMS74968.1 ,gb OQO78467.1 ,ref WP_005880047.1	65.692,7
161	stress response regulator Gls24 [Enterococcus durans]	gb AKX84772.1 ,gb EMS76996.1 ,gb EOT35451.1 ,gb EOU19160.1 ,gb OQO82729.1 ,ref WP_005875244.1	20.508,5
162	6-phosphofructokinase [Enterococcus durans]	gb AKX86644.1 ,gb AKZ47997.1 ,gb EMS77111.1 ,gb EOT34120.1 ,gb EOU26237.1 ,gb OQO80010.1	34.187,9
163	universal stress protein UspA [Enterococcus durans]	gb AKX86231.1 ,gb AKZ47601.1 ,gb EMS76048.1 ,gb EOT34514.1 ,gb EOU25751.1 ,gb OQO79639.1	17.246,0
164	ornithine carbamoyltransferase [Enterococcus durans]	gb AKX84989.1 ,gb AKZ48651.1 ,gb EMS76654.1 ,gb EOT36298.1 ,gb EOU18886.1 ,gb OQO79340.1	38.341,7
165	hypothetical protein LIANG_08385 [Enterococcus durans]	gb AKX86179.1 ,gb AKZ47551.1 ,gb EMS75204.1 ,ref WP_005879148.1	16.865,4
166	S-adenosylmethionine synthetase [Enterococcus durans]	gb AKX86039.1 ,gb AKZ47417.1 ,gb EMS75771.1 ,gb EOT26478.1 ,gb EOU22338.1 ,gb OQO81524.1	43.278,6
167	uridylyate kinase [Enterococcus durans]	gb AKX87068.1 ,gb AKZ49357.1 ,gb EMS76648.1 ,gb EOT36304.1 ,gb EOU18892.1 ,gb OQO79362.1	26.031,2
168	30S ribosomal protein S1 [Enterococcus durans]	gb AKX86712.1 ,gb AKZ48067.1 ,gb EMS75475.1 ,gb EOT34735.1 ,gb EOU19593.1 ,gb OQO81191.1	44.559,6
169	30S ribosomal protein S12 [Enterococcus durans]	gb AKX85821.1 ,gb AKZ47199.1 ,gb EMS74561.1 ,gb EOT33809.1 ,gb EOU25440.1 ,gb OQO82130.1	15.215,9
170	50S ribosomal protein L17 [Enterococcus durans]	gb AKX85783.1 ,gb AKZ47161.1 ,gb EMS74524.1 ,gb EOT33847.1 ,gb EOU25478.1 ,gb OQO82167.1	14.344,9

171	cell division protein FtsZ [Enterococcus durans]	gb AKX86518.1 ,gb EMS74412.1 ,gb EOT33976.1 ,gb EQU26093.1 ,gb OQO81852.1 ,ref WP_005881040.1	44.328,3
172	ATP FOF1 synthase subunit alpha [Enterococcus durans]	gb AKX85198.1 ,gb AKZ48860.1 ,gb EMS75647.1 ,gb EOT32261.1 ,gb EQU20062.1 ,gb OQO81424.1	56.397,4
173	2-oxoisovalerate dehydrogenase [Enterococcus durans]	gb AKX86820.1 ,gb AKZ48177.1 ,gb EMS76471.1 ,gb EOT34968.1 ,gb EQU19460.1 ,gb OQO82464.1	35.384,6
174	hypothetical protein LIANG_06205 [Enterococcus durans]	gb AKX85830.1 ,gb AKZ47209.1 ,gb EMS74571.1 ,gb EOT33799.1 ,gb EQU25430.1 ,gb OQO82120.1	37.834,8
175	hypothetical protein LIANG_11460 [Enterococcus durans]	gb AKX86705.1 ,gb AKZ48060.1 ,gb EMS75482.1 ,gb EOT34742.1 ,gb EQU19600.1 ,gb OQO81198.1	49.365,7
176	30S ribosomal protein S5 [Enterococcus durans]	gb AKX85793.1 ,gb AKZ47171.1 ,gb EMS74534.1 ,gb EOT33837.1 ,gb EQU25468.1 ,gb OQO82157.1	17.476,9
177	elongation factor G [Enterococcus durans]	gb AKX85819.1 ,gb AKZ47197.1 ,gb EMS74559.1 ,gb EOT33811.1 ,gb EQU25442.1 ,gb OQO82132.1	76.742,1
178	glyceraldehyde-3-phosphate dehydrogenase	gb AKX86905.1 ,gb AKZ48257.1 ,gb EMS75963.1 ,gb EOT35299.1 ,gb EQU19372.1 ,gb OQO82543.1	36.155,7
179	ribosome-recycling factor [Enterococcus durans]	gb AKX84983.1 ,gb AKZ48645.1 ,gb EMS76647.1 ,gb EOT36305.1 ,gb EQU18893.1 ,gb OQO79346.1	20.881,9
180	lactate dehydrogenase [Enterococcus durans]	gb AKX86027.1 ,gb AKZ47406.1 ,gb EMS77070.1 ,gb EOT29104.1 ,gb EQU16350.1 ,gb OQO79291.1	35.131,4
181	tagatose-bisphosphate aldolase [Enterococcus durans]	gb AKX86137.1 ,gb AKZ47509.1 ,gb EMS75410.1 ,ref WP_002290551.1 ,ref WP_005878819.1	36.445,1
182	osmotically inducible protein C [Enterococcus durans]	gb AKX86185.1 ,gb AKZ47557.1 ,gb EMS74585.1 ,gb OQO80135.1 ,ref WP_002292291.1	14.674,3
183	phosphoenolpyruvate-protein phosphotransferase	gb AKX86601.1 ,gb AKZ47958.1 ,ref WP_053108894.1	63.353,5
184	3-deoxy-7-phosphoheptulonate synthase [Enterococcus durans]	gb AKX85527.1 ,gb AKZ49178.1 ,gb EMS75341.1 ,gb EOT25786.1 ,gb EQU22495.1 ,gb OQO81675.1	37.495,2
185	enolase [Enterococcus durans]	gb AKX85212.1 ,gb AKZ48874.1 ,gb EMS75631.1 ,gb EOT32245.1 ,gb EQU20046.1 ,gb OQO81409.1	46.481,0
186	50S ribosomal protein L2 [Enterococcus durans]	gb AKX85807.1 ,gb AKZ47185.1 ,gb EMS74548.1 ,gb EOT33823.1 ,gb EQU25454.1 ,gb OQO82144.1	30.326,6
187	elongation factor Tu [Enterococcus durans]	gb AKX85818.1 ,gb AKZ47196.1 ,gb EMS74558.1 ,gb EOT33812.1 ,gb EQU25443.1 ,gb OQO82133.1	43.162,2
188	DNA-binding protein [Enterococcus durans]	gb AKX86714.1 ,gb AKZ48069.1 ,gb EMS75473.1 ,gb EOT34733.1 ,gb EQU19591.1 ,gb OQO81189.1	9.682,3
189	ATP synthase beta-subunit, partial [Enterococcus durans]	gb ADO14905.1 ,gb AKX85196.1 ,gb AKZ48858.1 ,gb EMS75649.1 ,gb EOT32263.1 ,gb EQU20064.1	51.167,8
190	osmotically inducible protein C [Enterococcus durans]	gb AKX86277.1 ,gb AKZ47647.1 ,gb EMS75167.1 ,gb EOT34377.1 ,gb EQU25801.1 ,gb OQO78110.1	14.438,9
191	trigger factor [Enterococcus durans]	gb AKX84928.1 ,gb AKZ48590.1 ,gb EMS74945.1 ,gb EOT36106.1 ,gb EQU18964.1 ,gb OQO78488.1	47.671,9
192	ferritin [Enterococcus durans]	gb AKX85904.1 ,gb AKZ47283.1 ,gb EMS74283.1 ,gb EOT28129.1 ,gb EQU16439.1 ,gb OQO79021.1	17.950,9
193	tyrosine--tRNA ligase [Enterococcus durans]	gb AKX85644.1 ,gb AKZ47022.1 ,gb EMS76936.1 ,gb EOT32743.1 ,gb EQU25646.1 ,gb OQO82310.1	47.335,7
194	50S ribosomal protein L13 [Enterococcus durans]	gb AKX87109.1 ,gb AKZ49295.1 ,gb EMS74286.1 ,gb EOT28126.1 ,gb EQU16436.1 ,gb OQO79028.1	15.652,5
195	NADPH:quinone reductase [Enterococcus durans]	gb AKX85133.1 ,gb AKZ48795.1 ,gb EMS74649.1 ,gb EOT31576.1 ,gb EQU18586.1 ,gb OQO81494.1	33.960,6
196	30S ribosomal protein S8 [Enterococcus durans]	gb AKX85796.1 ,gb AKZ47174.1 ,gb EMS74537.1 ,gb EOT33834.1 ,gb EQU25465.1 ,gb OQO82155.1	14.840,7
197	phosphoglycerate kinase [Enterococcus durans]	gb AKX85214.1 ,gb AKZ48876.1 ,gb EMS75629.1 ,gb EOT32243.1 ,gb EQU20044.1 ,gb OQO81407.1	41.855,5
198	50S ribosomal protein L7/L12 [Enterococcus durans]	gb AKX85495.1 ,gb AKZ49148.1 ,gb EMS75307.1 ,gb EOT25821.1 ,gb EQU22530.1 ,gb OQO81709.1	12.497,9
199	30S ribosomal protein S9 [Enterococcus durans]	gb AKX85907.1 ,gb AKZ47286.1 ,gb EMS74287.1 ,gb EOT28125.1 ,gb EQU16435.1 ,gb OQO79024.1	14.362,8

200	oxidoreductase [Enterococcus durans]	gb AKX84788.1 ,gb AKZ48447.1 ,gb EMS75711.1 ,gb EOT35435.1 ,gb EOU19143.1 ,gb OQO78703.1	31.934,8
201	triosephosphate isomerase [Enterococcus durans]	gb AKX85213.1 ,gb AKZ48875.1 ,gb EMS75630.1 ,gb OQO81408.1 ,ref WP_005878431.1	26.877,8
202	30S ribosomal protein S6 [Enterococcus durans]	gb AKX85856.1 ,gb AKZ47235.1 ,gb EMS76722.1 ,gb EOT33747.1 ,gb EOU25378.1 ,gb OQO82066.1	11.578,8
203	30S ribosomal protein S19 [Enterococcus durans]	gb AKX85806.1 ,gb AKZ47184.1 ,gb EMS74547.1 ,gb EOT33824.1 ,gb EOU25455.1 ,gb OQO82145.1	10.521,2
204	50S ribosomal protein L15 [Enterococcus durans]	gb AKX85791.1 ,gb AKZ47169.1 ,gb EMS74532.1 ,gb EOT33839.1 ,gb EOU25470.1 ,gb OQO82159.1	15.424,8
205	aspartate carbamoyltransferase catalytic subunit	gb AKZ48309.1 ,gb EMS74996.1 ,gb EOT35362.1 ,gb EOU19315.1 ,gb OQO82592.1 ,ref WP_005879675.1	34.904,9
206	GMP synthase [Enterococcus durans]	gb AKX85836.1 ,gb AKZ47215.1 ,gb EMS74578.1 ,gb EOT33792.1 ,gb EOU25423.1 ,gb OQO82113.1	57.759,7
207	30S ribosomal protein S11 [Enterococcus durans]	gb AKX85785.1 ,gb AKZ47163.1 ,gb EMS74526.1 ,gb EOT33845.1 ,gb EOU25476.1 ,gb OQO82165.1	13.736,0
208	50S ribosomal protein L21 [Enterococcus durans]	gb AKX86487.1 ,gb AKZ47845.1 ,gb EMS74445.1 ,gb EOT33943.1 ,gb EOU26060.1 ,gb OQO81885.1	11.174,6
209	elongation factor Tu [Enterococcus durans]	gb AKX86680.1 ,gb AKZ48035.1 ,gb EMS75506.1 ,gb EOT34767.1 ,gb EOU19625.1 ,gb OQO81219.1	43.213,0
210	inosine-5-monophosphate dehydrogenase [Enterococcus durans]	gb AKX85874.1 ,gb AKZ47255.1 ,gb EMS76701.1 ,gb EOT33726.1 ,gb EOU25357.1 ,gb OQO82046.1	53.029,8
211	glucose-6-phosphate isomerase [Enterococcus durans]	gb AKX85027.1 ,gb AKZ48688.1 ,gb EMS76330.1 ,gb EOT36447.1 ,gb EOU18787.1 ,gb OQO77954.1	49.752,2
212	GroES, partial [Enterococcus durans]	gb AAN32674.1 AF417585_1,gb AKX85467.1 ,gb AKZ49124.1 ,gb EMS74729.1 ,gb EOT29787.1	10.031,4
213	molecular chaperone GrpE [Enterococcus durans]	gb AKX84949.1 ,gb AKZ48611.1 ,gb EMS74969.1 ,gb EOT36158.1 ,gb EOU18940.1 ,gb OQO78466.1	21.149,6
214	glyceraldehyde-3-phosphate dehydrogenase	gb AKX85215.1 ,gb AKZ48877.1 ,gb EMS75628.1 ,gb EOT32242.1 ,gb EOU20043.1 ,gb OQO81406.1	35.774,4
215	pyruvate kinase [Enterococcus durans]	gb AKX86645.1 ,gb AKZ47998.1 ,gb EMS77112.1 ,gb EOT34121.1 ,gb EOU26238.1 ,gb OQO80009.1	63.662,1
216	30S ribosomal protein S10 [Enterococcus durans]	gb AKX85811.1 ,gb AKZ47189.1 ,gb EMS74552.1 ,gb EOT33819.1 ,gb EOU25450.1 ,gb OQO82140.1	18.006,8
217	uracil phosphoribosyltransferase [Enterococcus durans]	gb AKX86332.1 ,gb AKZ47698.1 ,gb EMS75108.1 ,gb EOT34441.1 ,gb EOU25865.1 ,gb OQO80536.1	22.888,5
218	molecular chaperone GroEL [Enterococcus durans]	gb AKX85466.1 ,gb AKZ49123.1 ,gb EMS74730.1 ,gb OQO81745.1 ,ref WP_005880136.1	57.148,7
219	50S ribosomal protein L29 [Enterococcus durans]	gb AKX85802.1 ,gb AKZ47180.1 ,gb EMS74543.1 ,gb EOT33828.1 ,gb EOU25459.1 ,gb OQO82149.1	7.344,0
220	50S ribosomal protein L22 [Enterococcus durans]	gb AKX85805.1 ,gb AKZ47183.1 ,gb EMS74546.1 ,gb EOT33825.1 ,gb EOU25456.1 ,gb OQO82146.1	12.450,7
221	arginine deiminase [Enterococcus durans]	gb AKX84990.1 ,gb AKZ48652.1 ,gb EMS76655.1 ,gb EOT36297.1 ,gb EOU18885.1 ,gb OQO79339.1	46.057,9
222	transketolase [Enterococcus durans]	gb AKX86919.1 ,gb AKZ48271.1 ,gb EMS76686.1 ,gb EOT35404.1 ,gb EOU19357.1 ,ref WP_005875855.1	72.147,2
223	acyl carrier protein [Enterococcus durans]	gb AKX86608.1 ,gb AKZ47964.1 ,gb EMS76493.1 ,gb EOT34083.1 ,gb EOU26200.1 ,gb OQO80044.1	8.562,8
224	phosphoglyceromutase [Enterococcus durans]	gb OQO82127.1 ,ref WP_081133709.1	25.914,4
225	fructose-bisphosphate aldolase [Enterococcus durans]	gb AKX85321.1 ,gb AKZ48980.1 ,gb EMS76746.1 ,gb EOT32130.1 ,gb EOU19931.1 ,gb OQO78736.1	30.809,7
226	Clp protease ClpX (plasmid) [Enterococcus durans]	gb AKX87246.1 ,gb EMS76214.1 ,gb EOT30301.1 ,gb EOU15544.1 ,ref WP_005876977.1	77.848,4
227	30S ribosomal protein S2 [Enterococcus durans]	gb AKX84985.1 ,gb AKZ48647.1 ,gb EMS76650.1 ,gb EOT36302.1 ,gb EOU18890.1 ,gb OQO79344.1	29.298,5
228	general stress protein [Enterococcus durans]	gb AKX85160.1 ,gb AKZ48823.1 ,gb EMS74678.1 ,gb EOT31758.1 ,gb EOU18551.1 ,gb OQO81463.1	21.119,5

229	molecular chaperone DnaK [Enterococcus durans]	gb AKX84948.1 ,gb AKZ48610.1 ,gb EMS74968.1 ,gb OQO78467.1 ,ref WP_005880047.1	65.692,7
230	phosphocarrier protein HPr [Enterococcus durans]	gb AKX86600.1 ,gb AKZ47957.1 ,gb EMS76502.1 ,gb EOT34074.1 ,gb EOU26191.1 ,gb OQO80053.1	9.312,5
231	stress response regulator Gls24 [Enterococcus durans]	gb AKX84772.1 ,gb EMS76996.1 ,gb EOT35451.1 ,gb EOU19160.1 ,gb OQO82729.1 ,ref WP_005875244.1	20.508,5
232	PTS cellobiose transporter subunit IIB [Enterococcus durans]	gb AKX86078.1 ,gb AKZ47452.1 ,gb EMS75805.1 ,gb EOT26119.1 ,gb EOU22378.1 ,gb OQO81560.1	11.549,6
233	6-phosphofructokinase [Enterococcus durans]	gb AKX86644.1 ,gb AKZ47997.1 ,gb EMS77111.1 ,gb EOT34120.1 ,gb EOU26237.1 ,gb OQO80010.1	34.187,9
234	30S ribosomal protein S3 [Enterococcus durans]	gb AKX85804.1 ,gb AKZ47182.1 ,gb EMS74545.1 ,gb EOT33826.1 ,gb EOU25457.1 ,gb OQO82147.1	24.406,0
235	ornithine carbamoyltransferase [Enterococcus durans]	gb AKX84989.1 ,gb AKZ48651.1 ,gb EMS76654.1 ,gb EOT36298.1 ,gb EOU18886.1 ,gb OQO79340.1	38.341,7
236	hypothetical protein LIANG_08385 [Enterococcus durans]	gb AKX86179.1 ,gb AKZ47551.1 ,gb EMS75204.1 ,ref WP_005879148.1	16.865,4
237	30S ribosomal protein S1 [Enterococcus durans]	gb AKX86712.1 ,gb AKZ48067.1 ,gb EMS75475.1 ,gb EOT34735.1 ,gb EOU19593.1 ,gb OQO81191.1	44.559,6
238	30S ribosomal protein S12 [Enterococcus durans]	gb AKX85821.1 ,gb AKZ47199.1 ,gb EMS74561.1 ,gb EOT33809.1 ,gb EOU25440.1 ,gb OQO82130.1	15.215,9
239	50S ribosomal protein L17 [Enterococcus durans]	gb AKX85783.1 ,gb AKZ47161.1 ,gb EMS74524.1 ,gb EOT33847.1 ,gb EOU25478.1 ,gb OQO82167.1	14.344,9
240	ATP FOF1 synthase subunit alpha [Enterococcus durans]	gb AKX85198.1 ,gb AKZ48860.1 ,gb EMS75647.1 ,gb EOT32261.1 ,gb EOU20062.1 ,gb OQO81424.1	56.397,4
241	hypothetical protein LIANG_06205 [Enterococcus durans]	gb AKX85830.1 ,gb AKZ47209.1 ,gb EMS74571.1 ,gb EOT33799.1 ,gb EOU25430.1 ,gb OQO82120.1	37.834,8
242	general stress protein [Enterococcus durans]	gb AKX85160.1 ,gb AKZ48823.1 ,gb EMS74678.1 ,gb EOT31758.1 ,gb EOU18551.1 ,gb OQO81463.1	21.119,5
243	30S ribosomal protein S9 [Enterococcus durans]	gb AKX85907.1 ,gb AKZ47286.1 ,gb EMS74287.1 ,gb EOT28125.1 ,gb EOU16435.1 ,gb OQO79024.1	14.362,8
244	hypothetical protein LIANG_06205 [Enterococcus durans]	gb AKX85830.1 ,gb AKZ47209.1 ,gb EMS74571.1 ,gb EOT33799.1 ,gb EOU25430.1 ,gb OQO82120.1	37.834,8
245	molecular chaperone GrpE [Enterococcus durans]	gb AKX84949.1 ,gb AKZ48611.1 ,gb EMS74969.1 ,gb EOT36158.1 ,gb EOU18940.1 ,gb OQO78466.1	21.149,6
246	30S ribosomal protein S12 [Enterococcus durans]	gb AKX85821.1 ,gb AKZ47199.1 ,gb EMS74561.1 ,gb EOT33809.1 ,gb EOU25440.1 ,gb OQO82130.1	15.215,9
247	hypothetical protein H318_07903	gb EMS75618.1 ,gb EOT32232.1 ,gb EOU20033.1 ,gb OQO81395.1 ,ref WP_005878408.1	16.010,1
248	citrate (Pro-3S)-lyase, beta subunit	gb EOT31306.1 ,gb EOU15562.1 ,ref WP_016177559.1	33.102,5
249	molecular chaperone DnaK [Enterococcus durans]	gb AKX84948.1 ,gb AKZ48610.1 ,gb EMS74968.1 ,gb OQO78467.1 ,ref WP_005880047.1	65.692,7
250	pheromone cAD1 precursor lipoprotein	gb EMS77019.1 ,gb EOT28144.1 ,gb EOU16454.1 ,ref WP_005875228.1 ,ref WP_01617776.1	33.478,6
251	ornithine carbamoyltransferase [Enterococcus durans]	gb AKX84989.1 ,gb AKZ48651.1 ,gb EMS76654.1 ,gb EOT36298.1 ,gb EOU18886.1 ,gb OQO79340.1	38.341,7
252	50S ribosomal protein L17 [Enterococcus durans]	gb AKX85783.1 ,gb AKZ47161.1 ,gb EMS74524.1 ,gb EOT33847.1 ,gb EOU25478.1 ,gb OQO82167.1	14.344,9
253	ATP FOF1 synthase subunit alpha [Enterococcus durans]	gb AKX85198.1 ,gb AKZ48860.1 ,gb EMS75647.1 ,gb EOT32261.1 ,gb EOU20062.1 ,gb OQO81424.1	56.397,4
254	ferritin [Enterococcus durans]	gb AKX85904.1 ,gb AKZ47283.1 ,gb EMS74283.1 ,gb EOT28129.1 ,gb EOU16439.1 ,gb OQO79021.1	17.950,9
255	pyruvate kinase [Enterococcus durans]	gb AKX86645.1 ,gb AKZ47998.1 ,gb EMS77112.1 ,gb EOT34121.1 ,gb EOU26238.1 ,gb OQO80009.1	63.662,1
256	hypothetical protein LIANG_08385 [Enterococcus durans]	gb AKX86179.1 ,gb AKZ47551.1 ,gb EMS75204.1 ,ref WP_005879148.1	16.865,4
257	S-adenosylmethionine synthetase [Enterococcus durans]	gb AKX86039.1 ,gb AKZ47417.1 ,gb EMS75771.1 ,gb EOT26478.1 ,gb EOU22338.1 ,gb OQO81524.1	43.278,6

258	cell division protein FtsZ [Enterococcus durans]	gb AKX86518.1 ,gb EMS74412.1 ,gb EOT33976.1 ,gb EOU26093.1 ,gb OQO81852.1 ,ref WP_005881040.1	44.328,3
259	6-phosphofructokinase [Enterococcus durans]	gb AKX86644.1 ,gb AKZ47997.1 ,gb EMS77111.1 ,gb EOT34120.1 ,gb EOU26237.1 ,gb OQO80010.1	34.187,9
260	glucose-6-phosphate isomerase [Enterococcus durans]	gb AKX85027.1 ,gb AKZ48688.1 ,gb EMS76330.1 ,gb EOT36447.1 ,gb EOU18787.1 ,gb OQO77954.1	49.752,2
261	phosphoenolpyruvate-protein phosphotransferase	gb AKX86601.1 ,gb AKZ47958.1 ,ref WP_053108894.1	63.353,5
262	Clp protease ClpX (plasmid) [Enterococcus durans]	gb AKX87246.1 ,gb EMS76214.1 ,gb EOT30301.1 ,gb EOU15544.1 ,ref WP_005876977.1	77.848,4
263	30S ribosomal protein S7 [Enterococcus durans]	gb AKX85820.1 ,gb AKZ47198.1 ,gb EMS74560.1 ,gb EOT33810.1 ,gb EOU25441.1 ,gb OQO82131.1	17.843,7
264	ATP synthase beta-subunit, partial [Enterococcus durans]	gb ADO14905.1 ,gb AKX85196.1 ,gb AKZ48858.1 ,gb EMS75649.1 ,gb EOT32263.1 ,gb EOU20064.1	51.167,8
265	arginine deiminase [Enterococcus durans]	gb AKX84990.1 ,gb AKZ48652.1 ,gb EMS76655.1 ,gb EOT36297.1 ,gb EOU18885.1 ,gb OQO79339.1	46.057,9
266	50S ribosomal protein L7/L12 [Enterococcus durans]	gb AKX85495.1 ,gb AKZ49148.1 ,gb EMS75307.1 ,gb EOT25821.1 ,gb EOU22530.1 ,gb OQO81709.1	12.497,9
267	general stress protein [Enterococcus durans]	gb AKX84785.1 ,gb AKZ48444.1 ,gb EMS75708.1 ,gb EOT35438.1 ,gb EOU19146.1 ,gb OQO78700.1	30.065,8
268	dihydrolipoamide dehydrogenase [Enterococcus durans]	gb AKX86822.1 ,gb AKZ48179.1 ,gb EMS76473.1 ,gb EOT34966.1 ,gb EOU19458.1 ,gb OQO82466.1	49.261,4
269	oxidoreductase [Enterococcus durans]	gb AKX84788.1 ,gb AKZ48447.1 ,gb EMS75711.1 ,gb EOT35435.1 ,gb EOU19143.1 ,gb OQO78703.1	31.934,8
270	PTS mannose transporter subunit IIAB [Enterococcus durans]	gb AKX86491.1 ,gb AKZ47851.1 ,gb EMS74439.1 ,gb EOT33949.1 ,gb EOU26066.1 ,gb OQO81879.1	35.253,2
271	glutamyl-tRNA synthetase [Enterococcus durans]	gb AKZ47353.1 ,gb EMS76247.1 ,gb EOT28433.1 ,gb EOU16409.1 ,gb OQO78070.1 ,ref WP_005876899.1	55.356,2
272	2-oxoisovalerate dehydrogenase [Enterococcus durans]	gb AKX86820.1 ,gb AKZ48177.1 ,gb EMS76471.1 ,gb EOT34968.1 ,gb EOU19460.1 ,gb OQO82464.1	35.384,6
273	30S ribosomal protein S6 [Enterococcus durans]	gb AKX85856.1 ,gb AKZ47235.1 ,gb EMS76722.1 ,gb EOT33747.1 ,gb EOU25378.1 ,gb OQO82066.1	11.578,8
274	30S ribosomal protein S10 [Enterococcus durans]	gb AKX85811.1 ,gb AKZ47189.1 ,gb EMS74552.1 ,gb EOT33819.1 ,gb EOU25450.1 ,gb OQO82140.1	18.006,8
275	GroES, partial [Enterococcus durans]	gb AAN32674.1 AF417585_1 ,gb AKX85467.1 ,gb AKZ49124.1 ,gb EMS74729.1 ,gb EOT29787.1	10.031,4
276	50S ribosomal protein L2 [Enterococcus durans]	gb AKX85807.1 ,gb AKZ47185.1 ,gb EMS74548.1 ,gb EOT33823.1 ,gb EOU25454.1 ,gb OQO82144.1	30.326,6
277	lactate dehydrogenase [Enterococcus durans]	gb AKX86027.1 ,gb AKZ47406.1 ,gb EMS77070.1 ,gb EOT29104.1 ,gb EOU16350.1 ,gb OQO79291.1	35.131,4
278	hypothetical protein H318_02255 [Enterococcus durans IPLA 655]	gb EMS76691.1 ,gb EOT35409.1 ,gb EOU19362.1 ,ref WP_016176718.1	228.261,1
279	ribosome-recycling factor [Enterococcus durans]	gb AKX84983.1 ,gb AKZ48645.1 ,gb EMS76647.1 ,gb EOT36305.1 ,gb EOU18893.1 ,gb OQO79346.1	20.881,9
280	30S ribosomal protein S19 [Enterococcus durans]	gb AKX85806.1 ,gb AKZ47184.1 ,gb EMS74547.1 ,gb EOT33824.1 ,gb EOU25455.1 ,gb OQO82145.1	10.521,2
281	30S ribosomal protein S2 [Enterococcus durans]	gb AKX84985.1 ,gb AKZ48647.1 ,gb EMS76650.1 ,gb EOT36302.1 ,gb EOU18890.1 ,gb OQO79344.1	29.298,5
282	50S ribosomal protein L4 [Enterococcus durans]	gb AKX85809.1 ,gb AKZ47187.1 ,gb EMS74550.1 ,gb EOT33821.1 ,gb EOU25452.1 ,gb OQO82142.1	22.462,4
283	galactose-6-phosphate isomerase [Enterococcus durans]	gb AKX86135.1 ,gb AKZ47507.1 ,gb EMS75412.1 ,ref WP_002345825.1	18.902,4
284	decarboxylase [Enterococcus durans]	gb AKX85643.1 ,gb AKZ47021.1 ,gb EMS76937.1 ,gb EOT32744.1 ,gb EOU25647.1 ,gb OQO82311.1	70.192,3
285	50S ribosomal protein L22 [Enterococcus durans]	gb AKX85805.1 ,gb AKZ47183.1 ,gb EMS74546.1 ,gb EOT33825.1 ,gb EOU25456.1 ,gb OQO82146.1	12.450,7
286	phosphoglycerate kinase [Enterococcus durans]	gb AKX85214.1 ,gb AKZ48876.1 ,gb EMS75629.1 ,gb EOT32243.1 ,gb EOU20044.1 ,gb OQO81407.1	41.855,5

287	molecular chaperone GroEL [Enterococcus durans]	gb AKX85466.1 ,gb AKZ49123.1 ,gb EMS74730.1 ,gb OQO81745.1 ,ref WP_005880136.1	57.148,7
288	dihydroxyacetone kinase [Enterococcus durans]	gb AKX85646.1 ,gb AKZ47024.1 ,gb EMS76934.1 ,gb EOT32741.1 ,gb EOU15552.1 ,gb EOU25644.1	21.837,3
289	tyrosine--tRNA ligase [Enterococcus durans]	gb AKX85644.1 ,gb AKZ47022.1 ,gb EMS76936.1 ,gb EOT32743.1 ,gb EOU25646.1 ,gb OQO82310.1	47.335,7
290	transcription elongation factor GreA [Enterococcus durans]	gb AKX86569.1 ,gb AKZ47926.1 ,gb EMS76534.1 ,gb EOT34040.1 ,gb EOU26157.1 ,gb OQO77684.1	17.458,1
291	osmotically inducible protein C [Enterococcus durans]	gb AKX86185.1 ,gb AKZ47557.1 ,gb EMS74585.1 ,gb OQO80135.1 ,ref WP_002292291.1	14.674,3
292	hypothetical protein OMS_02283, partial	gb EOT31214.1 ,gb EOU15594.1 ,ref WP_016252677.1 ,ref WP_034865697.1 ,ref WP_060789802.1	161.488,2
293	glyceraldehyde-3-phosphate dehydrogenase	gb AKX86905.1 ,gb AKZ48257.1 ,gb EMS75963.1 ,gb EOT35299.1 ,gb EOU19372.1 ,gb OQO82543.1	36.155,7
294	tagatose-bisphosphate aldolase [Enterococcus durans]	gb AKX86137.1 ,gb AKZ47509.1 ,gb EMS75410.1 ,ref WP_002290551.1 ,ref WP_005878819.1	36.445,1
295	triosephosphate isomerase [Enterococcus durans]	gb AKX85213.1 ,gb AKZ48875.1 ,gb EMS75630.1 ,gb OQO81408.1 ,ref WP_005878431.1	26.877,8
296	trigger factor [Enterococcus durans]	gb AKX84928.1 ,gb AKZ48590.1 ,gb EMS74945.1 ,gb EOT36106.1 ,gb EOU18964.1 ,gb OQO78488.1	47.671,9
297	50S ribosomal protein L29 [Enterococcus durans]	gb AKX85802.1 ,gb AKZ47180.1 ,gb EMS74543.1 ,gb EOT33828.1 ,gb EOU25459.1 ,gb OQO82149.1	7.344,0
298	50S ribosomal protein L31 type B [Enterococcus durans]	gb AKX85318.1 ,gb AKZ48977.1 ,gb EMS76749.1 ,gb EOT32133.1 ,gb EOU19934.1 ,gb OQO78733.1	9.936,9
299	NADH oxidase [Enterococcus durans]	gb AKZ48275.1 ,gb EMS76682.1 ,gb EOT35400.1 ,gb EOU19353.1 ,gb OQO82558.1	49.253,2
300	GMP synthase [Enterococcus durans]	gb AKX85836.1 ,gb AKZ47215.1 ,gb EMS74578.1 ,gb EOT33792.1 ,gb EOU25423.1 ,gb OQO82113.1	57.759,7
301	2,5-diketo-D-gluconic acid reductase [Enterococcus durans]	gb AKX86672.1 ,gb AKZ48028.1 ,gb EMS75514.1 ,gb OQO81227.1 ,ref WP_005878645.1	31.865,6
302	uracil phosphoribosyltransferase [Enterococcus durans]	gb AKX86332.1 ,gb AKZ47698.1 ,gb EMS75108.1 ,gb EOT34441.1 ,gb EOU25865.1 ,gb OQO80536.1	22.888,5
303	phosphoglyceromutase [Enterococcus durans]	gb OQO82127.1 ,ref WP_081133709.1	25.914,4
304	30S ribosomal protein S8 [Enterococcus durans]	gb AKX85796.1 ,gb AKZ47174.1 ,gb EMS74537.1 ,gb EOT33834.1 ,gb EOU25465.1 ,gb OQO82155.1	14.840,7
305	30S ribosomal protein S11 [Enterococcus durans]	gb AKX85785.1 ,gb AKZ47163.1 ,gb EMS74526.1 ,gb EOT33845.1 ,gb EOU25476.1 ,gb OQO82165.1	13.736,0
306	glyceraldehyde-3-phosphate dehydrogenase	gb AKX85215.1 ,gb AKZ48877.1 ,gb EMS75628.1 ,gb EOT32242.1 ,gb EOU20043.1 ,gb OQO81406.1	35.774,4
307	50S ribosomal protein L13 [Enterococcus durans]	gb AKX87109.1 ,gb AKZ49295.1 ,gb EMS74286.1 ,gb EOT28126.1 ,gb EOU16436.1 ,gb OQO79028.1	15.652,5
308	universal stress protein UspA [Enterococcus durans]	gb AKX86231.1 ,gb AKZ47601.1 ,gb EMS76048.1 ,gb EOT34514.1 ,gb EOU25751.1 ,gb OQO79639.1	17.246,0
309	hypothetical protein LIANG_08515 [Enterococcus durans]	gb AKX86201.1 ,gb AKZ47572.1 ,gb EMS76564.1 ,gb EOT32805.1 ,gb EOU25708.1 ,gb OQO80094.1	13.151,2
310	fructose-bisphosphate aldolase [Enterococcus durans]	gb AKX85321.1 ,gb AKZ48980.1 ,gb EMS76746.1 ,gb EOT32130.1 ,gb EOU19931.1 ,gb OQO78736.1	30.809,7
311	30S ribosomal protein S5 [Enterococcus durans]	gb AKX85793.1 ,gb AKZ47171.1 ,gb EMS74534.1 ,gb EOT33837.1 ,gb EOU25468.1 ,gb OQO82157.1	17.476,9
312	elongation factor Tu [Enterococcus durans]	gb AKX85818.1 ,gb AKZ47196.1 ,gb EMS74558.1 ,gb EOT33812.1 ,gb EOU25443.1 ,gb OQO82133.1	43.162,2
313	cold-shock protein [Enterococcus durans]	gb AKX86580.1 ,gb AKZ47937.1 ,gb EMS76523.1 ,gb EOT34050.1 ,gb EOU26167.1 ,gb OQO77812.1	7.204,7
314	elongation factor Tu [Enterococcus durans]	gb AKX86680.1 ,gb AKZ48035.1 ,gb EMS75506.1 ,gb EOT34767.1 ,gb EOU19625.1 ,gb OQO81219.1	43.213,0
315	30S ribosomal protein S1 [Enterococcus durans]	gb AKX86712.1 ,gb AKZ48067.1 ,gb EMS75475.1 ,gb EOT34735.1 ,gb EOU19593.1 ,gb OQO81191.1	44.559,6

316	enolase [Enterococcus durans]	gb AKX85212.1 ,gb AKZ48874.1 ,gb EMS75631.1 ,gb EOT32245.1 ,gb EOU20046.1 ,gb OQO81409.1	46.481,0
317	hypothetical protein LIANG_11460 [Enterococcus durans]	gb AKX86705.1 ,gb AKZ48060.1 ,gb EMS75482.1 ,gb EOT34742.1 ,gb EOU19600.1 ,gb OQO81198.1	49.365,7
318	hypothetical protein OMS_02998 [Enterococcus durans]	gb EOT25847.1 ,gb EOT31699.1 ,gb EOU18709.1 ,gb EOU22556.1 ,ref WP_016177529.1	12.102,2
319	50S ribosomal protein L15 [Enterococcus durans]	gb AKX85791.1 ,gb AKZ47169.1 ,gb EMS74532.1 ,gb EOT33839.1 ,gb EOU25470.1 ,gb OQO82159.1	15.424,8
320	general stress protein [Enterococcus durans]	gb AKX85160.1 ,gb AKZ48823.1 ,gb EMS74678.1 ,gb EOT31758.1 ,gb EOU18551.1 ,gb OQO81463.1	21.119,5
321	hypothetical protein LIANG_05820 [Enterococcus durans]	gb AKX85756.1 ,gb AKZ47134.1 ,gb EMS74497.1 ,gb EOT33673.1 ,gb EOU25508.1 ,gb OQO82198.1	13.205,8
322	hypothetical protein LIANG_06205 [Enterococcus durans]	gb AKX85830.1 ,gb AKZ47209.1 ,gb EMS74571.1 ,gb EOT33799.1 ,gb EOU25430.1 ,gb OQO82120.1	37.834,8
323	molecular chaperone GrpE [Enterococcus durans]	gb AKX84949.1 ,gb AKZ48611.1 ,gb EMS74969.1 ,gb EOT36158.1 ,gb EOU18940.1 ,gb OQO78466.1	21.149,6
324	30S ribosomal protein S12 [Enterococcus durans]	gb AKX85821.1 ,gb AKZ47199.1 ,gb EMS74561.1 ,gb EOT33809.1 ,gb EOU25440.1 ,gb OQO82130.1	15.215,9
325	hypothetical protein H318_07903 [Enterococcus durans IPLA 655]	gb EMS75618.1 ,gb EOT32232.1 ,gb EOU20033.1 ,gb OQO81395.1 ,ref WP_005878408.1	16.010,1
326	50S ribosomal protein L32 [Enterococcus durans]	gb AKX86648.1 ,gb AKZ48001.1 ,gb EMS77115.1 ,gb EOT34124.1 ,gb EOU26241.1 ,gb OQO80006.1	6.669,8
327	glycerol kinase [Enterococcus durans]	gb AKX86100.1 ,gb AKZ47472.1 ,gb EMS76961.1 ,gb EOT32768.1 ,gb EOU25671.1 ,gb OQO82334.1	55.577,9
328	30S ribosomal protein S11 [Enterococcus durans]	gb AKX85785.1 ,gb AKZ47163.1 ,gb EMS74526.1 ,gb EOT33845.1 ,gb EOU25476.1 ,gb OQO82165.1	13.736,0
329	citrate (Pro-3S)-lyase, beta subunit [Enterococcus durans]	gb EOT31306.1 ,gb EOU15562.1 ,ref WP_016177559.1	33.102,5
330	stress response regulator Gls24 [Enterococcus durans]	gb AKX84772.1 ,gb EMS76996.1 ,gb EOT35451.1 ,gb EOU19160.1 ,gb OQO82729.1 ,ref WP_005875244.1	20.508,5
331	cell division protein FtsH [Enterococcus durans]	gb AKX86034.1 ,gb EMS77079.1 ,gb EOT29095.1 ,gb EOU16341.1 ,gb OQO79299.1 ,ref WP_005875188.1	77.036,0
332	arginine deiminase [Enterococcus durans]	gb AKX84990.1 ,gb AKZ48652.1 ,gb EMS76655.1 ,gb EOT36297.1 ,gb EOU18885.1 ,gb OQO79339.1	46.057,9
333	ornithine carbamoyltransferase [Enterococcus durans]	gb AKX84989.1 ,gb AKZ48651.1 ,gb EMS76654.1 ,gb EOT36298.1 ,gb EOU18886.1 ,gb OQO79340.1	38.341,7
334	50S ribosomal protein L17 [Enterococcus durans]	gb AKX85783.1 ,gb AKZ47161.1 ,gb EMS74524.1 ,gb EOT33847.1 ,gb EOU25478.1 ,gb OQO82167.1	14.344,9
335	ATP FOF1 synthase subunit alpha [Enterococcus durans]	gb AKX85198.1 ,gb AKZ48860.1 ,gb EMS75647.1 ,gb EOT32261.1 ,gb EOU20062.1 ,gb OQO81424.1	56.397,4
336	ferritin [Enterococcus durans]	gb AKX85904.1 ,gb AKZ47283.1 ,gb EMS74283.1 ,gb EOT28129.1 ,gb EOU16439.1 ,gb OQO79021.1	17.950,9
337	pyruvate kinase [Enterococcus durans]	gb AKX86645.1 ,gb AKZ47998.1 ,gb EMS77112.1 ,gb EOT34121.1 ,gb EOU26238.1 ,gb OQO80009.1	63.662,1
338	hypothetical protein LIANG_08385 [Enterococcus durans]	gb AKX86179.1 ,gb AKZ47551.1 ,gb EMS75204.1 ,ref WP_005879148.1	16.865,4
339	30S ribosomal protein S13 [Enterococcus durans]	gb AKX85786.1 ,gb AKZ47164.1 ,gb EMS74527.1 ,gb EOT33844.1 ,gb EOU25475.1 ,gb OQO82164.1	13.534,1
340	cell division protein FtsZ [Enterococcus durans]	gb AKX86518.1 ,gb EMS74412.1 ,gb EOT33976.1 ,gb EOU26093.1 ,gb OQO81852.1 ,ref WP_005881040.1	44.328,3
341	6-phosphofructokinase [Enterococcus durans]	gb AKX86644.1 ,gb AKZ47997.1 ,gb EMS77111.1 ,gb EOT34120.1 ,gb EOU26237.1 ,gb OQO80010.1	34.187,9
342	molecular chaperone DnaK [Enterococcus durans]	gb AKX84948.1 ,gb AKZ48610.1 ,gb EMS74968.1 ,gb OQO78467.1 ,ref WP_005880047.1	65.692,7
343	glucose-6-phosphate isomerase [Enterococcus durans]	gb AKX85027.1 ,gb AKZ48688.1 ,gb EMS76330.1 ,gb EOT36447.1 ,gb EOU18787.1 ,gb OQO77954.1	49.752,2
344	elongation factor Tu [Enterococcus durans]	gb AKX85818.1 ,gb AKZ47196.1 ,gb EMS74558.1 ,gb EOT33812.1 ,gb EOU25443.1 ,gb OQO82133.1	43.162,2

345	cell division protein DivIVA [Enterococcus durans]	gb AKX86522.1 ,gb AKZ47882.1 ,gb EMS74407.1 ,gb EOT33981.1 ,gb EOU26098.1 ,gb OQO81847.1	26.724,9
346	phosphoenolpyruvate-protein phosphotransferase	gb AKX86601.1 ,gb AKZ47958.1 ,ref WP_053108894.1	63.353,5
347	Clp protease ClpX (plasmid) [Enterococcus durans]	gb AKX87246.1 ,gb EMS76214.1 ,gb EOT30301.1 ,gb EOU15544.1 ,ref WP_005876977.1	77.848,4
348	osmotically inducible protein C [Enterococcus durans]	gb AKX86277.1 ,gb AKZ47647.1 ,gb EMS75167.1 ,gb EOT34377.1 ,gb EOU25801.1 ,gb OQO78110.1	14.438,9
349	30S ribosomal protein S7 [Enterococcus durans]	gb AKX85820.1 ,gb AKZ47198.1 ,gb EMS74560.1 ,gb EOT33810.1 ,gb EOU25441.1 ,gb OQO82131.1	17.843,7
350	ATP synthase beta-subunit, partial [Enterococcus durans]	gb ADO14905.1 ,gb AKX85196.1 ,gb AKZ48858.1 ,gb EMS75649.1 ,gb EOT32263.1 ,gb EOU20064.1	51.167,8
351	50S ribosomal protein L21 [Enterococcus durans]	gb AKX86487.1 ,gb AKZ47845.1 ,gb EMS74445.1 ,gb EOT33943.1 ,gb EOU26060.1 ,gb OQO81885.1	11.174,6
352	50S ribosomal protein L7/L12 [Enterococcus durans]	gb AKX85495.1 ,gb AKZ49148.1 ,gb EMS75307.1 ,gb EOT25821.1 ,gb EOU22530.1 ,gb OQO81709.1	12.497,9
353	general stress protein [Enterococcus durans]	gb AKX84785.1 ,gb AKZ48444.1 ,gb EMS75708.1 ,gb EOT35438.1 ,gb EOU19146.1 ,gb OQO78700.1	30.065,8
354	dihydrolipoamide dehydrogenase [Enterococcus durans]	gb AKX86822.1 ,gb AKZ48179.1 ,gb EMS76473.1 ,gb EOT34966.1 ,gb EOU19458.1 ,gb OQO82466.1	49.261,4
355	oxidoreductase [Enterococcus durans]	gb AKX84788.1 ,gb AKZ48447.1 ,gb EMS75711.1 ,gb EOT35435.1 ,gb EOU19143.1 ,gb OQO78703.1	31.934,8
356	dihydrolipoamide acetyltransferase [Enterococcus durans]	gb AKX86821.1 ,gb AKZ48178.1 ,gb EMS76472.1 ,gb EOT34967.1 ,gb EOU19459.1 ,gb OQO82465.1	57.943,6
357	phosphocarrier protein HPr [Enterococcus durans]	gb AKX86600.1 ,gb AKZ47957.1 ,gb EMS76502.1 ,gb EOT34074.1 ,gb EOU26191.1 ,gb OQO80053.1	9.312,5
358	30S ribosomal protein S9 [Enterococcus durans]	gb AKX85907.1 ,gb AKZ47286.1 ,gb EMS74287.1 ,gb EOT28125.1 ,gb EOU16435.1 ,gb OQO79024.1	14.362,8
359	phosphotransacetylase [Enterococcus durans]	gb AKX86438.1 ,gb EMS75735.1 ,gb EOT34226.1 ,gb EOU26008.1 ,gb OQO81931.1 ,ref WP_005878037.1	35.410,5
360	2-oxoisovalerate dehydrogenase [Enterococcus durans]	gb AKX86820.1 ,gb AKZ48177.1 ,gb EMS76471.1 ,gb EOT34968.1 ,gb EOU19460.1 ,gb OQO82464.1	35.384,6
361	peptidylprolyl isomerase [Enterococcus durans]	gb AKX86215.1 ,gb AKZ47586.1 ,gb EMS76065.1 ,gb EOT34496.1 ,gb EOU25733.1 ,gb OQO79622.1	37.434,4
362	30S ribosomal protein S6 [Enterococcus durans]	gb AKX85856.1 ,gb AKZ47235.1 ,gb EMS76722.1 ,gb EOT33747.1 ,gb EOU25378.1 ,gb OQO82066.1	11.578,8
363	30S ribosomal protein S10 [Enterococcus durans]	gb AKX85811.1 ,gb AKZ47189.1 ,gb EMS74552.1 ,gb EOT33819.1 ,gb EOU25450.1 ,gb OQO82140.1	18.006,8
364	GroES, partial [Enterococcus durans]	gb AAN32674.1 AF417585_1,gb AKX85467.1 ,gb AKZ49124.1 ,gb EMS74729.1 ,gb EOT29787.1	10.031,4
365	50S ribosomal protein L2 [Enterococcus durans]	gb AKX85807.1 ,gb AKZ47185.1 ,gb EMS74548.1 ,gb EOT33823.1 ,gb EOU25454.1 ,gb OQO82144.1	30.326,6
366	lactate dehydrogenase [Enterococcus durans]	gb AKX86027.1 ,gb AKZ47406.1 ,gb EMS77070.1 ,gb EOT29104.1 ,gb EOU16350.1 ,gb OQO79291.1	35.131,4
367	phosphoglyceromutase [Enterococcus durans]	gb OQO82127.1 ,ref WP_081133709.1	25.914,4
368	ribosome-recycling factor [Enterococcus durans]	gb AKX84983.1 ,gb AKZ48645.1 ,gb EMS76647.1 ,gb EOT36305.1 ,gb EOU18893.1 ,gb OQO79346.1	20.881,9
369	trigger factor [Enterococcus durans]	gb AKX84928.1 ,gb AKZ48590.1 ,gb EMS74945.1 ,gb EOT36106.1 ,gb EOU18964.1 ,gb OQO78488.1	47.671,9
370	30S ribosomal protein S19 [Enterococcus durans]	gb AKX85806.1 ,gb AKZ47184.1 ,gb EMS74547.1 ,gb EOT33824.1 ,gb EOU25455.1 ,gb OQO82145.1	10.521,2
371	peptide ABC transporter substrate-binding protein	gb AKX85768.1 ,gb AKZ47145.1 ,gb EMS74510.1 ,gb EOT33660.1 ,gb EOU25495.1 ,ref WP_005880706.1	66.222,4
372	50S ribosomal protein L4 [Enterococcus durans]	gb AKX85809.1 ,gb AKZ47187.1 ,gb EMS74550.1 ,gb EOT33821.1 ,gb EOU25452.1 ,gb OQO82142.1	22.462,4
373	galactose-6-phosphate isomerase [Enterococcus durans]	gb AKX86135.1 ,gb AKZ47507.1 ,gb EMS75412.1 ,ref WP_002345825.1	18.902,4



374	decarboxylase [Enterococcus durans]	gb AKX85643.1 ,gb AKZ47021.1 ,gb EMS76937.1 ,gb EOT32744.1 ,gb EOU25647.1 ,gb OQO82311.1	70.192,3
375	50S ribosomal protein L22 [Enterococcus durans]	gb AKX85805.1 ,gb AKZ47183.1 ,gb EMS74546.1 ,gb EOT33825.1 ,gb EOU25456.1 ,gb OQO82146.1	12.450,7
376	phosphoglycerate kinase [Enterococcus durans]	gb AKX85214.1 ,gb AKZ48876.1 ,gb EMS75629.1 ,gb EOT32243.1 ,gb EOU20044.1 ,gb OQO81407.1	41.855,5
377	pyrrolidone-carboxylate peptidase [Enterococcus durans]	gb AKX86427.1 ,gb AKZ47791.1 ,gb EMS76129.1 ,gb EOT34212.1 ,gb EOU25995.1 ,gb OQO81945.1	22.969,3
378	molecular chaperone GroEL [Enterococcus durans]	gb AKX85466.1 ,gb AKZ49123.1 ,gb EMS74730.1 ,gb OQO81745.1 ,ref WP_005880136.1	57.148,7
379	tyrosine--tRNA ligase [Enterococcus durans]	gb AKX85644.1 ,gb AKZ47022.1 ,gb EMS76936.1 ,gb EOT32743.1 ,gb EOU25646.1 ,gb OQO82310.1	47.335,7
380	transcription elongation factor GreA [Enterococcus durans]	gb AKX86569.1 ,gb AKZ47926.1 ,gb EMS76534.1 ,gb EOT34040.1 ,gb EOU26157.1 ,gb OQO77684.1	17.458,1
381	osmotically inducible protein C [Enterococcus durans]	gb AKX86185.1 ,gb AKZ47557.1 ,gb EMS74585.1 ,gb OQO80135.1 ,ref WP_002292291.1	14.674,3
382	pheromone cAD1 precursor lipoprotein	gb EMS77019.1 ,gb EOT28144.1 ,gb EOU16454.1 ,ref WP_005875228.1 ,ref WP_016177786.1	33.478,6
383	enolase [Enterococcus durans]	gb AKX85212.1 ,gb AKZ48874.1 ,gb EMS75631.1 ,gb EOT32245.1 ,gb EOU20046.1 ,gb OQO81409.1	46.481,0
384	triosephosphate isomerase [Enterococcus durans]	gb AKX85213.1 ,gb AKZ48875.1 ,gb EMS75630.1 ,gb OQO81408.1 ,ref WP_005878431.1	26.877,8
385	dihydroxyacetone kinase [Enterococcus durans]	gb AKX85646.1 ,gb AKZ47024.1 ,gb EMS76934.1 ,gb EOT32741.1 ,gb EOU15552.1 ,gb EOU25644.1	21.837,3
386	50S ribosomal protein L29 [Enterococcus durans]	gb AKX85802.1 ,gb AKZ47180.1 ,gb EMS74543.1 ,gb EOT33828.1 ,gb EOU25459.1 ,gb OQO82149.1	7.344,0
387	NADH oxidase [Enterococcus durans]	gb AKZ48275.1 ,gb EMS76682.1 ,gb EOT35400.1 ,gb EOU19353.1 ,gb OQO82558.1 ,ref WP_005875847.1	49.253,2
388	tagatose-bisphosphate aldolase [Enterococcus durans]	gb AKX86137.1 ,gb AKZ47509.1 ,gb EMS75410.1 ,ref WP_002290551.1 ,ref WP_005878819.1	36.445,1
389	2,5-diketo-D-gluconic acid reductase [Enterococcus durans]	gb AKX86672.1 ,gb AKZ48028.1 ,gb EMS75514.1 ,gb OQO81227.1 ,ref WP_005878645.1	31.865,6
390	30S ribosomal protein S15 [Enterococcus durans]	gb AKX85715.1 ,gb EMS76858.1 ,gb EOT33619.1 ,gb EOU25559.1 ,gb OQO82242.1 ,ref WP_005875316.1	10.595,5
391	uracil phosphoribosyltransferase [Enterococcus durans]	gb AKX86332.1 ,gb AKZ47698.1 ,gb EMS75108.1 ,gb EOT34441.1 ,gb EOU25865.1 ,gb OQO80536.1	22.888,5
392	PTS cellobiose transporter subunit IIB [Enterococcus durans]	gb AKX86078.1 ,gb AKZ47452.1 ,gb EMS75805.1 ,gb EOT26119.1 ,gb EOU22378.1 ,gb OQO81560.1	11.549,6
393	30S ribosomal protein S8 [Enterococcus durans]	gb AKX85796.1 ,gb AKZ47174.1 ,gb EMS74537.1 ,gb EOT33834.1 ,gb EOU25465.1 ,gb OQO82155.1	14.840,7
394	GapA [Enterococcus durans]	gb AKX84774.1 ,gb AKZ48433.1 ,gb EMS76998.1 ,gb EOT35449.1 ,gb EOU19158.1 ,gb OQO82731.1	21.310,7
395	hypothetical protein LIANG_11460 [Enterococcus durans]	gb AKX86705.1 ,gb AKZ48060.1 ,gb EMS75482.1 ,gb EOT34742.1 ,gb EOU19600.1 ,gb OQO81198.1	49.365,7
396	glyceraldehyde-3-phosphate dehydrogenase	gb AKX85215.1 ,gb AKZ48877.1 ,gb EMS75628.1 ,gb EOT32242.1 ,gb EOU20043.1 ,gb OQO81406.1	35.774,4
397	50S ribosomal protein L13 [Enterococcus durans]	gb AKX87109.1 ,gb AKZ49295.1 ,gb EMS74286.1 ,gb EOT28126.1 ,gb EOU16436.1 ,gb OQO79028.1	15.652,5
398	universal stress protein UspA [Enterococcus durans]	gb AKX86231.1 ,gb AKZ47601.1 ,gb EMS76048.1 ,gb EOT34514.1 ,gb EOU25751.1 ,gb OQO79639.1	17.246,0
399	hypothetical protein LIANG_08515 [Enterococcus durans]	gb AKX86201.1 ,gb AKZ47572.1 ,gb EMS76564.1 ,gb EOT32805.1 ,gb EOU25708.1 ,gb OQO80094.1	13.151,2
400	formate acetyltransferase [Enterococcus durans]	gb AKX86748.1 ,gb AKZ48103.1 ,gb EMS75698.1 ,gb EOT34872.1 ,gb EOU19550.1 ,gb OQO81152.1	83.799,1
401	fructose-bisphosphate aldolase [Enterococcus durans]	gb AKX85321.1 ,gb AKZ48980.1 ,gb EMS76746.1 ,gb EOT32130.1 ,gb EOU19931.1 ,gb OQO78736.1	30.809,7
402	30S ribosomal protein S5 [Enterococcus durans]	gb AKX85793.1 ,gb AKZ47171.1 ,gb EMS74534.1 ,gb EOT33837.1 ,gb EOU25468.1 ,gb OQO82157.1	17.476,9

403	cold-shock protein [Enterococcus durans]	gb AKX86580.1 ,gb AKZ47937.1 ,gb EMS76523.1 ,gb EOT34050.1 ,gb EOU26167.1 ,gb OQO77812.1	7.204,7
404	elongation factor Tu [Enterococcus durans]	gb AKX86680.1 ,gb AKZ48035.1 ,gb EMS75506.1 ,gb EOT34767.1 ,gb EOU19625.1 ,gb OQO81219.1	43.213,0
405	50S ribosomal protein L30 [Enterococcus durans]	gb AKX85792.1 ,gb AKZ47170.1 ,gb EMS74533.1 ,gb EOT33838.1 ,gb EOU25469.1 ,gb OQO82158.1	6.427,5
406	30S ribosomal protein S1 [Enterococcus durans]	gb AKX86712.1 ,gb AKZ48067.1 ,gb EMS75475.1 ,gb EOT34735.1 ,gb EOU19593.1 ,gb OQO81191.1	44.559,6
407	50S ribosomal protein L15 [Enterococcus durans]	gb AKX85791.1 ,gb AKZ47169.1 ,gb EMS74532.1 ,gb EOT33839.1 ,gb EOU25470.1 ,gb OQO82159.1	15.424,8
408	30S ribosomal protein S3 [Enterococcus durans]	gb AKX85804.1 ,gb AKZ47182.1 ,gb EMS74545.1 ,gb EOT33826.1 ,gb EOU25457.1 ,gb OQO82147.1	24.406,0
409	general stress protein [Enterococcus durans]	gb AKX85160.1 ,gb AKZ48823.1 ,gb EMS74678.1 ,gb EOT31758.1 ,gb EOU18551.1 ,gb OQO81463.1	21.119,5
410	hypothetical protein LIANG_05820 [Enterococcus durans]	gb AKX85756.1 ,gb AKZ47134.1 ,gb EMS74497.1 ,gb EOT33673.1 ,gb EOU25508.1 ,gb OQO82198.1	13.205,8
411	hypothetical protein LIANG_06205 [Enterococcus durans]	gb AKX85830.1 ,gb AKZ47209.1 ,gb EMS74571.1 ,gb EOT33799.1 ,gb EOU25430.1 ,gb OQO82120.1	37.834,8
412	molecular chaperone GrpE [Enterococcus durans]	gb AKX84949.1 ,gb AKZ48611.1 ,gb EMS74969.1 ,gb EOT36158.1 ,gb EOU18940.1 ,gb OQO78466.1	21.149,6
413	30S ribosomal protein S12 [Enterococcus durans]	gb AKX85821.1 ,gb AKZ47199.1 ,gb EMS74561.1 ,gb EOT33809.1 ,gb EOU25440.1 ,gb OQO82130.1	15.215,9
414	50S ribosomal protein L13 [Enterococcus durans]	gb AKX87109.1 ,gb AKZ49295.1 ,gb EMS74286.1 ,gb EOT28126.1 ,gb EOU16436.1 ,gb OQO79028.1	15.652,5
415	glycerol kinase [Enterococcus durans]	gb AKX86100.1 ,gb AKZ47472.1 ,gb EMS76961.1 ,gb EOT32768.1 ,gb EOU25671.1 ,gb OQO82334.1	55.577,9
416	30S ribosomal protein S11 [Enterococcus durans]	gb AKX85785.1 ,gb AKZ47163.1 ,gb EMS74526.1 ,gb EOT33845.1 ,gb EOU25476.1 ,gb OQO82165.1	13.736,0
417	stress response regulator Gls24 [Enterococcus durans]	gb AKX84772.1 ,gb EMS76996.1 ,gb EOT35451.1 ,gb EOU19160.1 ,gb OQO82729.1 ,ref WP_005875244.1	20.508,5
418	pheromone cAD1 precursor lipoprotein [Enterococcus durans]	gb EMS77019.1 ,gb EOT28144.1 ,gb EOU16454.1 ,ref WP_005875228.1 ,ref WP_016177786.1	33.478,6
419	arginine deiminase [Enterococcus durans]	gb AKX84990.1 ,gb AKZ48652.1 ,gb EMS76655.1 ,gb EOT36297.1 ,gb EOU18885.1 ,gb OQO79339.1	46.057,9
420	ornithine carbamoyltransferase [Enterococcus durans]	gb AKX84989.1 ,gb AKZ48651.1 ,gb EMS76654.1 ,gb EOT36298.1 ,gb EOU18886.1 ,gb OQO79340.1	38.341,7
421	50S ribosomal protein L17 [Enterococcus durans]	gb AKX85783.1 ,gb AKZ47161.1 ,gb EMS74524.1 ,gb EOT33847.1 ,gb EOU25478.1 ,gb OQO82167.1	14.344,9
422	ATP F0F1 synthase subunit alpha [Enterococcus durans]	gb AKX85198.1 ,gb AKZ48860.1 ,gb EMS75647.1 ,gb EOT32261.1 ,gb EOU20062.1 ,gb OQO81424.1	56.397,4
423	ferritin [Enterococcus durans]	gb AKX85904.1 ,gb AKZ47283.1 ,gb EMS74283.1 ,gb EOT28129.1 ,gb EOU16439.1 ,gb OQO79021.1	17.950,9
424	pyruvate kinase [Enterococcus durans]	gb AKX86645.1 ,gb AKZ47998.1 ,gb EMS77112.1 ,gb EOT34121.1 ,gb EOU26238.1 ,gb OQO80009.1	63.662,1
425	hypothetical protein LIANG_08385 [Enterococcus durans]	gb AKX86179.1 ,gb AKZ47551.1 ,gb EMS75204.1 ,ref WP_005879148.1	16.865,4
426	hypothetical protein LIANG_11540 [Enterococcus durans]	gb AKX86721.1 ,gb AKZ48076.1 ,gb EMS75465.1 ,gb EOT34725.1 ,gb EOU19583.1 ,gb OQO81182.1	17.610,7
427	30S ribosomal protein S13 [Enterococcus durans]	gb AKX85786.1 ,gb AKZ47164.1 ,gb EMS74527.1 ,gb EOT33844.1 ,gb EOU25475.1 ,gb OQO82164.1	13.534,1
428	6-phosphofructokinase [Enterococcus durans]	gb AKX86644.1 ,gb AKZ47997.1 ,gb EMS77111.1 ,gb EOT34120.1 ,gb EOU26237.1 ,gb OQO80010.1	34.187,9
429	cell division protein FtsZ [Enterococcus durans]	gb AKX86518.1 ,gb EMS74412.1 ,gb EOT33976.1 ,gb EOU26093.1 ,gb OQO81852.1 ,ref WP_005881040.1	44.328,3
430	NAD(FAD)-dependent dehydrogenase [Enterococcus durans]	gb AKX85538.1 ,gb AKZ49189.1 ,gb EMS75354.1 ,gb EOT25774.1 ,gb EOU22483.1 ,ref WP_005879034.1	50.332,3
431	molecular chaperone DnaK [Enterococcus durans]	gb AKX84948.1 ,gb AKZ48610.1 ,gb EMS74968.1 ,gb OQO78467.1 ,ref WP_005880047.1	65.692,7

432	glucose-6-phosphate isomerase [Enterococcus durans]	gb AKX85027.1 ,gb AKZ48688.1 ,gb EMS76330.1 ,gb EOT36447.1 ,gb EOU18787.1 ,gb OQO77954.1	49.752,2
433	elongation factor Tu [Enterococcus durans]	gb AKX85818.1 ,gb AKZ47196.1 ,gb EMS74558.1 ,gb EOT33812.1 ,gb EOU25443.1 ,gb OQO82133.1	43.162,2
434	phosphoenolpyruvate-protein phosphotransferase	gb AKX86601.1 ,gb AKZ47958.1 ,ref WP_053108894.1	63.353,5
435	Clp protease ClpX (plasmid) [Enterococcus durans]	gb AKX87246.1 ,gb EMS76214.1 ,gb EOT30301.1 ,gb EOU15544.1 ,ref WP_005876977.1	77.848,4
436	30S ribosomal protein S7 [Enterococcus durans]	gb AKX85820.1 ,gb AKZ47198.1 ,gb EMS74560.1 ,gb EOT33810.1 ,gb EOU25441.1 ,gb OQO82131.1	17.843,7
437	ATP synthase beta-subunit, partial [Enterococcus durans]	gb ADO14905.1 ,gb AKX85196.1 ,gb AKZ48858.1 ,gb EMS75649.1 ,gb EOT32263.1 ,gb EOU20064.1	51.167,8
438	50S ribosomal protein L21 [Enterococcus durans]	gb AKX86487.1 ,gb AKZ47845.1 ,gb EMS74445.1 ,gb EOT33943.1 ,gb EOU26060.1 ,gb OQO81885.1	11.174,6
439	50S ribosomal protein L7/L12 [Enterococcus durans]	gb AKX85495.1 ,gb AKZ49148.1 ,gb EMS75307.1 ,gb EOT25821.1 ,gb EOU22530.1 ,gb OQO81709.1	12.497,9
440	general stress protein [Enterococcus durans]	gb AKX84785.1 ,gb AKZ48444.1 ,gb EMS75708.1 ,gb EOT35438.1 ,gb EOU19146.1 ,gb OQO78700.1	30.065,8
441	dihydrolipoamide dehydrogenase [Enterococcus durans]	gb AKX86822.1 ,gb AKZ48179.1 ,gb EMS76473.1 ,gb EOT34966.1 ,gb EOU19458.1 ,gb OQO82466.1	49.261,4
442	PTS cellobiose transporter subunit IIB [Enterococcus durans]	gb AKX86078.1 ,gb AKZ47452.1 ,gb EMS75805.1 ,gb EOT26119.1 ,gb EOU22378.1 ,gb OQO81560.1	11.549,6
443	oxidoreductase [Enterococcus durans]	gb AKX84788.1 ,gb AKZ48447.1 ,gb EMS75711.1 ,gb EOT35435.1 ,gb EOU19143.1 ,gb OQO78703.1	31.934,8
444	dihydrolipoamide acetyltransferase [Enterococcus durans]	gb AKX86821.1 ,gb AKZ48178.1 ,gb EMS76472.1 ,gb EOT34967.1 ,gb EOU19459.1 ,gb OQO82465.1	57.943,6
445	PTS mannose transporter subunit IIAB [Enterococcus durans]	gb AKX86491.1 ,gb AKZ47851.1 ,gb EMS74439.1 ,gb EOT33949.1 ,gb EOU26066.1 ,gb OQO81879.1	35.253,2
446	glutamyl-tRNA synthetase [Enterococcus durans]	gb AKZ47353.1 ,gb EMS76247.1 ,gb EOT28433.1 ,gb EOU16409.1 ,gb OQO78070.1 ,ref WP_005876899.1	55.356,2
447	30S ribosomal protein S9 [Enterococcus durans]	gb AKX85907.1 ,gb AKZ47286.1 ,gb EMS74287.1 ,gb EOT28125.1 ,gb EOU16435.1 ,gb OQO79024.1	14.362,8
448	uracil phosphoribosyltransferase [Enterococcus durans]	gb AKX86332.1 ,gb AKZ47698.1 ,gb EMS75108.1 ,gb EOT34441.1 ,gb EOU25865.1 ,gb OQO80536.1	22.888,5
449	2-oxoisovalerate dehydrogenase [Enterococcus durans]	gb AKX86820.1 ,gb AKZ48177.1 ,gb EMS76471.1 ,gb EOT34968.1 ,gb EOU19460.1 ,gb OQO82464.1	35.384,6
450	S-adenosylmethionine synthetase [Enterococcus durans]	gb AKX86039.1 ,gb AKZ47417.1 ,gb EMS75771.1 ,gb EOT26478.1 ,gb EOU22338.1 ,gb OQO81524.1	43.278,6
451	30S ribosomal protein S6 [Enterococcus durans]	gb AKX85856.1 ,gb AKZ47235.1 ,gb EMS76722.1 ,gb EOT33747.1 ,gb EOU25378.1 ,gb OQO82066.1	11.578,8
452	30S ribosomal protein S10 [Enterococcus durans]	gb AKX85811.1 ,gb AKZ47189.1 ,gb EMS74552.1 ,gb EOT33819.1 ,gb EOU25450.1 ,gb OQO82140.1	18.006,8
453	GroES, partial [Enterococcus durans]	gb AAN32674.1 AF417585_1 ,gb AKX85467.1 ,gb AKZ49124.1 ,gb EMS74729.1 ,gb EOT29787.1	10.031,4
454	50S ribosomal protein L2 [Enterococcus durans]	gb AKX85807.1 ,gb AKZ47185.1 ,gb EMS74548.1 ,gb EOT33823.1 ,gb EOU25454.1 ,gb OQO82144.1	30.326,6
455	lactate dehydrogenase [Enterococcus durans]	gb AKX86027.1 ,gb AKZ47406.1 ,gb EMS77070.1 ,gb EOT29104.1 ,gb EOU16350.1 ,gb OQO79291.1	35.131,4
456	glucokinase [Enterococcus durans]	gb AKX85004.1 ,gb AKZ48666.1 ,gb EMS75062.1 ,gb EOT36277.1 ,gb EOU18865.1 ,gb OQO79324.1	33.859,2
457	ribosome-recycling factor [Enterococcus durans]	gb AKX84983.1 ,gb AKZ48645.1 ,gb EMS76647.1 ,gb EOT36305.1 ,gb EOU18893.1 ,gb OQO79346.1	20.881,9
458	30S ribosomal protein S19 [Enterococcus durans]	gb AKX85806.1 ,gb AKZ47184.1 ,gb EMS74547.1 ,gb EOT33824.1 ,gb EOU25455.1 ,gb OQO82145.1	10.521,2
459	peptide ABC transporter substrate-binding protein	gb AKX85768.1 ,gb AKZ47145.1 ,gb EMS74510.1 ,gb EOT33660.1 ,gb EOU25495.1 ,ref WP_005880706.1	66.222,4
460	50S ribosomal protein L4 [Enterococcus durans]	gb AKX85809.1 ,gb AKZ47187.1 ,gb EMS74550.1 ,gb EOT33821.1 ,gb EOU25452.1 ,gb OQO82142.1	22.462,4

461	galactose-6-phosphate isomerase [Enterococcus durans]	gb AKX86135.1 ,gb AKZ47507.1 ,gb EMS75412.1 ,ref WP_002345825.1	18.902,4
462	decarboxylase [Enterococcus durans]	gb AKX85643.1 ,gb AKZ47021.1 ,gb EMS76937.1 ,gb EOT32744.1 ,gb EOU25647.1 ,gb OQO82311.1	70.192,3
463	50S ribosomal protein L22 [Enterococcus durans]	gb AKX85805.1 ,gb AKZ47183.1 ,gb EMS74546.1 ,gb EOT33825.1 ,gb EOU25456.1 ,gb OQO82146.1	12.450,7
464	phosphoglycerate kinase [Enterococcus durans]	gb AKX85214.1 ,gb AKZ48876.1 ,gb EMS75629.1 ,gb EOT32243.1 ,gb EOU20044.1 ,gb OQO81407.1	41.855,5
465	molecular chaperone GroEL [Enterococcus durans]	gb AKX85466.1 ,gb AKZ49123.1 ,gb EMS74730.1 ,gb OQO81745.1 ,ref WP_005880136.1	57.148,7
466	tyrosine--tRNA ligase [Enterococcus durans]	gb AKX85644.1 ,gb AKZ47022.1 ,gb EMS76936.1 ,gb EOT32743.1 ,gb EOU25646.1 ,gb OQO82310.1	47.335,7
467	30S ribosomal protein S3 [Enterococcus durans]	gb AKX85804.1 ,gb AKZ47182.1 ,gb EMS74545.1 ,gb EOT33826.1 ,gb EOU25457.1 ,gb OQO82147.1	24.406,0
468	osmotically inducible protein C [Enterococcus durans]	gb AKX86185.1 ,gb AKZ47557.1 ,gb EMS74585.1 ,gb OQO80135.1 ,ref WP_002292291.1	14.674,3
469	glyceraldehyde-3-phosphate dehydrogenase	gb AKX86905.1 ,gb AKZ48257.1 ,gb EMS75963.1 ,gb EOT35299.1 ,gb EOU19372.1 ,gb OQO82543.1	36.155,7
470	enolase [Enterococcus durans]	gb AKX85212.1 ,gb AKZ48874.1 ,gb EMS75631.1 ,gb EOT32245.1 ,gb EOU20046.1 ,gb OQO81409.1	46.481,0
471	triosephosphate isomerase [Enterococcus durans]	gb AKX85213.1 ,gb AKZ48875.1 ,gb EMS75630.1 ,gb OQO81408.1 ,ref WP_005878431.1	26.877,8
472	dihydroxyacetone kinase [Enterococcus durans]	gb AKX85646.1 ,gb AKZ47024.1 ,gb EMS76934.1 ,gb EOT32741.1 ,gb EOU15552.1 ,gb EOU25644.1	21.837,3
473	NADH oxidase [Enterococcus durans]	gb AKZ48275.1 ,gb EMS76682.1 ,gb EOT35400.1 ,gb EOU19353.1 ,gb OQO82558.1 ,ref WP_005875847.1	49.253,2
474	tagatose-bisphosphate aldolase [Enterococcus durans]	gb AKX86137.1 ,gb AKZ47509.1 ,gb EMS75410.1 ,ref WP_002290551.1 ,ref WP_005878819.1	36.445,1
475	phosphotransacetylase [Enterococcus durans]	gb AKX86438.1 ,gb EMS75735.1 ,gb EOT34226.1 ,gb EOU26008.1 ,gb OQO81931.1 ,ref WP_005878037.1	35.410,5
476	phosphoglyceromutase [Enterococcus durans]	gb OQO82127.1 ,ref WP_081133709.1	25.914,4
477	30S ribosomal protein S8 [Enterococcus durans]	gb AKX85796.1 ,gb AKZ47174.1 ,gb EMS74537.1 ,gb EOT33834.1 ,gb EOU25465.1 ,gb OQO82155.1	14.840,7
478	GapA [Enterococcus durans]	gb AKX84774.1 ,gb AKZ48433.1 ,gb EMS76998.1 ,gb EOT35449.1 ,gb EOU19158.1 ,gb OQO82731.1	21.310,7
479	hypothetical protein LIANG_11460 [Enterococcus durans]	gb AKX86705.1 ,gb AKZ48060.1 ,gb EMS75482.1 ,gb EOT34742.1 ,gb EOU19600.1 ,gb OQO81198.1	49.365,7
480	glyceraldehyde-3-phosphate dehydrogenase	gb AKX85215.1 ,gb AKZ48877.1 ,gb EMS75628.1 ,gb EOT32242.1 ,gb EOU20043.1 ,gb OQO81406.1	35.774,4
481	universal stress protein UspA [Enterococcus durans]	gb AKX86231.1 ,gb AKZ47601.1 ,gb EMS76048.1 ,gb EOT34514.1 ,gb EOU25751.1 ,gb OQO79639.1	17.246,0
482	hypothetical protein LIANG_08515 [Enterococcus durans]	gb AKX86201.1 ,gb AKZ47572.1 ,gb EMS76564.1 ,gb EOT32805.1 ,gb EOU25708.1 ,gb OQO80094.1	13.151,2
483	formate acetyltransferase [Enterococcus durans]	gb AKX86748.1 ,gb AKZ48103.1 ,gb EMS75698.1 ,gb EOT34872.1 ,gb EOU19550.1 ,gb OQO81152.1	83.799,1
484	fructose-bisphosphate aldolase [Enterococcus durans]	gb AKX85321.1 ,gb AKZ48980.1 ,gb EMS76746.1 ,gb EOT32130.1 ,gb EOU19931.1 ,gb OQO78736.1	30.809,7
485	30S ribosomal protein S5 [Enterococcus durans]	gb AKX85793.1 ,gb AKZ47171.1 ,gb EMS74534.1 ,gb EOT33837.1 ,gb EOU25468.1 ,gb OQO82157.1	17.476,9
486	cold-shock protein [Enterococcus durans]	gb AKX86580.1 ,gb AKZ47937.1 ,gb EMS76523.1 ,gb EOT34050.1 ,gb EOU26167.1 ,gb OQO77812.1	7.204,7
487	elongation factor Tu [Enterococcus durans]	gb AKX86680.1 ,gb AKZ48035.1 ,gb EMS75506.1 ,gb EOT34767.1 ,gb EOU19625.1 ,gb OQO81219.1	43.213,0
488	50S ribosomal protein L30 [Enterococcus durans]	gb AKX85792.1 ,gb AKZ47170.1 ,gb EMS74533.1 ,gb EOT33838.1 ,gb EOU25469.1 ,gb OQO82158.1	6.427,5
489	30S ribosomal protein S1 [Enterococcus durans]	gb AKX86712.1 ,gb AKZ48067.1 ,gb EMS75475.1 ,gb EOT34735.1 ,gb EOU19593.1 ,gb OQO81191.1	44.559,6

490	50S ribosomal protein L15 [Enterococcus durans]	gb AKX85791.1 ,gb AKZ47169.1 ,gb EMS74532.1 ,gb EOT33839.1 ,gb EOU25470.1 ,gb OQO82159.1	15.424,8
491	general stress protein [Enterococcus durans]	gb AKX85160.1 ,gb AKZ48823.1 ,gb EMS74678.1 ,gb EOT31758.1 ,gb EOU18551.1 ,gb OQO81463.1	21.119,5
492	hypothetical protein LIANG_05820 [Enterococcus durans]	gb AKX85756.1 ,gb AKZ47134.1 ,gb EMS74497.1 ,gb EOT33673.1 ,gb EOU25508.1 ,gb OQO82198.1	13.205,8
493	molecular chaperone GrpE [Enterococcus durans]	gb AKX84949.1 ,gb AKZ48611.1 ,gb EMS74969.1 ,gb EOT36158.1 ,gb EOU18940.1 ,gb OQO78466.1	21.149,6
494	30S ribosomal protein S12 [Enterococcus durans]	gb AKX85821.1 ,gb AKZ47199.1 ,gb EMS74561.1 ,gb EOT33809.1 ,gb EOU25440.1 ,gb OQO82130.1	15.215,9
495	50S ribosomal protein L13 [Enterococcus durans]	gb AKX87109.1 ,gb AKZ49295.1 ,gb EMS74286.1 ,gb EOT28126.1 ,gb EOU16436.1 ,gb OQO79028.1	15.652,5
496	30S ribosomal protein S11 [Enterococcus durans]	gb AKX85785.1 ,gb AKZ47163.1 ,gb EMS74526.1 ,gb EOT33845.1 ,gb EOU25476.1 ,gb OQO82165.1	13.736,0
497	osmotically inducible protein C [Enterococcus durans]	gb AKX86185.1 ,gb AKZ47557.1 ,gb EMS74585.1 ,gb OQO80135.1 ,ref WP_002292291.1	14.674,3
498	cell division protein FtsH [Enterococcus durans]	gb AKX86034.1 ,gb EMS77079.1 ,gb EOT29095.1 ,gb EOU16341.1 ,gb OQO79299.1 ,ref WP_005875188.1	77.036,0
499	arginine deiminase [Enterococcus durans]	gb AKX84990.1 ,gb AKZ48652.1 ,gb EMS76655.1 ,gb EOT36297.1 ,gb EOU18885.1 ,gb OQO79339.1	46.057,9
500	50S ribosomal protein L17 [Enterococcus durans]	gb AKX85783.1 ,gb AKZ47161.1 ,gb EMS74524.1 ,gb EOT33847.1 ,gb EOU25478.1 ,gb OQO82167.1	14.344,9
501	ATP FOF1 synthase subunit alpha [Enterococcus durans]	gb AKX85198.1 ,gb AKZ48860.1 ,gb EMS75647.1 ,gb EOT32261.1 ,gb EOU20062.1 ,gb OQO81424.1	56.397,4
502	gb AKX86463.1 -DECOY	gb AKX86463.1 -DECOY,gb AKZ47822.1 -DECOY	0,0
503	ferritin [Enterococcus durans]	gb AKX85904.1 ,gb AKZ47283.1 ,gb EMS74283.1 ,gb EOT28129.1 ,gb EOU16439.1 ,gb OQO79021.1	17.950,9
504	pyruvate kinase [Enterococcus durans]	gb AKX86645.1 ,gb AKZ47998.1 ,gb EMS77112.1 ,gb EOT34121.1 ,gb EOU26238.1 ,gb OQO80009.1	63.662,1
505	hypothetical protein LIANG_08385 [Enterococcus durans]	gb AKX86179.1 ,gb AKZ47551.1 ,gb EMS75204.1 ,ref WP_005879148.1	16.865,4
506	S-adenosylmethionine synthetase [Enterococcus durans]	gb AKX86039.1 ,gb AKZ47417.1 ,gb EMS75771.1 ,gb EOT26478.1 ,gb EOU22338.1 ,gb OQO81524.1	43.278,6
507	elongation factor G [Enterococcus durans]	gb AKX85819.1 ,gb AKZ47197.1 ,gb EMS74559.1 ,gb EOT33811.1 ,gb EOU25442.1 ,gb OQO82132.1	76.742,1
508	6-phosphofructokinase [Enterococcus durans]	gb AKX86644.1 ,gb AKZ47997.1 ,gb EMS77111.1 ,gb EOT34120.1 ,gb EOU26237.1 ,gb OQO80010.1	34.187,9
509	cell division protein FtsZ [Enterococcus durans]	gb AKX86518.1 ,gb EMS74412.1 ,gb EOT33976.1 ,gb EOU26093.1 ,gb OQO81852.1 ,ref WP_005881040.1	44.328,3
510	NAD(FAD)-dependent dehydrogenase [Enterococcus durans]	gb AKX85538.1 ,gb AKZ49189.1 ,gb EMS75354.1 ,gb EOT25774.1 ,gb EOU22483.1 ,ref WP_005879034.1	50.332,3
511	molecular chaperone DnaK [Enterococcus durans]	gb AKX84948.1 ,gb AKZ48610.1 ,gb EMS74968.1 ,gb OQO78467.1 ,ref WP_005880047.1	65.692,7
512	glucose-6-phosphate isomerase [Enterococcus durans]	gb AKX85027.1 ,gb AKZ48688.1 ,gb EMS76330.1 ,gb EOT36447.1 ,gb EOU18787.1 ,gb OQO77954.1	49.752,2
513	glucokinase [Enterococcus durans]	gb AKX85004.1 ,gb AKZ48666.1 ,gb EMS75062.1 ,gb EOT36277.1 ,gb EOU18865.1 ,gb OQO79324.1	33.859,2
514	cell division protein DivIVA [Enterococcus durans]	gb AKX86522.1 ,gb AKZ47882.1 ,gb EMS74407.1 ,gb EOT33981.1 ,gb EOU26098.1 ,gb OQO81847.1	26.724,9
515	Clp protease ClpX (plasmid) [Enterococcus durans]	gb AKX87246.1 ,gb EMS76214.1 ,gb EOT30301.1 ,gb EOU15544.1 ,ref WP_005876977.1	77.848,4
516	osmotically inducible protein C [Enterococcus durans]	gb AKX86277.1 ,gb AKZ47647.1 ,gb EMS75167.1 ,gb EOT34377.1 ,gb EOU25801.1 ,gb OQO78110.1	14.438,9
517	30S ribosomal protein S7 [Enterococcus durans]	gb AKX85820.1 ,gb AKZ47198.1 ,gb EMS74560.1 ,gb EOT33810.1 ,gb EOU25441.1 ,gb OQO82131.1	17.843,7

518	phosphoenolpyruvate-protein phosphotransferase [Enterococcus durans]	gb AKX86601.1 ,gb AKZ47958.1 ,ref WP_053108894.1	63.353,5
519	ATP synthase beta-subunit, partial [Enterococcus durans]	gb ADO14905.1 ,gb AKX85196.1 ,gb AKZ48858.1 ,gb EMS75649.1 ,gb EOT32263.1 ,gb EOU20064.1	51.167,8
520	50S ribosomal protein L21 [Enterococcus durans]	gb AKX86487.1 ,gb AKZ47845.1 ,gb EMS74445.1 ,gb EOT33943.1 ,gb EOU26060.1 ,gb OQO81885.1	11.174,6
521	50S ribosomal protein L7/L12 [Enterococcus durans]	gb AKX85495.1 ,gb AKZ49148.1 ,gb EMS75307.1 ,gb EOT25821.1 ,gb EOU22530.1 ,gb OQO81709.1	12.497,9
522	branched-chain amino acid aminotransferase	gb AKX86897.1 ,gb AKZ48249.1 ,gb EMS76178.1 ,gb EOT35275.1 ,gb EOU19381.1 ,gb OQO82535.1	37.297,0
523	adenylosuccinate synthetase [Enterococcus durans]	gb AKX85849.1 ,gb AKZ47228.1 ,gb EMS75973.1 ,gb EOT33753.1 ,gb EOU25384.1 ,gb OQO82072.1	47.817,8
524	ornithine carbamoyltransferase [Enterococcus durans]	gb AKX84989.1 ,gb AKZ48651.1 ,gb EMS76654.1 ,gb EOT36298.1 ,gb EOU18886.1 ,gb OQO79340.1	38.341,7
525	dihydrolipoamide dehydrogenase [Enterococcus durans]	gb AKX86822.1 ,gb AKZ48179.1 ,gb EMS76473.1 ,gb EOT34966.1 ,gb EOU19458.1 ,gb OQO82466.1	49.261,4
526	general stress protein [Enterococcus durans]	gb AKX84785.1 ,gb AKZ48444.1 ,gb EMS75708.1 ,gb EOT35438.1 ,gb EOU19146.1 ,gb OQO78700.1	30.065,8
527	oxidoreductase [Enterococcus durans]	gb AKX84788.1 ,gb AKZ48447.1 ,gb EMS75711.1 ,gb EOT35435.1 ,gb EOU19143.1 ,gb OQO78703.1	31.934,8
528	dihydrolipoamide acetyltransferase [Enterococcus durans]	gb AKX86821.1 ,gb AKZ48178.1 ,gb EMS76472.1 ,gb EOT34967.1 ,gb EOU19459.1 ,gb OQO82465.1	57.943,6
529	glutamyl-tRNA synthetase [Enterococcus durans]	gb AKZ47353.1 ,gb EMS76247.1 ,gb EOT28433.1 ,gb EOU16409.1 ,gb OQO78070.1 ,ref WP_005876899.1	55.356,2
530	phosphocarrier protein HPr [Enterococcus durans]	gb AKX86600.1 ,gb AKZ47957.1 ,gb EMS76502.1 ,gb EOT34074.1 ,gb EOU26191.1 ,gb OQO80053.1	9.312,5
531	30S ribosomal protein S9 [Enterococcus durans]	gb AKX85907.1 ,gb AKZ47286.1 ,gb EMS74287.1 ,gb EOT28125.1 ,gb EOU16435.1 ,gb OQO79024.1	14.362,8
532	peptidylprolyl isomerase [Enterococcus durans]	gb AKX86215.1 ,gb AKZ47586.1 ,gb EMS76065.1 ,gb EOT34496.1 ,gb EOU25733.1 ,gb OQO79622.1	37.434,4
533	30S ribosomal protein S6 [Enterococcus durans]	gb AKX85856.1 ,gb AKZ47235.1 ,gb EMS76722.1 ,gb EOT33747.1 ,gb EOU25378.1 ,gb OQO82066.1	11.578,8
534	30S ribosomal protein S10 [Enterococcus durans]	gb AKX85811.1 ,gb AKZ47189.1 ,gb EMS74552.1 ,gb EOT33819.1 ,gb EOU25450.1 ,gb OQO82140.1	18.006,8
535	GroES, partial [Enterococcus durans]	gb AAN32674.1 AF417585_1 ,gb AKX85467.1 ,gb AKZ49124.1 ,gb EMS74729.1 ,gb EOT29787.1	10.031,4
536	50S ribosomal protein L2 [Enterococcus durans]	gb AKX85807.1 ,gb AKZ47185.1 ,gb EMS74548.1 ,gb EOT33823.1 ,gb EOU25454.1 ,gb OQO82144.1	30.326,6
537	lactate dehydrogenase [Enterococcus durans]	gb AKX86027.1 ,gb AKZ47406.1 ,gb EMS77070.1 ,gb EOT29104.1 ,gb EOU16350.1 ,gb OQO79291.1	35.131,4
538	phosphoglyceromutase [Enterococcus durans]	gb OQO82127.1 ,ref WP_081133709.1	25.914,4
539	ribosome-recycling factor [Enterococcus durans]	gb AKX84983.1 ,gb AKZ48645.1 ,gb EMS76647.1 ,gb EOT36305.1 ,gb EOU18893.1 ,gb OQO79346.1	20.881,9
540	30S ribosomal protein S2 [Enterococcus durans]	gb AKX84985.1 ,gb AKZ48647.1 ,gb EMS76650.1 ,gb EOT36302.1 ,gb EOU18890.1 ,gb OQO79344.1	29.298,5
541	30S ribosomal protein S13 [Enterococcus durans]	gb AKX85786.1 ,gb AKZ47164.1 ,gb EMS74527.1 ,gb EOT33844.1 ,gb EOU25475.1 ,gb OQO82164.1	13.534,1
542	transketolase [Enterococcus durans]	gb AKX86919.1 ,gb AKZ48271.1 ,gb EMS76686.1 ,gb EOT35404.1 ,gb EOU19357.1 ,ref WP_005875855.1	72.147,2
543	50S ribosomal protein L4 [Enterococcus durans]	gb AKX85809.1 ,gb AKZ47187.1 ,gb EMS74550.1 ,gb EOT33821.1 ,gb EOU25452.1 ,gb OQO82142.1 ,	22.462,4
544	galactose-6-phosphate isomerase [Enterococcus durans]	gb AKX86135.1 ,gb AKZ47507.1 ,gb EMS75412.1 ,ref WP_002345825.1	18.902,4
545	decarboxylase [Enterococcus durans]	gb AKX85643.1 ,gb AKZ47021.1 ,gb EMS76937.1 ,gb EOT32744.1 ,gb EOU25647.1 ,gb OQO82311.1	70.192,3

546	50S ribosomal protein L22 [Enterococcus durans]	gb AKX85805.1 ,gb AKZ47183.1 ,gb EMS74546.1 ,gb EOT33825.1 ,gb EOU25456.1 ,gb OQO82146.1	12.450,7
547	phosphoglycerate kinase [Enterococcus durans]	gb AKX85214.1 ,gb AKZ48876.1 ,gb EMS75629.1 ,gb EOT32243.1 ,gb EOU20044.1 ,gb OQO81407.1	41.855,5
548	pyrrolidone-carboxylate peptidase [Enterococcus durans]	gb AKX86427.1 ,gb AKZ47791.1 ,gb EMS76129.1 ,gb EOT34212.1 ,gb EOU25995.1 ,gb OQO81945.1	22.969,3
549	3-deoxy-7-phosphoheptulonate synthase [Enterococcus durans]	gb AKX85527.1 ,gb AKZ49178.1 ,gb EMS75341.1 ,gb EOT25786.1 ,gb EOU22495.1 ,gb OQO81675.1	37.495,2
550	molecular chaperone GroEL [Enterococcus durans]	gb AKX85466.1 ,gb AKZ49123.1 ,gb EMS74730.1 ,gb OQO81745.1 ,ref WP_005880136.1	57.148,7
551	dihydroxyacetone kinase [Enterococcus durans]	gb AKX85646.1 ,gb AKZ47024.1 ,gb EMS76934.1 ,gb EOT32741.1 ,gb EOU15552.1 ,gb EOU25644.1	21.762,5
552	tyrosine--tRNA ligase [Enterococcus durans]	gb AKX85644.1 ,gb AKZ47022.1 ,gb EMS76936.1 ,gb EOT32743.1 ,gb EOU25646.1 ,gb OQO82310.1	47.335,7
553	30S ribosomal protein S3 [Enterococcus durans]	gb AKX85804.1 ,gb AKZ47182.1 ,gb EMS74545.1 ,gb EOT33826.1 ,gb EOU25457.1 ,gb OQO82147.1	24.406,0
554	glyceraldehyde-3-phosphate dehydrogenase	gb AKX86905.1 ,gb AKZ48257.1 ,gb EMS75963.1 ,gb EOT35299.1 ,gb EOU19372.1 ,gb OQO82543.1 ,	36.155,7
555	enolase [Enterococcus durans]	gb AKX85212.1 ,gb AKZ48874.1 ,gb EMS75631.1 ,gb EOT32245.1 ,gb EOU20046.1 ,gb OQO81409.1	46.481,0
556	triosephosphate isomerase [Enterococcus durans]	gb AKX85213.1 ,gb AKZ48875.1 ,gb EMS75630.1 ,gb OQO81408.1 ,ref WP_005878431.1	26.877,8
557	trigger factor [Enterococcus durans]	gb AKX84928.1 ,gb AKZ48590.1 ,gb EMS74945.1 ,gb EOT36106.1 ,gb EOU18964.1 ,gb OQO78488.1	47.671,9
558	NADH oxidase [Enterococcus durans]	gb AKZ48275.1 ,gb EMS76682.1 ,gb EOT35400.1 ,gb EOU19353.1 ,gb OQO82558.1 ,ref WP_005875847.1	49.253,2
559	NADPH:quinone reductase [Enterococcus durans]	gb AKX85133.1 ,gb AKZ48795.1 ,gb EMS74649.1 ,gb EOT31576.1 ,gb EOU18586.1 ,gb OQO81494.1	33.960,6
560	tagatose-bisphosphate aldolase [Enterococcus durans]	gb AKX86137.1 ,gb AKZ47509.1 ,gb EMS75410.1 ,ref WP_002290551.1 ,ref WP_005878819.1	36.445,1
561	30S ribosomal protein S15 [Enterococcus durans]	gb AKX85715.1 ,gb EMS76858.1 ,gb EOT33619.1 ,gb EOU25559.1 ,gb OQO82242.1 ,ref WP_005875316.1	10.595,5
562	uracil phosphoribosyltransferase [Enterococcus durans]	gb AKX86332.1 ,gb AKZ47698.1 ,gb EMS75108.1 ,gb EOT34441.1 ,gb EOU25865.1 ,gb OQO80536.1	22.888,5
563	PTS cellobiose transporter subunit IIB [Enterococcus durans]	gb AKX86078.1 ,gb AKZ47452.1 ,gb EMS75805.1 ,gb EOT26119.1 ,gb EOU22378.1 ,gb OQO81560.1	11.549,6
564	30S ribosomal protein S8 [Enterococcus durans]	gb AKX85796.1 ,gb AKZ47174.1 ,gb EMS74537.1 ,gb EOT33834.1 ,gb EOU25465.1 ,gb OQO82155.1	14.840,7
565	hypothetical protein LIANG_11460 [Enterococcus durans]	gb AKX86705.1 ,gb AKZ48060.1 ,gb EMS75482.1 ,gb EOT34742.1 ,gb EOU19600.1 ,gb OQO81198.1	49.365,7
566	glyceraldehyde-3-phosphate dehydrogenase	gb AKX85215.1 ,gb AKZ48877.1 ,gb EMS75628.1 ,gb EOT32242.1 ,gb EOU20043.1 ,gb OQO81406.1	35.774,4
567	serine hydroxymethyltransferase [Enterococcus durans]	gb AKX86330.1 ,gb AKZ47696.1 ,gb EMS75110.1 ,gb EOT34438.1 ,gb EOU25862.1 ,gb OQO80538.1	44.881,8
568	universal stress protein UspA [Enterococcus durans]	gb AKX86231.1 ,gb AKZ47601.1 ,gb EMS76048.1 ,gb EOT34514.1 ,gb EOU25751.1 ,gb OQO79639.1	17.246,0
569	hypothetical protein LIANG_08515 [Enterococcus durans]	gb AKX86201.1 ,gb AKZ47572.1 ,gb EMS76564.1 ,gb EOT32805.1 ,gb EOU25708.1 ,gb OQO80094.1	13.151,2
570	fructose-bisphosphate aldolase [Enterococcus durans]	gb AKX85321.1 ,gb AKZ48980.1 ,gb EMS76746.1 ,gb EOT32130.1 ,gb EOU19931.1 ,gb OQO78736.1	30.809,7
571	30S ribosomal protein S5 [Enterococcus durans]	gb AKX85793.1 ,gb AKZ47171.1 ,gb EMS74534.1 ,gb EOT33837.1 ,gb EOU25468.1 ,gb OQO82157.1	17.476,9
572	elongation factor Tu [Enterococcus durans]	gb AKX85818.1 ,gb AKZ47196.1 ,gb EMS74558.1 ,gb EOT33812.1 ,gb EOU25443.1 ,gb OQO82133.1	43.162,2
573	cold-shock protein [Enterococcus durans]	gb AKX86580.1 ,gb AKZ47937.1 ,gb EMS76523.1 ,gb EOT34050.1 ,gb EOU26167.1 ,gb OQO77812.1	7.204,7
574	elongation factor Tu [Enterococcus durans]	gb AKX86680.1 ,gb AKZ48035.1 ,gb EMS75506.1 ,gb EOT34767.1 ,gb EOU19625.1 ,gb OQO81219.1	43.213,0

575	50S ribosomal protein L30 [Enterococcus durans]	gb AKX85792.1 ,gb AKZ47170.1 ,gb EMS74533.1 ,gb EOT33838.1 ,gb EOU25469.1 ,gb OQO82158.1 ,	6.427,5
576	30S ribosomal protein S1 [Enterococcus durans]	gb AKX86712.1 ,gb AKZ48067.1 ,gb EMS75475.1 ,gb EOT34735.1 ,gb EOU19593.1 ,gb OQO81191.1	44.559,6
577	stress response regulator Gls24 [Enterococcus durans]	gb AKX84772.1 ,gb EMS76996.1 ,gb EOT35451.1 ,gb EOU19160.1 ,gb OQO82729.1 ,ref WP_005875244.1	20.508,5
578	hypothetical protein OMS_02998 [Enterococcus durans]	gb EOT25847.1 ,gb EOT31699.1 ,gb EOU18709.1 ,gb EOU22556.1 ,ref WP_016177529.1	12.102,2
579	50S ribosomal protein L15 [Enterococcus durans]	gb AKX85791.1 ,gb AKZ47169.1 ,gb EMS74532.1 ,gb EOT33839.1 ,gb EOU25470.1 ,gb OQO82159.1	15.424,8
580	general stress protein [Enterococcus durans]	gb AKX85160.1 ,gb AKZ48823.1 ,gb EMS74678.1 ,gb EOT31758.1 ,gb EOU18551.1 ,gb OQO81463.1	21.119,5
581	30S ribosomal protein S9 [Enterococcus durans]	gb AKX85907.1 ,gb AKZ47286.1 ,gb EMS74287.1 ,gb EOT28125.1 ,gb EOU16435.1 ,gb OQO79024.1	14.362,8
582	30S ribosomal protein S13 [Enterococcus durans]	gb AKX85786.1 ,gb AKZ47164.1 ,gb EMS74527.1 ,gb EOT33844.1 ,gb EOU25475.1 ,gb OQO82164.1	13.534,1
583	stress response regulator Gls24 [Enterococcus durans]	gb AKX84772.1 ,gb EMS76996.1 ,gb EOT35451.1 ,gb EOU19160.1 ,gb OQO82729.1 ,ref WP_005875244.1	20.508,5
584	hypothetical protein H318_07903 [Enterococcus durans IPLA 655]	gb EMS75618.1 ,gb EOT32232.1 ,gb EOU20033.1 ,gb OQO81395.1 ,ref WP_005878408.1	16.010,1
585	30S ribosomal protein S11 [Enterococcus durans]	gb AKX85785.1 ,gb AKZ47163.1 ,gb EMS74526.1 ,gb EOT33845.1 ,gb EOU25476.1 ,gb OQO82165.1	13.736,0
586	50S ribosomal protein L17 [Enterococcus durans]	gb AKX85783.1 ,gb AKZ47161.1 ,gb EMS74524.1 ,gb EOT33847.1 ,gb EOU25478.1 ,gb OQO82167.1	14.344,9
587	ATP FOF1 synthase subunit alpha [Enterococcus durans]	gb AKX85198.1 ,gb AKZ48860.1 ,gb EMS75647.1 ,gb EOT32261.1 ,gb EOU20062.1 ,gb OQO81424.1	56.397,4
588	gb AKX86463.1 -DECOY	gb AKX86463.1 -DECOY,gb AKZ47822.1 -DECOY	0,0
589	ferritin [Enterococcus durans]	gb AKX85904.1 ,gb AKZ47283.1 ,gb EMS74283.1 ,gb EOT28129.1 ,gb EOU16439.1 ,gb OQO79021.1	17.950,9
590	pyruvate kinase [Enterococcus durans]	gb AKX86645.1 ,gb AKZ47998.1 ,gb EMS77112.1 ,gb EOT34121.1 ,gb EOU26238.1 ,gb OQO80009.1	63.662,1
591	hypothetical protein LIANG_08385 [Enterococcus durans]	gb AKX86179.1 ,gb AKZ47551.1 ,gb EMS75204.1 ,ref WP_005879148.1	16.865,4
592	S-adenosylmethionine synthetase [Enterococcus durans]	gb AKX86039.1 ,gb AKZ47417.1 ,gb EMS75771.1 ,gb EOT26478.1 ,gb EOU22338.1 ,gb OQO81524.1	43.278,6
593	elongation factor G [Enterococcus durans]	gb AKX85819.1 ,gb AKZ47197.1 ,gb EMS74559.1 ,gb EOT33811.1 ,gb EOU25442.1 ,gb OQO82132.1	76.742,1
594	hypothetical protein LIANG_03665 [Enterococcus durans]	gb AKX85374.1 ,gb AKZ49033.1 ,gb EMS76367.1 ,gb EOT29603.1 ,gb EOU22725.1 ,gb OQO78273.1	12.186,8
595	6-phosphofructokinase [Enterococcus durans]	gb AKX86644.1 ,gb AKZ47997.1 ,gb EMS77111.1 ,gb EOT34120.1 ,gb EOU26237.1 ,gb OQO80010.1	34.187,9
596	cell division protein FtsZ [Enterococcus durans]	gb AKX86518.1 ,gb EMS74412.1 ,gb EOT33976.1 ,gb EOU26093.1 ,gb OQO81852.1 ,ref WP_005881040.1	44.328,3
597	NAD(FAD)-dependent dehydrogenase [Enterococcus durans]	gb AKX85538.1 ,gb AKZ49189.1 ,gb EMS75354.1 ,gb EOT25774.1 ,gb EOU22483.1 ,ref WP_005879034.1	50.332,3
598	molecular chaperone DnaK [Enterococcus durans]	gb AKX84948.1 ,gb AKZ48610.1 ,gb EMS74968.1 ,gb OQO78467.1 ,ref WP_005880047.1	65.692,7
599	glucose-6-phosphate isomerase [Enterococcus durans]	gb AKX85027.1 ,gb AKZ48688.1 ,gb EMS76330.1 ,gb EOT36447.1 ,gb EOU18787.1 ,gb OQO77954.1	49.752,2
600	elongation factor Tu [Enterococcus durans]	gb AKX85818.1 ,gb AKZ47196.1 ,gb EMS74558.1 ,gb EOT33812.1 ,gb EOU25443.1 ,gb OQO82133.1	43.162,2
601	cell division protein DivIVA [Enterococcus durans]	gb AKX86522.1 ,gb AKZ47882.1 ,gb EMS74407.1 ,gb EOT33981.1 ,gb EOU26098.1 ,gb OQO81847.1	26.724,9
602	phosphoenolpyruvate-protein phosphotransferase	gb AKX86601.1 ,gb AKZ47958.1 ,ref WP_053108894.1	63.353,5
603	peptidase M29 [Enterococcus durans]	gb AKX86541.1 ,gb AKZ47900.1 ,ref WP_053108834.1	45.096,0



604	Clp protease ClpX (plasmid) [Enterococcus durans]	gb AKX87246.1 ,gb EMS76214.1 ,gb EOT30301.1 ,gb EOU15544.1 ,ref WP_005876977.1	77.848,4
605	osmotically inducible protein C [Enterococcus durans]	gb AKX86277.1 ,gb AKZ47647.1 ,gb EMS75167.1 ,gb EOT34377.1 ,gb EOU25801.1 ,gb OQO78110.1	14.438,9
606	30S ribosomal protein S7 [Enterococcus durans]	gb AKX85820.1 ,gb AKZ47198.1 ,gb EMS74560.1 ,gb EOT33810.1 ,gb EOU25441.1 ,gb OQO82131.1	17.843,7
607	ATP synthase beta-subunit, partial [Enterococcus durans]	gb ADO14905.1 ,gb AKX85196.1 ,gb AKZ48858.1 ,gb EMS75649.1 ,gb EOT32263.1 ,gb EOU20064.1	51.167,8
608	arginine deiminase [Enterococcus durans]	gb AKX84990.1 ,gb AKZ48652.1 ,gb EMS76655.1 ,gb EOT36297.1 ,gb EOU18885.1 ,gb OQO79339.1	46.057,9
609	50S ribosomal protein L7/L12 [Enterococcus durans]	gb AKX85495.1 ,gb AKZ49148.1 ,gb EMS75307.1 ,gb EOT25821.1 ,gb EOU22530.1 ,gb OQO81709.1	12.497,9
610	adenylosuccinate synthetase [Enterococcus durans]	gb AKX85849.1 ,gb AKZ47228.1 ,gb EMS75973.1 ,gb EOT33753.1 ,gb EOU25384.1 ,gb OQO82072.1	47.817,8
611	ornithine carbamoyltransferase [Enterococcus durans]	gb AKX84989.1 ,gb AKZ48651.1 ,gb EMS76654.1 ,gb EOT36298.1 ,gb EOU18886.1 ,gb OQO79340.1	38.341,7
612	PTS cellobiose transporter subunit IIB [Enterococcus durans]	gb AKX86078.1 ,gb AKZ47452.1 ,gb EMS75805.1 ,gb EOT26119.1 ,gb EOU22378.1 ,gb OQO81560.1	11.549,6
613	oxidoreductase [Enterococcus durans]	gb AKX84788.1 ,gb AKZ48447.1 ,gb EMS75711.1 ,gb EOT35435.1 ,gb EOU19143.1 ,gb OQO78703.1	31.934,8
614	dihydrolipoamide acetyltransferase [Enterococcus durans]	gb AKX86821.1 ,gb AKZ48178.1 ,gb EMS76472.1 ,gb EOT34967.1 ,gb EOU19459.1 ,gb OQO82465.1	57.943,6
615	glutamyl-tRNA synthetase [Enterococcus durans]	gb AKZ47353.1 ,gb EMS76247.1 ,gb EOT28433.1 ,gb EOU16409.1 ,gb OQO78070.1 ,ref WP_005876899.1	55.356,2
616	2-oxoisovalerate dehydrogenase [Enterococcus durans]	gb AKX86820.1 ,gb AKZ48177.1 ,gb EMS76471.1 ,gb EOT34968.1 ,gb EOU19460.1 ,gb OQO82464.1	35.384,6
617	N-acetylglucosamine-6-phosphate deacetylase	gb AKX86783.1 ,gb AKZ48142.1 ,gb EMS76427.1 ,gb EOT35012.1 ,gb EOU19504.1 ,gb OQO82426.1	41.332,0
618	peptidylprolyl isomerase [Enterococcus durans]	gb AKX86215.1 ,gb AKZ47586.1 ,gb EMS76065.1 ,gb EOT34496.1 ,gb EOU25733.1 ,gb OQO79622.1	37.434,4
619	30S ribosomal protein S6 [Enterococcus durans]	gb AKX85856.1 ,gb AKZ47235.1 ,gb EMS76722.1 ,gb EOT33747.1 ,gb EOU25378.1 ,gb OQO82066.1	11.578,8
620	30S ribosomal protein S10 [Enterococcus durans]	gb AKX85811.1 ,gb AKZ47189.1 ,gb EMS74552.1 ,gb EOT33819.1 ,gb EOU25450.1 ,gb OQO82140.1	18.006,8
621	GroES, partial [Enterococcus durans]	gb AAN32674.1 AF417585_1 ,gb AKX85467.1 ,gb AKZ49124.1 ,gb EMS74729.1 ,gb EOT29787.1	10.031,4
622	lactate dehydrogenase [Enterococcus durans]	gb AKX86027.1 ,gb AKZ47406.1 ,gb EMS77070.1 ,gb EOT29104.1 ,gb EOU16350.1 ,gb OQO79291.1	35.131,4
623	uridylylate kinase [Enterococcus durans]	gb AKX87068.1 ,gb AKZ49357.1 ,gb EMS76648.1 ,gb EOT36304.1 ,gb EOU18892.1 ,gb OQO79362.1	26.031,2
624	ribosome-recycling factor [Enterococcus durans]	gb AKX84983.1 ,gb AKZ48645.1 ,gb EMS76647.1 ,gb EOT36305.1 ,gb EOU18893.1 ,gb OQO79346.1	20.881,9
625	50S ribosomal protein L3 [Enterococcus durans]	gb AKX85810.1 ,gb AKZ47188.1 ,gb EMS74551.1 ,gb EOT33820.1 ,gb EOU25451.1 ,gb OQO82141.1	22.808,2
626	30S ribosomal protein S2 [Enterococcus durans]	gb AKX84985.1 ,gb AKZ48647.1 ,gb EMS76650.1 ,gb EOT36302.1 ,gb EOU18890.1 ,gb OQO79344.1	29.298,5
627	transketolase [Enterococcus durans]	gb AKX86919.1 ,gb AKZ48271.1 ,gb EMS76686.1 ,gb EOT35404.1 ,gb EOU19357.1 ,ref WP_005875855.1	72.147,2
628	peptide ABC transporter substrate-binding protein [Enterococcus durans]	gb AKX85768.1 ,gb AKZ47145.1 ,gb EMS74510.1 ,gb EOT33660.1 ,gb EOU25495.1 ,ref WP_005880706.1	66.222,4
629	50S ribosomal protein L4 [Enterococcus durans]	gb AKX85809.1 ,gb AKZ47187.1 ,gb EMS74550.1 ,gb EOT33821.1 ,gb EOU25452.1 ,gb OQO82142.1	22.462,4
630	galactose-6-phosphate isomerase [Enterococcus durans]	gb AKX86135.1 ,gb AKZ47507.1 ,gb EMS75412.1 ,ref WP_002345825.1	18.902,4
631	decarboxylase [Enterococcus durans]	gb AKX85643.1 ,gb AKZ47021.1 ,gb EMS76937.1 ,gb EOT32744.1 ,gb EOU25647.1 ,gb OQO82311.1	70.192,3

632	50S ribosomal protein L22 [Enterococcus durans]	gb AKX85805.1 ,gb AKZ47183.1 ,gb EMS74546.1 ,gb EOT33825.1 ,gb EOU25456.1 ,gb OQO82146.1	12.450,7
633	phosphoglycerate kinase [Enterococcus durans]	gb AKX85214.1 ,gb AKZ48876.1 ,gb EMS75629.1 ,gb EOT32243.1 ,gb EOU20044.1 ,gb OQO81407.1	41.855,5
634	pyrrolidone-carboxylate peptidase [Enterococcus durans]	gb AKX86427.1 ,gb AKZ47791.1 ,gb EMS76129.1 ,gb EOT34212.1 ,gb EOU25995.1 ,gb OQO81945.1	22.969,3
635	3-deoxy-7-phosphoheptulonate synthase [Enterococcus durans]	gb AKX85527.1 ,gb AKZ49178.1 ,gb EMS75341.1 ,gb EOT25786.1 ,gb EOU22495.1 ,gb OQO81675.1	37.495,2
636	molecular chaperone GroEL [Enterococcus durans]	gb AKX85466.1 ,gb AKZ49123.1 ,gb EMS74730.1 ,gb OQO81745.1 ,ref WP_005880136.1	57.148,7
637	molecular chaperone GrpE [Enterococcus durans]	gb AKX84949.1 ,gb AKZ48611.1 ,gb EMS74969.1 ,gb EOT36158.1 ,gb EOU18940.1 ,gb OQO78466.1	21.149,6
638	tyrosine--tRNA ligase [Enterococcus durans]	gb AKX85644.1 ,gb AKZ47022.1 ,gb EMS76936.1 ,gb EOT32743.1 ,gb EOU25646.1 ,gb OQO82310.1	47.335,7
639	30S ribosomal protein S3 [Enterococcus durans]	gb AKX85804.1 ,gb AKZ47182.1 ,gb EMS74545.1 ,gb EOT33826.1 ,gb EOU25457.1 ,gb OQO82147.1	24.406,0
640	osmotically inducible protein C [Enterococcus durans]	gb AKX86185.1 ,gb AKZ47557.1 ,gb EMS74585.1 ,gb OQO80135.1 ,ref WP_002292291.1	14.674,3
641	glyceraldehyde-3-phosphate dehydrogenase	gb AKX86905.1 ,gb AKZ48257.1 ,gb EMS75963.1 ,gb EOT35299.1 ,gb EOU19372.1 ,gb OQO82543.1	36.155,7
642	enolase [Enterococcus durans]	gb AKX85212.1 ,gb AKZ48874.1 ,gb EMS75631.1 ,gb EOT32245.1 ,gb EOU20046.1 ,gb OQO81409.1	46.481,0
643	hypothetical protein LIANG_10220 [Enterococcus durans]	gb AKX86497.1 ,gb AKZ47857.1 ,gb EMS74433.1 ,gb EOT33955.1 ,gb EOU26072.1 ,gb OQO81873.1	15.537,3
644	triosephosphate isomerase [Enterococcus durans]	gb AKX85213.1 ,gb AKZ48875.1 ,gb EMS75630.1 ,gb OQO81408.1 ,ref WP_005878431.1	26.877,8
645	trigger factor [Enterococcus durans]	gb AKX84928.1 ,gb AKZ48590.1 ,gb EMS74945.1 ,gb EOT36106.1 ,gb EOU18964.1 ,gb OQO78488.1	47.671,9
646	50S ribosomal protein L29 [Enterococcus durans]	gb AKX85802.1 ,gb AKZ47180.1 ,gb EMS74543.1 ,gb EOT33828.1 ,gb EOU25459.1 ,gb OQO82149.1	7.344,0
647	GMP synthase [Enterococcus durans]	gb AKX85836.1 ,gb AKZ47215.1 ,gb EMS74578.1 ,gb EOT33792.1 ,gb EOU25423.1 ,gb OQO82113.1	57.759,7
648	tagatose-bisphosphate aldolase [Enterococcus durans]	gb AKX86137.1 ,gb AKZ47509.1 ,gb EMS75410.1 ,ref WP_002290551.1 ,ref WP_005878819.1	36.445,1
649	uracil phosphoribosyltransferase [Enterococcus durans]	gb AKX86332.1 ,gb AKZ47698.1 ,gb EMS75108.1 ,gb EOT34441.1 ,gb EOU25865.1 ,gb OQO80536.1	22.888,5
650	phosphoglyceromutase [Enterococcus durans]	gb OQO82127.1 ,ref WP_081133709.1	25.914,4
651	30S ribosomal protein S8 [Enterococcus durans]	gb AKX85796.1 ,gb AKZ47174.1 ,gb EMS74537.1 ,gb EOT33834.1 ,gb EOU25465.1 ,gb OQO82155.1	14.840,7
652	30S ribosomal protein S12 [Enterococcus durans]	gb AKX85821.1 ,gb AKZ47199.1 ,gb EMS74561.1 ,gb EOT33809.1 ,gb EOU25440.1 ,gb OQO82130.1	15.215,9
653	50S ribosomal protein L6 [Enterococcus durans]	gb AKX85795.1 ,gb AKZ47173.1 ,gb EMS74536.1 ,gb EOT33835.1 ,gb EOU25466.1 ,gb OQO82156.1	19.209,3
654	glyceraldehyde-3-phosphate dehydrogenase	gb AKX85215.1 ,gb AKZ48877.1 ,gb EMS75628.1 ,gb EOT32242.1 ,gb EOU20043.1 ,gb OQO81406.1	35.774,4
655	serine hydroxymethyltransferase [Enterococcus durans]	gb AKX86330.1 ,gb AKZ47696.1 ,gb EMS75110.1 ,gb EOT34438.1 ,gb EOU25862.1 ,gb OQO80538.1	44.881,8
656	50S ribosomal protein L13 [Enterococcus durans]	gb AKX87109.1 ,gb AKZ49295.1 ,gb EMS74286.1 ,gb EOT28126.1 ,gb EOU16436.1 ,gb OQO79028.1	15.652,5
657	universal stress protein UspA [Enterococcus durans]	gb AKX86231.1 ,gb AKZ47601.1 ,gb EMS76048.1 ,gb EOT34514.1 ,gb EOU25751.1 ,gb OQO79639.1	17.246,0
658	50S ribosomal protein L18 [Enterococcus durans]	gb AKX85794.1 ,gb AKZ47172.1 ,gb EMS74535.1 ,gb EOT33836.1 ,gb EOU25467.1 ,gb OQO82367.1	13.323,3
659	hypothetical protein LIANG_08515 [Enterococcus durans]	gb AKX86201.1 ,gb AKZ47572.1 ,gb EMS76564.1 ,gb EOT32805.1 ,gb EOU25708.1 ,gb OQO80094.1	13.151,2
660	fructose-bisphosphate aldolase [Enterococcus durans]	gb AKX85321.1 ,gb AKZ48980.1 ,gb EMS76746.1 ,gb EOT32130.1 ,gb EOU19931.1 ,gb OQO78736.1	30.809,7

661	30S ribosomal protein S5 [Enterococcus durans]	gb AKX85793.1 ,gb AKZ47171.1 ,gb EMS74534.1 ,gb EOT33837.1 ,gb EOU25468.1 ,gb OQO82157.1	17.476,9
662	cold-shock protein [Enterococcus durans]	gb AKX86580.1 ,gb AKZ47937.1 ,gb EMS76523.1 ,gb EOT34050.1 ,gb EOU26167.1 ,gb OQO77812.1	7.204,7
663	elongation factor Tu [Enterococcus durans]	gb AKX86680.1 ,gb AKZ48035.1 ,gb EMS75506.1 ,gb EOT34767.1 ,gb EOU19625.1 ,gb OQO81219.1	43.213,0
664	50S ribosomal protein L30 [Enterococcus durans]	gb AKX85792.1 ,gb AKZ47170.1 ,gb EMS74533.1 ,gb EOT33838.1 ,gb EOU25469.1 ,gb OQO82158.1	6.427,5
665	30S ribosomal protein S1 [Enterococcus durans]	gb AKX86712.1 ,gb AKZ48067.1 ,gb EMS75475.1 ,gb EOT34735.1 ,gb EOU19593.1 ,gb OQO81191.1	44.559,6
666	hypothetical protein LIANG_11460 [Enterococcus durans]	gb AKX86705.1 ,gb AKZ48060.1 ,gb EMS75482.1 ,gb EOT34742.1 ,gb EOU19600.1 ,gb OQO81198.1	49.365,7
667	50S ribosomal protein L15 [Enterococcus durans]	gb AKX85791.1 ,gb AKZ47169.1 ,gb EMS74532.1 ,gb EOT33839.1 ,gb EOU25470.1 ,gb OQO82159.1	15.424,8
668	general stress protein [Enterococcus durans]	gb AKX85160.1 ,gb AKZ48823.1 ,gb EMS74678.1 ,gb EOT31758.1 ,gb EOU18551.1 ,gb OQO81463.1	21.119,5
669	30S ribosomal protein S9 [Enterococcus durans]	gb AKX85907.1 ,gb AKZ47286.1 ,gb EMS74287.1 ,gb EOT28125.1 ,gb EOU16435.1 ,gb OQO79024.1	14.362,8
670	inosine-5-monophosphate dehydrogenase [Enterococcus durans]	gb AKX85874.1 ,gb AKZ47255.1 ,gb EMS76701.1 ,gb EOT33726.1 ,gb EOU25357.1 ,gb OQO82046.1	53.029,8
671	hypothetical protein LIANG_06205 [Enterococcus durans]	gb AKX85830.1 ,gb AKZ47209.1 ,gb EMS74571.1 ,gb EOT33799.1 ,gb EOU25430.1 ,gb OQO82120.1	37.834,8
672	molecular chaperone GrpE [Enterococcus durans]	gb AKX84949.1 ,gb AKZ48611.1 ,gb EMS74969.1 ,gb EOT36158.1 ,gb EOU18940.1 ,gb OQO78466.1	21.149,6
673	stress response regulator Gls24 [Enterococcus durans]	gb AKX84772.1 ,gb EMS76996.1 ,gb EOT35451.1 ,gb EOU19160.1 ,gb OQO82729.1 ,ref WP_005875244.1	20.508,5
674	hypothetical protein H318_07903 [Enterococcus durans IPLA 655]	gb EMS75618.1 ,gb EOT32232.1 ,gb EOU20033.1 ,gb OQO81395.1 ,ref WP_005878408.1	16.010,1
675	molecular chaperone DnaK [Enterococcus durans]	gb AKX84948.1 ,gb AKZ48610.1 ,gb EMS74968.1 ,gb OQO78467.1 ,ref WP_005880047.1	65.692,7
676	hypothetical protein LIANG_03400 [Enterococcus durans]	gb AKX87092.1 ,gb AKZ49376.1 ,gb EMS76736.1 ,gb EOT32119.1 ,gb EOU19920.1 ,gb OQO78771.1	7.017,2
677	osmotically inducible protein C [Enterococcus durans]	gb AKX86185.1 ,gb AKZ47557.1 ,gb EMS74585.1 ,gb OQO80135.1 ,ref WP_002292291.1	14.674,3
678	glutamine synthetase [Enterococcus durans]	gb AKX85323.1 ,gb AKZ48982.1 ,gb EMS76744.1 ,gb EOT32128.1 ,gb EOU19929.1 ,gb OQO78738.1	50.675,0
679	50S ribosomal protein L17 [Enterococcus durans]	gb AKX85783.1 ,gb AKZ47161.1 ,gb EMS74524.1 ,gb EOT33847.1 ,gb EOU25478.1 ,gb OQO82167.1	14.344,9
680	ATP F0F1 synthase subunit alpha [Enterococcus durans]	gb AKX85198.1 ,gb AKZ48860.1 ,gb EMS75647.1 ,gb EOT32261.1 ,gb EOU20062.1 ,gb OQO81424.1	56.397,4
681	gb AKX86463.1 -DECOY	gb AKX86463.1 -DECOY,gb AKZ47822.1 -DECOY	0,0
682	ferritin [Enterococcus durans]	gb AKX85904.1 ,gb AKZ47283.1 ,gb EMS74283.1 ,gb EOT28129.1 ,gb EOU16439.1 ,gb OQO79021.1	17.950,9
683	pyruvate kinase [Enterococcus durans]	gb AKX86645.1 ,gb AKZ47998.1 ,gb EMS77112.1 ,gb EOT34121.1 ,gb EOU26238.1 ,gb OQO80009.1	63.662,1
684	hypothetical protein LIANG_08385 [Enterococcus durans]	gb AKX86179.1 ,gb AKZ47551.1 ,gb EMS75204.1 ,ref WP_005879148.1	16.865,4
685	S-adenosylmethionine synthetase [Enterococcus durans]	gb AKX86039.1 ,gb AKZ47417.1 ,gb EMS75771.1 ,gb EOT26478.1 ,gb EOU22338.1 ,gb OQO81524.1	43.278,6
686	elongation factor G [Enterococcus durans]	gb AKX85819.1 ,gb AKZ47197.1 ,gb EMS74559.1 ,gb EOT33811.1 ,gb EOU25442.1 ,gb OQO82132.1	76.742,1
687	30S ribosomal protein S13 [Enterococcus durans]	gb AKX85786.1 ,gb AKZ47164.1 ,gb EMS74527.1 ,gb EOT33844.1 ,gb EOU25475.1 ,gb OQO82164.1	13.534,1
688	6-phosphofructokinase [Enterococcus durans]	gb AKX86644.1 ,gb AKZ47997.1 ,gb EMS77111.1 ,gb EOT34120.1 ,gb EOU26237.1 ,gb OQO80010.1	34.187,9
689	cell division protein FtsZ [Enterococcus durans]	gb AKX86518.1 ,gb EMS74412.1 ,gb EOT33976.1 ,gb EOU26093.1 ,gb OQO81852.1 ,ref WP_005881040.1	44.328,3

690	NAD(FAD)-dependent dehydrogenase [Enterococcus durans]	gb AKX85538.1 ,gb AKZ49189.1 ,gb EMS75354.1 ,gb EOT25774.1 ,gb EOU22483.1 ,ref WP_005879034.1	50.332,3
691	glucose-6-phosphate isomerase [Enterococcus durans]	gb AKX85027.1 ,gb AKZ48688.1 ,gb EMS76330.1 ,gb EOT36447.1 ,gb EOU18787.1 ,gb OQO77954.1	49.752,2
692	elongation factor Tu [Enterococcus durans]	gb AKX85818.1 ,gb AKZ47196.1 ,gb EMS74558.1 ,gb EOT33812.1 ,gb EOU25443.1 ,gb OQO82133.1	43.162,2
693	cell division protein DivIVA [Enterococcus durans]	gb AKX86522.1 ,gb AKZ47882.1 ,gb EMS74407.1 ,gb EOT33981.1 ,gb EOU26098.1 ,gb OQO81847.1	26.724,9
694	cold-shock protein [Enterococcus durans]	gb AKX85335.1 ,gb AKZ48994.1 ,gb EMS76731.1 ,gb EOT32114.1 ,gb EOU19915.1 ,gb OQO78750.1	7.258,7
695	hypothetical protein LIANG_03665 [Enterococcus durans]	gb AKX85374.1 ,gb AKZ49033.1 ,gb EMS76367.1 ,gb EOT29603.1 ,gb EOU22725.1 ,gb OQO78273.1	12.186,8
696	Clp protease ClpX (plasmid) [Enterococcus durans]	gb AKX87246.1 ,gb EMS76214.1 ,gb EOT30301.1 ,gb EOU15544.1 ,ref WP_005876977.1	77.848,4
697	osmotically inducible protein C [Enterococcus durans]	gb AKX86277.1 ,gb AKZ47647.1 ,gb EMS75167.1 ,gb EOT34377.1 ,gb EOU25801.1 ,gb OQO78110.1	14.438,9
698	30S ribosomal protein S7 [Enterococcus durans]	gb AKX85820.1 ,gb AKZ47198.1 ,gb EMS74560.1 ,gb EOT33810.1 ,gb EOU25441.1 ,gb OQO82131.1	17.843,7
699	phosphoenolpyruvate-protein phosphotransferase	gb AKX86601.1 ,gb AKZ47958.1 ,ref WP_053108894.1	63.353,5
700	30S ribosomal protein S11 [Enterococcus durans]	gb AKX85785.1 ,gb AKZ47163.1 ,gb EMS74526.1 ,gb EOT33845.1 ,gb EOU25476.1 ,gb OQO82165.1 ,	13.736,0
701	ATP synthase beta-subunit, partial [Enterococcus durans]	gb ADO14905.1 ,gb AKX85196.1 ,gb AKZ48858.1 ,gb EMS75649.1 ,gb EOT32263.1 ,gb EOU20064.1	51.167,8
702	arginine deiminase [Enterococcus durans]	gb AKX84990.1 ,gb AKZ48652.1 ,gb EMS76655.1 ,gb EOT36297.1 ,gb EOU18885.1 ,gb OQO79339.1	46.057,9
703	50S ribosomal protein L7/L12 [Enterococcus durans]	gb AKX85495.1 ,gb AKZ49148.1 ,gb EMS75307.1 ,gb EOT25821.1 ,gb EOU22530.1 ,gb OQO81709.1	12.497,9
704	branched-chain amino acid aminotransferase	gb AKX86897.1 ,gb AKZ48249.1 ,gb EMS76178.1 ,gb EOT35275.1 ,gb EOU19381.1 ,gb OQO82535.1	37.297,0
705	adenylosuccinate synthetase [Enterococcus durans]	gb AKX85849.1 ,gb AKZ47228.1 ,gb EMS75973.1 ,gb EOT33753.1 ,gb EOU25384.1 ,gb OQO82072.1	47.817,8
706	ornithine carbamoyltransferase [Enterococcus durans]	gb AKX84989.1 ,gb AKZ48651.1 ,gb EMS76654.1 ,gb EOT36298.1 ,gb EOU18886.1 ,gb OQO79340.1	38.341,7
707	dihydrolipoamide dehydrogenase [Enterococcus durans]	gb AKX86822.1 ,gb AKZ48179.1 ,gb EMS76473.1 ,gb EOT34966.1 ,gb EOU19458.1 ,gb OQO82466.1	49.261,4
708	aspartate carbamoyltransferase catalytic subunit	gb AKZ48309.1 ,gb EMS74996.1 ,gb EOT35362.1 ,gb EOU19315.1 ,gb OQO82592.1 ,ref WP_005879675.1	34.904,9
709	general stress protein [Enterococcus durans]	gb AKX84785.1 ,gb AKZ48444.1 ,gb EMS75708.1 ,gb EOT35438.1 ,gb EOU19146.1 ,gb OQO78700.1	30.065,8
710	oxidoreductase [Enterococcus durans]	gb AKX84788.1 ,gb AKZ48447.1 ,gb EMS75711.1 ,gb EOT35435.1 ,gb EOU19143.1 ,gb OQO78703.1	31.934,8
711	dihydrolipoamide acetyltransferase [Enterococcus durans]	gb AKX86821.1 ,gb AKZ48178.1 ,gb EMS76472.1 ,gb EOT34967.1 ,gb EOU19459.1 ,gb OQO82465.1	57.943,6
712	glutamyl-tRNA synthetase [Enterococcus durans]	gb AKZ47353.1 ,gb EMS76247.1 ,gb EOT28433.1 ,gb EOU16409.1 ,gb OQO78070.1 ,ref WP_005876899.1	55.356,2
713	phosphocarrier protein HPr [Enterococcus durans]	gb AKX86600.1 ,gb AKZ47957.1 ,gb EMS76502.1 ,gb EOT34074.1 ,gb EOU26191.1 ,gb OQO80053.1	9.312,5
714	glyceraldehyde-3-phosphate dehydrogenase	gb AKX86905.1 ,gb AKZ48257.1 ,gb EMS75963.1 ,gb EOT35299.1 ,gb EOU19372.1 ,gb OQO82543.1	36.155,7
715	2-oxoisovalerate dehydrogenase [Enterococcus durans]	gb AKX86820.1 ,gb AKZ48177.1 ,gb EMS76471.1 ,gb EOT34968.1 ,gb EOU19460.1 ,gb OQO82464.1	35.384,6
716	N-acetylglucosamine-6-phosphate deacetylase	gb AKX86783.1 ,gb AKZ48142.1 ,gb EMS76427.1 ,gb EOT35012.1 ,gb EOU19504.1 ,gb OQO82426.1	41.332,0
717	30S ribosomal protein S6 [Enterococcus durans]	gb AKX85856.1 ,gb AKZ47235.1 ,gb EMS76722.1 ,gb EOT33747.1 ,gb EOU25378.1 ,gb OQO82066.1	11.578,8
718	30S ribosomal protein S10 [Enterococcus durans]	gb AKX85811.1 ,gb AKZ47189.1 ,gb EMS74552.1 ,gb EOT33819.1 ,gb EOU25450.1 ,gb OQO82140.1	18.006,8

719	GroES, partial [Enterococcus durans]	gb AAN32674.1 AF417585_1,gb AKX85467.1 ,gb AKZ49124.1 ,gb EMS74729.1 ,gb EOT29787.1	10.031,4
720	50S ribosomal protein L2 [Enterococcus durans]	gb AKX85807.1 ,gb AKZ47185.1 ,gb EMS74548.1 ,gb EOT33823.1 ,gb EOU25454.1 ,gb OQO82144.1	30.326,6
721	lactate dehydrogenase [Enterococcus durans]	gb AKX86027.1 ,gb AKZ47406.1 ,gb EMS77070.1 ,gb EOT29104.1 ,gb EOU16350.1 ,gb OQO79291.1	35.131,4
722	phosphoglyceromutase [Enterococcus durans]	gb OQO82127.1 ,ref WP_081133709.1	25.914,4
723	ribosome-recycling factor [Enterococcus durans]	gb AKX84983.1 ,gb AKZ48645.1 ,gb EMS76647.1 ,gb EOT36305.1 ,gb EOU18893.1 ,gb OQO79346.1	20.881,9
724	trigger factor [Enterococcus durans]	gb AKX84928.1 ,gb AKZ48590.1 ,gb EMS74945.1 ,gb EOT36106.1 ,gb EOU18964.1 ,gb OQO78488.1	47.671,9
725	30S ribosomal protein S2 [Enterococcus durans]	gb AKX84985.1 ,gb AKZ48647.1 ,gb EMS76650.1 ,gb EOT36302.1 ,gb EOU18890.1 ,gb OQO79344.1	29.298,5
726	transketolase [Enterococcus durans]	gb AKX86919.1 ,gb AKZ48271.1 ,gb EMS76686.1 ,gb EOT35404.1 ,gb EOU19357.1 ,ref WP_005875855.1	72.147,2
727	DNA-binding protein [Enterococcus durans]	gb AKX86714.1 ,gb AKZ48069.1 ,gb EMS75473.1 ,gb EOT34733.1 ,gb EOU19591.1 ,gb OQO81189.1	9.682,3
728	peptide ABC transporter substrate-binding protein	gb AKX85768.1 ,gb AKZ47145.1 ,gb EMS74510.1 ,gb EOT33660.1 ,gb EOU25495.1 ,ref WP_005880706.1	66.222,4
729	50S ribosomal protein L4 [Enterococcus durans]	gb AKX85809.1 ,gb AKZ47187.1 ,gb EMS74550.1 ,gb EOT33821.1 ,gb EOU25452.1 ,gb OQO82142.1	22.462,4
730	galactose-6-phosphate isomerase [Enterococcus durans]	gb AKX86135.1 ,gb AKZ47507.1 ,gb EMS75412.1 ,ref WP_002345825.1	18.902,4
731	decarboxylase [Enterococcus durans]	gb AKX85643.1 ,gb AKZ47021.1 ,gb EMS76937.1 ,gb EOT32744.1 ,gb EOU25647.1 ,gb OQO82311.1	70.192,3
732	50S ribosomal protein L22 [Enterococcus durans]	gb AKX85805.1 ,gb AKZ47183.1 ,gb EMS74546.1 ,gb EOT33825.1 ,gb EOU25456.1 ,gb OQO82146.1	12.450,7
733	phosphoglycerate kinase [Enterococcus durans]	gb AKX85214.1 ,gb AKZ48876.1 ,gb EMS75629.1 ,gb EOT32243.1 ,gb EOU20044.1 ,gb OQO81407.1	41.855,5
734	pyrrolidone-carboxylate peptidase [Enterococcus durans]	gb AKX86427.1 ,gb AKZ47791.1 ,gb EMS76129.1 ,gb EOT34212.1 ,gb EOU25995.1 ,gb OQO81945.1	22.969,3
735	3-deoxy-7-phosphoheptulonate synthase [Enterococcus durans]	gb AKX85527.1 ,gb AKZ49178.1 ,gb EMS75341.1 ,gb EOT25786.1 ,gb EOU22495.1 ,gb OQO81675.1	37.495,2
736	molecular chaperone GroEL [Enterococcus durans]	gb AKX85466.1 ,gb AKZ49123.1 ,gb EMS74730.1 ,gb OQO81745.1 ,ref WP_005880136.1	57.148,7
737	tyrosine--tRNA ligase [Enterococcus durans]	gb AKX85644.1 ,gb AKZ47022.1 ,gb EMS76936.1 ,gb EOT32743.1 ,gb EOU25646.1 ,gb OQO82310.1	47.335,7
738	30S ribosomal protein S3 [Enterococcus durans]	gb AKX85804.1 ,gb AKZ47182.1 ,gb EMS74545.1 ,gb EOT33826.1 ,gb EOU25457.1 ,gb OQO82147.1	24.406,0
739	pheromone cAD1 precursor lipoprotein	gb EMS77019.1 ,gb EOT28144.1 ,gb EOU16454.1 ,ref WP_005875228.1 ,ref WP_016177786.1	33.478,6
740	enolase [Enterococcus durans]	gb AKX85212.1 ,gb AKZ48874.1 ,gb EMS75631.1 ,gb EOT32245.1 ,gb EOU20046.1 ,gb OQO81409.1	46.481,0
741	carbamoyl phosphate synthase large subunit	gb AKX86955.1 ,gb AKZ48312.1 ,gb EMS74993.1 ,gb EOT35359.1 ,gb EOU19312.1 ,gb OQO82595.1	117.433,8
742	hypothetical protein LIANG_10220 [Enterococcus durans]	gb AKX86497.1 ,gb AKZ47857.1 ,gb EMS74433.1 ,gb EOT33955.1 ,gb EOU26072.1 ,gb OQO81873.1	15.537,3
743	triosephosphate isomerase [Enterococcus durans]	gb AKX85213.1 ,gb AKZ48875.1 ,gb EMS75630.1 ,gb OQO81408.1 ,ref WP_005878431.1	26.877,8
744	dihydroxyacetone kinase [Enterococcus durans]	gb AKX85646.1 ,gb AKZ47024.1 ,gb EMS76934.1 ,gb EOT32741.1 ,gb EOU15552.1 ,gb EOU25644.1	21.837,3
745	50S ribosomal protein L29 [Enterococcus durans]	gb AKX85802.1 ,gb AKZ47180.1 ,gb EMS74543.1 ,gb EOT33828.1 ,gb EOU25459.1 ,gb OQO82149.1	7.344,0
746	general stress protein [Enterococcus durans]	gb AKX85521.1 ,gb AKZ49172.1 ,gb EMS75334.1 ,gb EOT25793.1 ,gb EOU22502.1 ,gb OQO81682.1	18.736,7
747	GMP synthase [Enterococcus durans]	gb AKX85836.1 ,gb AKZ47215.1 ,gb EMS74578.1 ,gb EOT33792.1 ,gb EOU25423.1 ,gb OQO82113.1	57.759,7

748	tagatose-bisphosphate aldolase [Enterococcus durans]	gb AKX86137.1 ,gb AKZ47509.1 ,gb EMS75410.1 ,ref WP_002290551.1 ,ref WP_005878819.1	36.445,1
749	2,5-diketo-D-gluconic acid reductase [Enterococcus durans]	gb AKX86672.1 ,gb AKZ48028.1 ,gb EMS75514.1 ,gb OQO81227.1 ,ref WP_005878645.1	31.865,6
750	30S ribosomal protein S15 [Enterococcus durans]	gb AKX85715.1 ,gb EMS76858.1 ,gb EOT33619.1 ,gb EOU25559.1 ,gb OQO82242.1 ,ref WP_005875316.1	10.595,5
751	uracil phosphoribosyltransferase [Enterococcus durans]	gb AKX86332.1 ,gb AKZ47698.1 ,gb EMS75108.1 ,gb EOT34441.1 ,gb EOU25865.1 ,gb OQO80536.1	22.888,5
752	PTS cellobiose transporter subunit IIB [Enterococcus durans]	gb AKX86078.1 ,gb AKZ47452.1 ,gb EMS75805.1 ,gb EOT26119.1 ,gb EOU22378.1 ,gb OQO81560.1	11.549,6
753	30S ribosomal protein S8 [Enterococcus durans]	gb AKX85796.1 ,gb AKZ47174.1 ,gb EMS74537.1 ,gb EOT33834.1 ,gb EOU25465.1 ,gb OQO82155.1	14.840,7
754	30S ribosomal protein S12 [Enterococcus durans]	gb AKX85821.1 ,gb AKZ47199.1 ,gb EMS74561.1 ,gb EOT33809.1 ,gb EOU25440.1 ,gb OQO82130.1	15.215,9
755	hypothetical protein LIANG_11460 [Enterococcus durans]	gb AKX86705.1 ,gb AKZ48060.1 ,gb EMS75482.1 ,gb EOT34742.1 ,gb EOU19600.1 ,gb OQO81198.1	49.365,7
756	superoxide dismutase, partial [Enterococcus durans]	emb CAB64967.1 ,emb CAB64968.1 ,emb CAB64969.1 ,emb CAB64970.1 ,gb ABV72039.1 ,gb AKX86579.1	22.657,9
757	glyceraldehyde-3-phosphate dehydrogenase	gb AKX85215.1 ,gb AKZ48877.1 ,gb EMS75628.1 ,gb EOT32242.1 ,gb EOU20043.1 ,gb OQO81406.1	35.774,4
758	serine hydroxymethyltransferase [Enterococcus durans]	gb AKX86330.1 ,gb AKZ47696.1 ,gb EMS75110.1 ,gb EOT34438.1 ,gb EOU25862.1 ,gb OQO80538.1	44.881,8
759	50S ribosomal protein L13 [Enterococcus durans]	gb AKX87109.1 ,gb AKZ49295.1 ,gb EMS74286.1 ,gb EOT28126.1 ,gb EOU16436.1 ,gb OQO79028.1	15.652,5
760	universal stress protein UspA [Enterococcus durans]	gb AKX86231.1 ,gb AKZ47601.1 ,gb EMS76048.1 ,gb EOT34514.1 ,gb EOU25751.1 ,gb OQO79639.1	17.246,0
761	50S ribosomal protein L18 [Enterococcus durans]	gb AKX85794.1 ,gb AKZ47172.1 ,gb EMS74535.1 ,gb EOT33836.1 ,gb EOU25467.1 ,gb OQO82367.1	13.323,3
762	50S ribosomal protein L21 [Enterococcus durans]	gb AKX86487.1 ,gb AKZ47845.1 ,gb EMS74445.1 ,gb EOT33943.1 ,gb EOU26060.1 ,gb OQO81885.1	11.174,6
763	fructose-bisphosphate aldolase [Enterococcus durans]	gb AKX85321.1 ,gb AKZ48980.1 ,gb EMS76746.1 ,gb EOT32130.1 ,gb EOU19931.1 ,gb OQO78736.1	30.809,7
764	acyl carrier protein [Enterococcus durans]	gb AKX86608.1 ,gb AKZ47964.1 ,gb EMS76493.1 ,gb EOT34083.1 ,gb EOU26200.1 ,gb OQO80044.1	8.562,8
765	30S ribosomal protein S5 [Enterococcus durans]	gb AKX85793.1 ,gb AKZ47171.1 ,gb EMS74534.1 ,gb EOT33837.1 ,gb EOU25468.1 ,gb OQO82157.1	17.476,9
766	hypothetical protein LIANG_02015 [Enterococcus durans]	gb AKX85093.1 ,gb AKZ48753.1 ,gb EMS76784.1 ,gb EOT31621.1 ,gb EOU18631.1 ,gb OQO81358.1	41.652,5
767	cold-shock protein [Enterococcus durans]	gb AKX86580.1 ,gb AKZ47937.1 ,gb EMS76523.1 ,gb EOT34050.1 ,gb EOU26167.1 ,gb OQO77812.1	7.204,7
768	elongation factor Tu [Enterococcus durans]	gb AKX86680.1 ,gb AKZ48035.1 ,gb EMS75506.1 ,gb EOT34767.1 ,gb EOU19625.1 ,gb OQO81219.1	43.213,0
769	50S ribosomal protein L30 [Enterococcus durans]	gb AKX85792.1 ,gb AKZ47170.1 ,gb EMS74533.1 ,gb EOT33838.1 ,gb EOU25469.1 ,gb OQO82158.1	6.427,5
770	30S ribosomal protein S1 [Enterococcus durans]	gb AKX86712.1 ,gb AKZ48067.1 ,gb EMS75475.1 ,gb EOT34735.1 ,gb EOU19593.1 ,gb OQO81191.1	44.559,6
771	50S ribosomal protein L15 [Enterococcus durans]	gb AKX85791.1 ,gb AKZ47169.1 ,gb EMS74532.1 ,gb EOT33839.1 ,gb EOU25470.1 ,gb OQO82159.1	15.424,8

**Supplementary Table 2. Scaffold validated protein identifications, quantitative analysis and GO functional annotation of Glucose, FOS and GOS treated *Enterococcus durans* LAB18S samples.** Protein name annotations, and statistical analysis of NSAF values are shown.

Number	Protein Description	Accession Number	Molecular Weight	p-value <sup>1</sup>	NSAF Values <sup>2</sup>								
					Glu1	Glu2	Glu3	Fos1	Fos2	Fos3	Gos1	Gos2	Gos3
1	ornithine carbamoyltransferase	gb AKX84989.1  (+6)	38 kDa	0.025	0.039	0.0381	0.0378	0.066	0.0419	0.054	0.0302	0.0364	0.0256
2	glyceraldehyde-3-phosphate dehydrogenase	gb AKX85215.1  (+6)	36 kDa	0.0082	0.0416	0.0706	0.063	0.0248	0.0256	0.0306	0.0614	0.0741	0.0581
3	Clp protease ClpX (plasmid)	gb AKX87246.1  (+6)	78 kDa	0.038	0.0125	0.0217	0.0149	0.0301	0.0242	0.0376	0.0242	0.0162	0.0171
4	elongation factor Tu	gb AKX86680.1  (+6)	43 kDa	0.018	0.0127	0.0268	0.0266	0.00895	0.0024	0.00258	0.0151	0.012	0.0135
5	glyceraldehyde-3-phosphate dehydrogenase	gb AKX86905.1  (+8)	36 kDa	0.011	0.0112	0.0175	0.00694	0.00351	0	0.00303	0.0127	0.0141	0.0139
6	tagatose-bisphosphate aldolase	gb AKX86137.1  (+4)	36 kDa	0.0021	0.00579	0	0.00357	0.00361	0	0.00312	0.0183	0.0204	0.0123
7	glucose-6-phosphate isomerase	gb AKX85027.1  (+7)	50 kDa	0.049	0.00842	0.00787	0.0026	0.00263	0.00211	0.00227	0.00571	0.00848	0.00893
8	oxidoreductase	gb AKX84788.1  (+7)	32 kDa	0.035	0.00847	0.0119	0.0118	0.00793	0.00637	0.0103	0.00574	0.0032	0.00674
9	2-oxoisovalerate dehydrogenase	gb AKX86820.1  (+6)	35 kDa	0.025	0.00581	0.00361	0	0.00725	0.0175	0.0125	0	0.00292	0.00411
10	ribosome-recycling factor	gb AKX84983.1  (+6)	21 kDa	0.043	0.0102	0.0191	0.0252	0.00637	0.00512	0.0055	0.00461	0.0103	0.0108
11	transketolase	gb AKX86919.1  (+6)	72 kDa	0.0042	0.00284	0.00531	0.00527	0	0	0	0.00128	0.00143	0.00301
12	hypothetical protein LIANG_08515 3-deoxy-7-phosphoheptulonate synthase	gb AKX86201.1  (+6)	13 kDa	0.0058	0.0111	0	0	0.0209	0.0251	0.018	0.00754	0	0
13	hypothetical protein LIANG_06205	gb AKX85527.1  (+7)	37 kDa	0.015	0.00742	0.00693	0.00344	0	0	0	0	0.0028	0.00394
14	hypothetical protein LIANG_06205	gb AKX85830.1  (+6)	38 kDa	0.025	0.00174	0.00649	0.00644	0.00325	0.00261	0.00281	0	0	0
15	30S ribosomal protein S10	gb AKX85811.1  (+7)	12 kDa	0.017	0.0123	0.0115	0.0114	0.0116	0.00928	0.00998	0.00835	0.00931	0.00654
16	30S ribosomal protein S19	gb AKX85806.1  (+6)	11 kDa	0.0071	0	0	0.0127	0.0384	0.0206	0.0221	0	0	0
17	cell division protein DivIVA aspartate carbamoyltransferase catalytic subunit	gb AKX86522.1  (+6)	27 kDa	0.037	0.00268	0	0	0	0.00403	0	0.00725	0.00404	0.00568
18	pyruvate kinase	gb AKZ48309.1  (+6)	35 kDa	0.0089	0.00408	0.00763	0.00378	0	0	0	0	0	0.00217
19	pyruvate kinase	gb AKX86645.1  (+7)	64 kDa	0.56	0.0201	0.0297	0.0196	0.0238	0.0159	0.0223	0.023	0.0272	0.0236
20	phosphoglycerate kinase	gb AKX85214.1  (+6)	42 kDa	0.12	0.0254	0.0267	0.0441	0.0327	0.0215	0.0283	0.043	0.0455	0.0371
21	tyrosine--tRNA ligase	gb AKX85644.1  (+8)	47 kDa	0.82	0.0256	0.00843	0.0251	0.00564	0.0181	0.0244	0.0102	0.00908	0.0255
22	elongation factor Tu	gb AKX85818.1  (+6)	43 kDa	0.58	0.0287	0.0149	0.0118	0.0179	0.024	0.0232	0.0324	0.0192	0.022
23	lactate dehydrogenase	gb AKX86027.1  (+6)	35 kDa	0.48	0.0233	0.0363	0.018	0.0218	0.0263	0.0126	0.0316	0.0322	0.0206

24	arginine deiminase	gb AKX84990.1  (+6)	46 kDa	0.59	0.0108	0.0144	0.0171	0.0144	0.0208	0.0124	0.0146	0.0116	0.013
25	fructose-bisphosphate aldolase	gb AKX85321.1  (+6)	31 kDa	0.34	0.0218	0.0285	0.0282	0.0245	0.0197	0.0211	0.0206	0.0296	0.0277
26	molecular chaperone DnaK	gb AKX84948.1  (+5)	66 kDa	0.44	0.0124	0.0116	0.00955	0.0155	0.00931	0.00834	0.00698	0.0109	0.00765
27	ferritin	gb AKX85904.1  (+6)	18 kDa	0.64	0.0487	0.0227	0.0226	0.038	0.0366	0.0394	0.0385	0.0367	0.0344
28	triosephosphate isomerase	gb AKX85213.1  (+4)	27 kDa	0.25	0.0226	0.0234	0.00929	0.0141	0.00754	0.0162	0.0272	0.0227	0.0159
29	decarboxylase	gb AKX85643.1  (+8)	70 kDa	0.21	0.00605	0.00188	0	0.00378	0.00758	0.00816	0.0041	0.00304	0.00535
30	50S ribosomal protein L17	gb AKX85783.1  (+6)	14 kDa	0.59	0.025	0.0186	0.0462	0.0468	0.0225	0.0323	0.0135	0.0301	0.0265
31	cell division protein FtsZ	gb AKX86518.1  (+6)	44 kDa	0.053	0.00914	0.00569	0	0.0114	0.0138	0.0148	0.00619	0.0115	0.00969
32	phosphoenolpyruvate-protein phosphotransferase	gb AKX86601.1  (+2)	63 kDa	0.18	0.00656	0.00409	0	0.0041	0.00329	0.00531	0.00445	0.00991	0.00928
33	30S ribosomal protein S8	gb AKX85796.1  (+6)	15 kDa	0.72	0.0143	0.0089	0.0353	0.0179	0.0287	0.00771	0.0323	0.0288	0.0152
34	enolase	gb AKX85212.1  (+6)	46 kDa	0.12	0.0466	0.0544	0.062	0.0573	0.0482	0.0518	0.0611	0.0703	0.0602
35	molecular chaperone GroEL	gb AKX85466.1  (+5)	57 kDa	0.2	0.00698	0.00869	0.00431	0.00653	0.0035	0.00188	0.00473	0.00175	0.0037
36	hypothetical protein LIANG_11460	gb AKX86705.1  (+8)	49 kDa	0.43	0.00846	0	0.00523	0.00264	0.00212	0	0.00382	0.00213	0.0015
37	50S ribosomal protein L7/L12	gb AKX85495.1  (+8)	13 kDa	0.36	0.0153	0	0.0284	0.00958	0	0.0166	0.0277	0.0154	0.0217
38	30S ribosomal protein S1	gb AKX86712.1  (+7)	45 kDa	0.44	0.0046	0.0086	0	0.00575	0.00923	0.00745	0.0125	0.00695	0.00488
39	30S ribosomal protein S9	gb AKX85907.1  (+6)	14 kDa	0.54	0.0194	0.0271	0.0179	0	0.0291	0.00783	0.0131	0.0219	0.0154
40	30S ribosomal protein S2	gb AKX84985.1  (+6)	29 kDa	0.43	0.00484	0.00452	0.0224	0.00906	0	0	0.00655	0.0073	0.00513
41	universal stress protein UspA	gb AKX86231.1  (+6)	17 kDa	0.5	0.02	0.015	0	0.015	0.0121	0.00648	0.0163	0.0181	0.017
42	elongation factor G	gb AKX85819.1  (+6)	77 kDa	0.14	0.00362	0	0.00168	0	0	0	0.00123	0.00273	0.00192
43	30S ribosomal protein S12 [	gb AKX85821.1  (+7)	15 kDa	0.11	0.00918	0.0171	0.017	0.0086	0.0138	0.0223	0.00622	0.00693	0.00487
44	GroES, partial	gb AAN32674.1  (+7) gb ADO14905.1	9 kDa	0.43	0.0219	0.0273	0.0136	0.0274	0.033	0.0237	0.0198	0.0331	0.0233
45	ATP synthase beta-subunit, partial	(+7)	26 kDa	0.21	0.0105	0.00492	0.00975	0.00493	0.00396	0.00426	0.0143	0.00794	0.00558
46	30S ribosomal protein S6	gb AKX85856.1  (+6)	12 kDa	0.46	0.0127	0.0356	0.0235	0.0476	0.0191	0.0206	0.0172	0.0192	0.0135
47	6-phosphofructokinase	gb AKX86644.1  (+7)	34 kDa	0.61	0.00786	0.00734	0.00364	0.00368	0.00296	0.00636	0.00266	0.00593	0.00834
48	galactose-6-phosphate isomerase	gb AKX86135.1  (+3)	19 kDa	0.075	0	0	0	0.0207	0	0.00595	0.0199	0.0111	0.0156
49	stress response regulator Gls24	gb AKX84772.1  (+6)	21 kDa	0.39	0.0068	0.0254	0.0126	0	0.0102	0.011	0.00921	0.0103	0.0108
50	general stress protein	gb AKX85160.1  (+9)	21 kDa	0.3	0.00319	0.00596	0.00592	0.012	0.0048	0.0103	0.00433	0.00964	0.00339
51	ATP FOF1 synthase subunit alpha	gb AKX85198.1  (+6)	56 kDa	0.64	0.00364	0.00227	0.0045	0.00227	0.00183	0.00196	0.00823	0.00183	0.00129



52	uracil phosphoribosyltransferase	gb AKX86332.1  (+6) gb OQO82127.1	23 kDa	0.27	0.00602	0	0.00558	0.00564	0.00453	0.00487	0.00815	0.00454	0.00958
53	phosphoglyceromutase	(+1)	26 kDa	0.69	0.00276	0	0.00511	0.00517	0.00415	0.00446	0	0.00416	0.00878
54	osmotically inducible protein C	gb AKX86185.1  (+4)	15 kDa	0.81	0.00469	0.00877	0.0348	0.00879	0.00706	0.0152	0.0191	0.0142	0.00498
55	cold-shock protein	gb AKX86580.1  (+6)	7 kDa	0.058	0.0191	0.0178	0	0.0357	0.0287	0.0463	0.0258	0.0288	0.0101
56	molecular chaperone GrpE	gb AKX84949.1  (+6)	21 kDa	0.31	0	0	0.0187	0.0189	0.0101	0.00544	0	0	0.00357
57	30S ribosomal protein S7	gb AKX85820.1  (+6)	18 kDa	0.97	0.00403	0.0151	0	0.00755	0.00607	0.00652	0.00546	0.0122	0.00428
58	30S ribosomal protein S11	gb AKX85785.1  (+7)	14 kDa	0.25	0.00975	0.0182	0.00903	0.00913	0.00734	0.00789	0.00661	0.00736	0.0103
59	50S ribosomal protein L2	gb AKX85807.1  (+6)	30 kDa	0.37	0.00681	0	0.00841	0.00425	0.00683	0.00367	0.00308	0	0.00241
60	50S ribosomal protein L30	gb AKX85792.1  (+6)	6 kDa	0.26	0.0107	0.0199	0	0	0.0321	0.0518	0	0	0.0113
61	trigger factor	gb AKX84928.1  (+7)	48 kDa	0.96	0.00294	0.00274	0.00272	0.00551	0.00221	0	0.00199	0.00222	0.00312
62	hypothetical protein LIANG_08385	gb AKX86179.1  (+3)	17 kDa	0.33	0.00422	0.0237	0.0156	0.00791	0.00635	0.00683	0.00572	0.0127	0.00448
63	50S ribosomal protein L4	gb AKX85809.1  (+7)	22 kDa	0.55	0.00608	0.00568	0	0	0.00457	0	0	0.00459	0
64	50S ribosomal protein L22	gb AKX85805.1  (+6)	12 kDa	0.54	0.00547	0.0102	0.0101	0	0.0165	0.0177	0.00741	0.00825	0
65	NAD(FAD)-dependent dehydrogenase	gb AKX85538.1  (+6)	50 kDa	0.97	0.00411	0	0	0	0	0.00443	0.00186	0.00207	0.00145
66	30S ribosomal protein S5	gb AKX85793.1  (+6)	17 kDa	0.19	0.00379	0.0142	0.014	0.0071	0.0057	0.00613	0.00513	0.00572	0.00402
67	glutamyl-tRNA synthetase	gb AKZ47353.1  (+6)	55 kDa	0.33	0.00259	0	0	0.00485	0	0.00209	0.00175	0.00391	0.00412
68	NADH oxidase	gb AKZ48275.1  (+7)	49 kDa	0.1	0	0	0	0.00262	0.0021	0.00452	0.00379	0	0
69	50S ribosomal protein L13	gb AKX87109.1  (+6)	16 kDa	0.93	0.00428	0.00799	0.00793	0.00802	0.00644	0.00692	0.0116	0.00646	0
70	50S ribosomal protein L15	gb AKX85791.1  (+6)	15 kDa	0.45	0.00431	0.00805	0.00798	0.00807	0.00648	0.00697	0.00584	0.0065	0.00457
71	30S ribosomal protein S13	gb AKX85786.1  (+6)	14 kDa	0.92	0.0104	0.00971	0	0	0.0156	0.00841	0.00704	0.00785	0.011
72	osmotically inducible protein C	gb AKX86277.1  (+6)	14 kDa	0.7	0.00473	0.00883	0.00876	0	0.0142	0	0.00641	0	0.00502
73	peptide ABC transporter substrate-binding protein	gb AKX85768.1  (+5)	66 kDa	0.26	0	0	0	0	0.00319	0.00171	0	0.0016	0.00225
74	general stress protein	gb AKX84785.1  (+8)	30 kDa	0.13	0.0023	0	0	0.00863	0.00693	0.00373	0	0	0.00733
75	30S ribosomal protein S3	gb AKX85804.1  (+6)	24 kDa	0.87	0.00289	0	0.0107	0	0.00434	0.00467	0.00391	0.00435	0.00306
76	dihydrolipoamide acetyltransferase	gb AKX86821.1  (+8)	58 kDa	0.31	0.0023	0	0	0	0.00173	0.00373	0	0	0
77	GMP synthase	gb AKX85836.1  (+7)	58 kDa	0.94	0.00362	0	0	0.00226	0	0	0	0.00182	0.00128
78	50S ribosomal protein L29	gb AKX85802.1  (+6)	7 kDa	0.5	0.0101	0.0379	0.0188	0.019	0.0153	0	0	0.0153	0.0215
79	30S ribosomal protein S15	gb AKX85715.1  (+5)	11 kDa	0.88	0.0141	0.0132	0	0	0.0213	0	0.0191	0	0.015

80	PTS cellobiose transporter subunit IIB	gb AKX86078.1  (+6)	12 kDa	0.37	0	0	0	0	0.00893	0.0288	0.0161	0	0
81	S-adenosylmethionine synthetase	gb AKX86039.1  (+7)	43 kDa	0.32	0.00158	0.00296	0	0	0	0.00256	0.00429	0.00239	0.00168
82	glutamine synthetase	gb AKX85323.1  (+9)	51 kDa	0.6	0.00282	0	0	0	0	0	0	0	0.00449
83	phosphocarrier protein HPr	gb AKX86600.1  (+6)	9 kDa	0.6	0.00715	0	0.0397	0	0.0108	0	0.0194	0	0.00758
84	hypothetical protein LIANG_02015	gb AKX85093.1  (+7)	42 kDa	0.51	0.00684	0	0	0	0	0	0	0	0.00181
85	dihydrolipoamide dehydrogenase	gb AKX86822.1  (+9)	49 kDa	0.25	0.00134	0	0	0	0	0	0.00182	0	0.00143
86	formate acetyltransferase	gb AKX86748.1  (+9)	84 kDa	0.17	0	0	0	0	0.00382	0.00137	0	0	0
87	DNA-binding protein	gb AKX86714.1  (+6)	10 kDa	0.57	0.0138	0	0	0	0	0	0	0	0.0293
88	50S ribosomal protein L21	gb AKX86487.1  (+6)	11 kDa	0.82	0.0123	0	0.0114	0	0.00928	0.00998	0.00835	0	0.00654
89	adenylosuccinate synthetase	gb AKX85849.1  (+7)	48 kDa	0.26	0.00146	0.00546	0	0	0	0	0.00198	0.00221	0.00155
90	hypothetical protein H318_07903	gb EMS75618.1  (+6)	16 kDa	0.5	0.00463	0	0	0.0173	0.00696	0	0	0.014	0.0049
91	carbamate kinase	gb AKX84988.1  (+7)	34 kDa	0.084	0.00597	0.00744	0	0	0	0	0	0	0
92	dihydroxyacetone kinase	gb AKX85646.1  (+9)	22 kDa	0.06	0.00315	0	0	0.00589	0.00473	0.00509	0.00426	0	0.00334
93	pyrrolidone-carboxylate peptidase	gb AKX86427.1  (+6)	23 kDa	0.46	0.00294	0.00549	0	0	0.00442	0	0.00398	0.00444	0.00312
94	2,5-diketo-D-gluconic acid reductase	gb AKX86672.1  (+5)	32 kDa	0.18	0.00222	0	0	0.00833	0.00669	0	0	0	0.00236
95	peptidylprolyl isomerase pheromone cAD1 precursor lipoprotein	gb AKX86215.1  (+7)	37 kDa	0.31	0	0	0	0	0.00555	0	0.005	0.00557	0
96	PTS mannose transporter subunit IIB	gb EMS77019.1  (+4)	33 kDa	0.42	0	0	0	0	0	0	0	0	0.00214
97	phosphotransacetylase	gb AKX86491.1  (+6)	35 kDa	0.081	0	0	0	0.0073	0	0.0063	0	0	0
98	phosphotransacetylase	gb AKX86438.1  (+5)	35 kDa	0.42	0	0	0	0	0.00871	0	0	0	0
100	hypothetical protein LIANG_10220 N-acetylglucosamine-6-phosphate deacetylase	gb AKX86497.1  (+7)	16 kDa	0.24	0.00446	0	0	0	0	0	0	0.00673	0.00473
101	cell division protein FtsH	gb AKX86783.1  (+7)	41 kDa	0.59	0.00329	0	0	0	0	0	0	0	0.00175
102	hypothetical protein LIANG_03665	gb AKX86034.1  (+6)	77 kDa	0.63	0	0	0	0	0.00271	0	0.00244	0	0
103	hypothetical protein LIANG_05820	gb AKX85374.1  (+7)	12 kDa	0.59	0.0113	0	0	0	0	0	0	0	0.00601
104	hypothetical protein LIANG_05820	gb AKX85756.1  (+6)	13 kDa	0.56	0	0	0	0	0	0.0173	0.00722	0	0
105	NADPH:quinone reductase	gb AKX85133.1  (+8)	34 kDa	0.62	0.00202	0	0	0	0	0	0.00273	0	0
106	general stress protein	gb AKX85521.1  (+6)	19 kDa	0.42	0	0	0	0	0	0	0	0	0.00392
107	glucokinase	gb AKX85004.1  (+7)	34 kDa	0.63	0	0	0	0	0	0.00318	0.00266	0	0
108	serine hydroxymethyltransferase	gb AKX86330.1  (+8)	45 kDa	0.42	0	0	0	0	0	0	0.00206	0	0

109	cold-shock protein branched-chain amino acid aminotransferase	gb AKX85335.1  (+6)	7 kDa	0.59	0.0191	0	0	0	0	0	0	0	0.0101
110	50S ribosomal protein L6	gb AKX86897.1  (+6)	37 kDa	0.63	0.00187	0	0	0	0	0	0	0	0.00198
111	GapA	gb AKX85795.1  (+6)	19 kDa	0.62	0.00707	0	0	0	0	0	0	0.00533	0
112	acyl carrier protein	gb AKX84774.1  (+6)	21 kDa	0.12	0	0	0	0	0.0101	0.00544	0	0	0
113	hypothetical protein LIANG_03400	gb AKX86608.1  (+6)	9 kDa	0.26	0.0085	0	0.0157	0	0	0	0	0	0.00901
114	transcription elongation factor GreA	gb AKX87092.1  (+7)	7 kDa	0.42	0	0	0	0	0	0	0	0	0.0226
115	citrate (Pro-3S)-lyase, beta subunit carbamoyl phosphate synthase large subunit	gb AKX86569.1  (+6)	17 kDa	0.42	0	0	0	0.0148	0	0	0	0	0
116	inosine-5-monophosphate dehydrogenase	gb EOT31306.1  (+2)	33 kDa	0.084	0	0	0	0.00391	0.00314	0	0	0	0
117	peptidase M29	gb AKX86955.1  (+7)	117 kDa	0.63	0.000593	0	0	0	0	0	0	0	0.000629
118	superoxide dismutase, partial	gb AKX85874.1  (+6)	53 kDa	0.42	0	0	0	0	0	0	0	0	0.00135
119	50S ribosomal protein L31 type B	gb AKX86541.1  (+2) emb CAB64967.1  (+12)	45 kDa	0.42	0.00153	0	0	0	0	0	0	0	0
120	50S ribosomal protein L31 type B	gb AKX85318.1  (+6)	10 kDa	0.42	0	0	0	0	0	0	0	0	0
121	50S ribosomal protein L18	gb AKX8794.1  (+6)	13 kDa	0.42	0	0	0	0	0	0	0	0	0.00565
122	uridylyate kinase	gb AKX87068.1  (+7)	26 kDa	0.61	0.00262	0	0	0	0	0	0	0.00396	0
123	pyruvate oxidase	gb AKZ47224.1  (+7)	64 kDa	0.42	0	0.00204	0	0	0	0	0	0	0
124	cysteine synthase	gb AKX86921.1  (+7)	33 kDa	0.42	0.00408	0	0	0	0	0	0	0	0
125	isomerase	gb AKX86085.1  (+6)	65 kDa	0.42	0.00112	0	0	0	0	0	0	0	0
126	hypothetical protein LIANG_11540	gb AKX86721.1  (+6)	18 kDa	0.42	0	0	0	0	0	0.00674	0	0	0
127	hypothetical protein OMS_02998	gb EOT25847.1  (+5)	12 kDa	0.62	0	0	0	0.0113	0	0	0.00819	0	0
128	glycerol kinase	gb AKX86100.1  (+7)	56 kDa	0.42	0	0	0	0	0.0019	0	0	0	0
129	NAD synthetase	gb AKX85456.1  (+7)	31 kDa	0.42	0.00229	0	0	0	0	0	0	0	0
130	hypothetical protein OMS_02283, partial	gb EOT31214.1  (+4) gb AKX84844.1  (+15)	140 kDa	0.42	0	0	0	0.000868	0	0	0	0	0
131	Clp protease ClpB	gb AKX85810.1  (+6)	98 kDa	0.42	0.000724	0	0	0	0	0	0	0	0
132	50S ribosomal protein L3	gb AKX85810.1  (+6)	23 kDa	0.42	0	0	0	0	0	0	0	0.00454	0
133	hypothetical protein H318_02255	gb EMS76691.1  (+3)	208 kDa	0.42	0	0	0	0.000648	0	0	0	0	0
134	50S ribosomal protein L32	gb AKX86648.1  (+7)	7 kDa	0.42	0	0	0	0	0.016	0	0	0	0
135													

<sup>1</sup> Grey marked cells indicate p-values lower than 0.05.

<sup>2</sup> GLU 1, 2 and 3 = biological replicas from glucose treated samples; FOS 1, 2 and 3 = biological replicates from FOS treated samples; GOS 1, 2 and 3 = biological replicates from GOS treated samples.

**Supplementary Table 3. Scaffold validated protein identifications, quantitative analysis and GO functional annotation of Glucose, FOS and GOS treated *Enterococcus durans* LAB18S samples.** Gene Ontology functional annotation of identified and Scaffold validated proteins

Number	Protein name	Accession number	Biological Process		Molecular Function		Cellular Component	
			GO code	GO term	GO code	GO term	GO code	GO term
1	Enolase	gb AKX85212.1	GO:0009094	L-phenylalanine biosynthetic process	GO:0000287	magnesium ion binding	GO:0000015	phosphopyruvate hydratase complex
			GO:0006571	tyrosine biosynthetic process	GO:0004634	phosphopyruvate hydratase activity		
			GO:0006096	glycolytic process				
			GO:0006094	gluconeogenesis				
			GO:0000162	tryptophan biosynthetic process				
2	glyceraldehyde-3-phosphate dehydrogenase	gb AKX85215.1	GO:0006006	glucose metabolic process	GO:0016620	oxidoreductase activity		
			GO:0055114	oxidation-reduction process	GO:0050661	NADP binding		
					GO:0051287	NAD binding		
3	ATP-dependent Clp protease ATP-binding	gb AKX87246.1			GO:0005515	protein binding		
					GO:0005524	ATP binding		
4	pyruvate kinase	gb AKX86645.1	GO:0006094	gluconeogenesis	GO:0000287	magnesium ion binding		
			GO:0006096	glycolytic process	GO:0004743	pyruvate kinase activity		
			GO:0006144	purine nucleobase metabolic process	GO:0030955	potassium ion binding		
			GO:0015976	carbon utilization				
5	ornithine carbamoyltransferase	gb AKX84989.1	GO:0006525	arginine metabolic process	GO:0004585	ornithine carbamoyltransferase activity	GO:0009348	ornithine carbamoyltransferase complex
			GO:0006560	proline metabolic process	GO:0016597	amino acid binding		
			GO:0006591	ornithine metabolic process				
6	elongation factor Tu	gb AKX85214.1	GO:0006448	regulation of translational elongation	GO:0003746	translation elongation factor activity	GO:0005840	ribosome
					GO:0003924	GTPase activity		
					GO:0005525	GTP binding		
7	L-lactate dehydrogenase	gb AKX85644.1	GO:0006094	gluconeogenesis	GO:0004459	L-lactate dehydrogenase activity	GO:0005737	cytoplasm

8	Arginine deiminase	gb AKX85818.1	GO:0006096	glycolytic process				
			GO:0006534	cysteine metabolic process				
			GO:0055114	oxidation-reduction process				
			GO:0006527	arginine catabolic process	GO:0016990	arginine deiminase activity		
			GO:0006560	proline metabolic process				
			GO:0006000	fructose metabolic process	GO:0004332	fructose-bisphosphate aldolase activity		
			GO:0006013	mannose metabolic process	GO:0008270	zinc ion binding		
9	fructose-bisphosphate aldolase	gb AKX86027.1	GO:0006020	inositol metabolic process				
			GO:0006094	gluconeogenesis				
			GO:0006096	glycolytic process				
			GO:0006098	pentose-phosphate shunt				
			GO:0015976	carbon utilization				
			GO:0030388	fructose 1,6-bisphosphate metabolic process				
			GO:0006457	protein folding	GO:0005524	ATP binding		
10	molecular chaperone DnaK	gb AKX84990.1			GO:0051082	unfolded protein binding		
			GO:0006879	cellular iron ion homeostasis	GO:0008199	ferric iron binding		
			GO:0006950	response to stress	GO:0016722	oxidoreductase activity		
11	DNA starvation/stationary phase protection	gb AKX84948.1	GO:0055114	oxidation-reduction process				
			GO:0006000	fructose metabolic process	GO:0004807	triose-phosphate isomerase activity		
			GO:0006013	mannose metabolic process				
			GO:0006020	inositol metabolic process				
			GO:0006094	gluconeogenesis				
12	triose-phosphate isomerase	gb AKX86680.1	GO:0006096	glycolytic process				
			GO:0015976	carbon utilization				
			GO:0046486	glycerolipid metabolic process				
			GO:0019752	carboxylic acid metabolic process	GO:0016831	carboxy-lyase activity		
			GO:0019752	carboxylic acid metabolic process	GO:0030170	pyridoxal phosphate binding		
13	tyrosine decarboxylase	gb AKX85904.1						
14	50S ribosomal protein L17	gb AKX85213.1	GO:0006412	translation	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome

15	cell division protein FtsZ	gb AKX85643.1	GO:0042254	ribosome biogenesis	GO:0003924	GTPase activity	O:0005737	cytoplasm
					GO:0005525	GTP binding		
16	glyceraldehyde-3-phosphate dehydrogenase	gb AKX85783.1	GO:0006006	glucose metabolic process	GO:0016620	oxidoreductase activity		
			GO:0055114	oxidation-reduction process	GO:0050661	NADP binding		
					GO:0051287	NAD binding		
17	tagatose 1,6-diphosphate aldolase	gb AKX86518.1	GO:0006012	galactose metabolic process	GO:0009024	tagatose-6-phosphate kinase activity		
			GO:0019512	lactose catabolic process	GO:0016829	lyase activity		
18	phosphoenolpyruvate--protein phosphotransferase	gb AKX86905.1	GO:0009401	phosphoenolpyruvate-dependent sugar phosphotransferase system	GO:0008965	phosphotransferase activity		
			GO:0016310	phosphorylation				
			GO:0005982	starch metabolic process	GO:0004347	glucose-6-phosphate isomerase activity		
19	glucose-6-phosphate isomerase	gb AKX86137.1	GO:0005985	sucrose metabolic process				
			GO:0006094	gluconeogenesis				
			GO:0006096	glycolytic process				
			GO:0006098	pentose-phosphate shunt				
20	30S ribosomal protein S8	gb AKX86601.1	GO:0006412	translation	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome
			GO:0042254	ribosome biogenesis				
21	short chain dehydrogenase/reductase family oxidoreductase	gb AKX85027.1			GO:0016491	oxidoreductase activity		
22	chaperonin	gb AKX85796.1	GO:0042026	protein refolding	GO:0005524	ATP binding	GO:0005737	cytoplasm
23	M20/M25/M40 family metallo-hydrolase	gb AKX84788.1	GO:0008152	metabolic process	GO:0008270	zinc ion binding		
					GO:0016805	dipeptidase activity		
24	50S ribosomal protein L7/L12	gb AKX85466.1	GO:0006412	translation	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome
			GO:0042254	ribosome biogenesis				
25	30S ribosomal protein S1	gi 915772437 gb AKX86705.1			GO:0003676	nucleic acid binding		
26	30S ribosomal protein S9	gb AKX85495.1	GO:0006412	translation	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome
			GO:0042254	ribosome biogenesis				
27	alpha-ketoacid dehydrogenase subunit beta	gb AKX86712.1	GO:0008152	metabolic process	GO:0003824	catalytic activity		

28	30S ribosomal protein S2	gb AKX85907.1	GO:0006412 translation GO:0042254 ribosome biogenesis	GO:0003735 structural constituent of ribosome	GO:0015935 small ribosomal subunit
29	universal stress protein	gb AKX86820.1	GO:0006950 response to stress		
30	elongation factor G	gb AKX84985.1	GO:0006448 regulation of translational elongation	GO:0003746 translation elongation factor activity GO:0003924 GTPase activity GO:0005525 GTP binding	GO:0005840 ribosome
31	30S ribosomal protein S12	gb AKX86231.1	GO:0006412 translation GO:0042254 ribosome biogenesis	GO:0003735 structural constituent of ribosome	GO:0015935 small ribosomal subunit
32	co-chaperone GroES	gb AKX85819.1	GO:0006457 protein folding		GO:0005737 cytoplasm
33	Ribosome recycling factor	gb AKX85821.1	GO:0006412 translation		
34	F0F1 ATP synthase subunit beta	gb AAN32674.1	GO:0046034 ATP metabolic process GO:1902600 proton transmembrane transport	GO:0005524 ATP binding	
35	transketolase	gb AKX84983.1	GO:0006098 pentose-phosphate shunt GO:0015976 carbon utilization	GO:0004802 transketolase activity	
36	30S ribosomal protein S6	gb ADO14905.1	GO:0006412 translation GO:0042254 ribosome biogenesis GO:0006000 fructose metabolic process GO:0006002 fructose 6-phosphate metabolic process	GO:0003735 structural constituent of ribosome GO:0019843 rRNA binding GO:0003872 6-phosphofructokinase activity GO:0005524 ATP binding	GO:0005840 ribosome GO:0005945 6-phosphofructokinase complex
37	6-phosphofructokinase	gb AKX86919.1	GO:0006012 galactose metabolic process GO:0006013 mannose metabolic process GO:0006094 gluconeogenesis GO:0006096 glycolytic process GO:0006098 pentose-phosphate shunt		
38	galactose-6-phosphate isomerase subunit lacB	gb AKX85856.1	GO:0005990 lactose catabolic process GO:0006012 galactose metabolic process	GO:0050044 galactose-6-phosphate isomerase activity	
39	general stress protein	gb AKX86135.1			GO:0016020 membrane integral component of membrane GO:0016021 membrane

40	ATP synthase subunit alpha	gb AKX84772.1	GO:0015986	ATP synthesis coupled proton transport	GO:0005524	ATP binding	GO:0045261	proton-transporting ATP synthase complex
					GO:0046933	proton-transporting ATP synthase activity		
41	uracil phosphoribosyltransferase	gb AKX85160.1	GO:0006223	uracil salvage	GO:0004845	uracil phosphoribosyltransferase activity		
			GO:0009116	nucleoside metabolic process				
42	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	gb AKX85198.1	GO:0006094	gluconeogenesis	GO:0004619	phosphoglycerate mutase activity		
			GO:0006096	glycolytic process				
43	Organic hydroperoxide resistance protein	gb AKX86332.1	GO:0006979	response to oxidative stress				
44	cold-shock protein	gb OQO82127.1	GO:0006355	regulation of transcription, DNA-templated	GO:0003677	DNA binding		
			GO:0006457	protein folding	GO:0000774	adenyl-nucleotide exchange factor activity		
45	co-chaperone GrpE	gb AKX86580.1				protein homodimerization activity		
					GO:0042803	chaperone binding		
			GO:0006412	translation	GO:0003723	RNA binding	GO:0015935	small ribosomal subunit
46	30S ribosomal protein S7	gb AKX86201.1				structural constituent of ribosome		
			GO:0042254	ribosome biogenesis	GO:0003735	ribosome structural constituent of ribosome		
47	30S ribosomal protein S11	gb AKX84949.1	GO:0006412	translation	GO:0003735	ribosome	GO:0005840	ribosome
			GO:0042254	ribosome biogenesis				
			GO:0006412	translation	GO:0003723	RNA binding structural constituent of ribosome	GO:0015934	large ribosomal subunit
48	50S ribosomal protein L2	gb AKX85820.1				ribosome		
			GO:0042254	ribosome biogenesis	GO:0003735	transferase activity		
49	3-deoxy-7-phosphoheptulonate synthase	gb AKX85785.1	GO:0009073	aromatic amino acid family biosynthetic process	GO:0016832	aldehyde-lyase activity		
						structural constituent of ribosome		
50	50S ribosomal protein L30	gb AKX85807.1	GO:0006412	translation	GO:0003735	ribosome	GO:0015934	large ribosomal subunit
			GO:0042254	ribosome biogenesis				
51	trigger factor	gb AKX85527.1	GO:0006457	protein folding				
			GO:0015031	protein transport				



52	50S ribosomal protein L4	gb AKX84928.1	GO:0006412 GO:0042254	translation ribosome biogenesis	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome
53	50S ribosomal protein L22	gb AKX86179.1	GO:0006412 GO:0042254	translation ribosome biogenesis	GO:0003735	structural constituent of ribosome	GO:0015934	large ribosomal subunit
54	NAD(FAD)-dependent dehydrogenase	gb AKX85809.1	GO:0045454 GO:0055114	cell redox homeostasis oxidation-reduction process	GO:0016491	oxidoreductase activity flavin adenine dinucleotide binding		
55	30S ribosomal protein S5	gb AKX85805.1	GO:0006412 GO:0042254	translation ribosome biogenesis	GO:0003723	RNA binding structural constituent of ribosome	GO:0015935	small ribosomal subunit
56	BMP family ABC transporter substrate-binding protein	gi 915771270 gb AKX85538.1	GO:0006424	glutamyl-tRNA aminoacylation	GO:0000049	tRNA binding glutamate-tRNA ligase activity	GO:0005886	plasma membrane glutamate-tRNA ligase complex
57	glutamate--tRNA ligase	gb AKX85793.1	GO:0015994	chlorophyll metabolic process	GO:0004818	ATP binding	GO:0009332	
58	NADH oxidase	gb AKX85830.1	GO:0045454 GO:0055114	cell redox homeostasis oxidation-reduction process	GO:0016491	zinc ion binding oxidoreductase activity flavin adenine dinucleotide binding	GO:0050660	
59	50S ribosomal protein L13	gb AKZ47353.1	GO:0006412 GO:0042254	translation ribosome biogenesis	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome
60	50S ribosomal protein L15	gb AKZ48275.1	GO:0006412 GO:0042254	translation ribosome biogenesis	GO:0003735	structural constituent of ribosome	GO:0015934	large ribosomal subunit
61	30S ribosomal protein S10	gb AKX87109.1	GO:0006412	translation	GO:0003723	RNA binding	GO:0005840	ribosome

			GO:0042254	ribosome biogenesis	GO:0003735	structural constituent of ribosome		
62	30S ribosomal protein S13	gb AKX85791.1	GO:0006412	translation	GO:0003723	RNA binding structural constituent of ribosome	GO:0005840	ribosome
			GO:0042254	ribosome biogenesis	GO:0003735	structural constituent of ribosome		
63	osmotically inducible protein C	gb AKX85811.1	GO:0006979	response to oxidative stress				
64	extracellular solute-binding protein	gb AKX85786.1	GO:0055085	transmembrane transport			GO:0043190	ATP-binding cassette (ABC) transporter complex
			GO:0006412	translation	GO:0003723	RNA binding structural constituent of ribosome	GO:0015935	small ribosomal subunit
65	30S ribosomal protein S3	gb AKX85768.1	GO:0042254	ribosome biogenesis	GO:0003735	structural constituent of ribosome		
66	dihydropolyllysine-residue acetyltransferase	gb AKX84785.1	GO:0008152	metabolic process	GO:0016746	transferase activity		
			GO:0006412	translation	GO:0003723	RNA binding structural constituent of ribosome	GO:0015935	small ribosomal subunit
67	30S ribosomal protein S19	gb AKX85804.1	GO:0042254	ribosome biogenesis	GO:0003735	GMP synthase (glutamine-hydrolyzing) activity		
68	GMP synthase	gb AKX86821.1	GO:0006144	purine nucleobase metabolic process	GO:0003922	ATP binding	GO:0016462	pyrophosphatase activity
			GO:0006177	GMP biosynthetic process	GO:0005524	ATP binding		
			GO:0006536	glutamate metabolic process				
69	50S ribosomal protein L29	gb AKX85806.1	GO:0006412	translation	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome
			GO:0042254	ribosome biogenesis				
70	30S ribosomal protein S15	gb AKX85836.1	GO:0006412	translation	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome
			GO:0042254	ribosome biogenesis				
71	PTS sugar transporter subunit IIB	gb AKX85802.1	GO:0009401	phosphoenolpyruvate-dependent sugar phosphotransferase system	GO:0008982	protein-N(Pi)-phosphohistidine-sugar phosphotransferase activity	GO:0009357	protein-N(Pi)-phosphohistidine-sugar phosphotransferase complex

72	methionine adenosyltransferase	gb AKX85715.1	GO:0006555 GO:0006556	methionine metabolic process S-adenosylmethionine biosynthetic process	GO:0004478 GO:0005524	methionine adenosyltransferase activity ATP binding glutamate-ammonia ligase activity		
73	type I glutamate--ammonia ligase	gb AKX86078.1	GO:0006542 GO:0009252 GO:0009399	glutamine biosynthetic process peptidoglycan biosynthetic process nitrogen fixation	GO:0004356		GO:0005737	cytoplasm
74	phosphocarrier protein HPr	gb AKX86039.1	GO:0009401	phosphoenolpyruvate-dependent sugar phosphotransferase system	GO:0016740	transferase activity	GO:0005737	cytoplasm
75	cell division protein DivIVA	gb AKX86600.1	GO:0007049 GO:0051301	cell cycle cell division			GO:0005737	cytoplasm
76	dihydrolipoyl dehydrogenase	gb AKX85093.1	GO:0006094 GO:0006096 GO:0006099 GO:0006118 GO:0006544 GO:0006563 GO:0006566 GO:0045454	gluconeogenesis glycolytic process tricarboxylic acid cycle obsolete electron transport glycine metabolic process L-serine metabolic process threonine metabolic process cell redox homeostasis	GO:0004148 GO:0009055	dihydrolipoyl dehydrogenase activity flavin adenine dinucleotide binding		
77	formate C-acetyltransferase	gb AKX86522.1	GO:0006090 GO:0006006 GO:0042967	glucose metabolic process pyruvate metabolic process obsolete acyl-carrier-protein biosynthetic process	GO:0008861	formate C-acetyltransferase activity	GO:0005737	cytoplasm
78	DNA-binding protein HU	gb AKX86822.1	GO:0006412	translation	GO:0003677 GO:0003723	DNA binding RNA binding	GO:0005840	ribosome
79	50S ribosomal protein L21	gb AKX86748.1	GO:0042254	ribosome biogenesis	GO:0003735	structural constituent of ribosome		

80	adenylosuccinate synthase	gb AKX86714.1	GO:0006144	purine nucleobase metabolic process	GO:0004019	adenylosuccinate synthase activity		
			GO:0006164	purine nucleotide biosynthetic process	GO:0005525	GTP binding		
			GO:0006522	alanine metabolic process				
			GO:0006531	aspartate metabolic process				
81	carbamate kinase	gb AKX85849.1	GO:0006144	purine nucleobase metabolic process	GO:0008804	carbamate kinase activity		
			GO:0006525	arginine metabolic process				
			GO:0006560	proline metabolic process cellular amino acid biosynthetic process				
			GO:0008652	phosphorylation				
82	dihydroxyacetone kinase subunit L	gb EMS75618.1	GO:0006071	glycerol metabolic process	GO:0004371	glycerone kinase activity		
			GO:0046486	glycerolipid metabolic process				
83	pyrrolidone-carboxylate peptidase	gb AKX84988.1	GO:0006508	proteolysis	GO:0008234	cysteine-type peptidase activity	GO:0005829	cytosol
84	aldo/keto reductase	gb AKX85646.1	GO:0055114	oxidation-reduction process	GO:0016491	pyroglutamyl-peptidase activity oxidoreductase activity		
85	peptidylprolyl isomerase	gb AKX86427.1			GO:0003755	peptidyl-prolyl cis-trans isomerase activity		
86	aspartate carbamoyltransferase	gb AKX86672.1	GO:0006207	pyrimidine nucleobase biosynthetic process	GO:0004070	aspartate carbamoyltransferase activity	GO:0009347	aspartate carbamoyltransferase complex
			GO:0006522	alanine metabolic process	GO:0016597	amino acid binding		
			GO:0006531	aspartate metabolic process				
87	FMN-binding protein	gb AKX86215.1			GO:0010181	FMN binding protein-N(Pi)-phosphohistidine-sugar phosphotransferase activity	GO:0016020	membrane
88	PTS mannose transporter subunit IIAB	gb AKZ48309.1	GO:0009401	phosphoenolpyruvate-dependent sugar phosphotransferase system	GO:0008982		GO:0009357	protein-N(Pi)-phosphohistidine-sugar phosphotransferase complex
							GO:0016021	integral component of membrane
89	phosphate acetyltransferase	gb EMS77019.1	GO:0008152	metabolic process	GO:0016407	acetyltransferase activity		
			GO:0042967	obsolete acyl-carrier-protein biosynthetic process				

90	N-acetylglucosamine-6-phosphate deacetylase	gb AKX86438.1	GO:0006044	N-acetylglucosamine metabolic process	GO:0008448	N-acetylglucosamine-6-phosphate deacetylase activity		
91	cell division protein FtsH	gb AKX86497.1	GO:0006508	proteolysis	GO:0004222	metalloendopeptidase activity	GO:0016021	integral component of membrane
92	NADPH:quinone reductase	gb AKX85374.1	GO:0055114	oxidation-reduction process	GO:0008270	zinc ion binding		
93	type 1 glutamine amidotransferase	gb AKX85756.1	GO:0006508	proteolysis	GO:0008233	peptidase activity		
			GO:0006541	glutamine metabolic process	GO:0016740	transferase activity		
			GO:0005982	starch metabolic process	GO:0004340	glucokinase activity	GO:0005737	cytoplasm
			GO:0005985	sucrose metabolic process				
94	glucokinase	gb AKX85133.1	GO:0006012	galactose metabolic process				
			GO:0006094	gluconeogenesis				
			GO:0006096	glycolytic process				
			GO:0019872	streptomycin biosynthetic process				
			GO:0051156	glucose 6-phosphate metabolic process				
95	serine hydroxymethyltransferase	gb AKX85521.1	GO:0019264	glycine biosynthetic process from serine	GO:0004372	glycine hydroxymethyltransferase activity		
			GO:0035999	tetrahydrofolate interconversion	GO:0030170	pyridoxal phosphate binding		
96	cold-shock protein	gb AKX85004.1	GO:0006355	regulation of transcription, DNA-templated	GO:0003677	DNA binding		
			GO:0006550	isoleucine catabolic process	GO:0004084	branched-chain-amino-acid transaminase activity		
			GO:0006552	leucine catabolic process				
97	branched-chain amino acid aminotransferase	gb AKX86330.1	GO:0006574	valine catabolic process				
			GO:0009097	isoleucine biosynthetic process				
			GO:0009098	leucine biosynthetic process				
			GO:0009099	valine biosynthetic process				
			GO:0015940	pantothenate biosynthetic process				

98	50S ribosomal protein L6	gb AKX85335.1	GO:0006412	translation	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome
			GO:0042254	ribosome biogenesis	GO:0019843	rRNA binding		
99	alkaline shock response membrane anchor protein AmaP	gb AKX86897.1	GO:0006508	proteolysis	GO:0008233	peptidase activity	GO:0016020 GO:0016021	membrane integral component of membrane
100	acyl carrier protein	gb AKX85795.1	GO:0006633	fatty acid biosynthetic process				
101	transcription elongation factor GreA	gb AKX86608.1	GO:0032784	regulation of DNA-templated transcription, elongation	GO:0003677	DNA binding		
					GO:0070063	RNA polymerase binding		
102	citrate (pro-3S)-lyase subunit beta	gb AKX87092.1	GO:0006084	acetyl-CoA metabolic process	GO:0008816	citryl-CoA lyase activity	GO:0009346	citrate lyase complex
			GO:0006099	tricarboxylic acid cycle				
103	carbamoyl-phosphate synthase large subunit	gb AKX86569.1	GO:0006807	nitrogen compound metabolic process	GO:0005524	ATP binding		
					GO:0046872	metal ion binding		
104	IMP dehydrogenase	gb EOT31306.1	GO:0006144	purine nucleobase metabolic process	GO:0003938	IMP dehydrogenase activity	GO:0042720	mitochondrial inner membrane peptidase complex
			GO:0006164	purine nucleotide biosynthetic process				
			GO:0055114	oxidation-reduction process				
105	peptidase M29	gb AKX86955.1	GO:0006508	proteolysis	GO:0004177	aminopeptidase activity		
106	superoxide dismutase	gb AKX85874.1	GO:0006801	superoxide metabolic process	GO:0004784	superoxide dismutase activity		
			GO:0055114	oxidation-reduction process	GO:0046872	metal ion binding		
107	50S ribosomal protein L31	gb AKX86541.1	GO:0006412	translation	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome
			GO:0042254	ribosome biogenesis				
108	50S ribosomal protein L18	gi 6687509	GO:0006412	translation	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome
			GO:0042254	ribosome biogenesis				
109	UMP kinase	gb AKX85318.1	GO:0006221	pyrimidine nucleotide biosynthetic process	GO:0033862	UMP kinase activity	GO:0005737	cytoplasm
110	Pyruvate oxidase	gb AKX85794.1	GO:0006090	pyruvate metabolic process	GO:0000287	magnesium ion binding		

			GO:0055114	oxidation-reduction process	GO:0030976	thiamine pyrophosphate binding		
					GO:0047112	pyruvate oxidase activity		
111	cysteine synthase A	gb AKX87068.1	GO:0006535	cysteine biosynthetic process from serine	GO:0004124	cysteine synthase activity	GO:0009333	cysteine synthase complex
112	oleate hydratase	gb AKZ47224.1	GO:0006631	fatty acid metabolic process	GO:0050151	oleate hydratase activity		
			GO:0005975	carbohydrate metabolic process	GO:0071949	FAD binding		
113	glycerol kinase	gb AKX86721.1	GO:0006072	glycerol-3-phosphate metabolic process	GO:0004370	glycerol kinase activity		
			GO:0046486	glycerolipid metabolic process				
			GO:0006769	nicotinamide metabolic process	GO:0003952	NAD+ synthase (glutamine-hydrolyzing) activity		
114	NH(3)-dependent NAD(+) synthetase	gb EOT25847.1	GO:0009435	NAD biosynthetic process	GO:0005524	ATP binding		
			GO:0046497	nicotinate nucleotide metabolic process	GO:0008795	NAD+ synthase activity		
115	LPXTG cell wall anchor domain-containing protein	gb AKX86100.1	GO:0006508	proteolysis	GO:0004252	serine-type endopeptidase activity	GO:0005618	cell wall
							GO:0016020	membrane
116	ATP-dependent chaperone ClpB	gb AKX85456.1	GO:0009408	response to heat	GO:0005524	ATP binding	GO:0005737	cytoplasm
			GO:0019538	protein metabolic process				
			GO:0042026	protein refolding				
117	50S ribosomal protein L3	gb EOT31214.1	GO:0006412	translation	GO:0003735	structural constituent of ribosome	GO:0005840	ribosome
			GO:0042254	ribosome biogenesis				

## ARTIGO CIENTÍFICO 3

---

**Comparative proteomic analysis reveals metabolic variability of probiotic *Enterococcus durans* during aerobic and anaerobic cultivation.**

Submetido no periódico Journal of Proteomics em 11 de Dezembro de 2019, fator impacto 4.909.



**Comparative proteomic analysis reveals metabolic variability of  
probiotic *Enterococcus durans* during aerobic and anaerobic cultivation.**

Carolina Baldisserotto Comerlato<sup>1</sup>

Xu Zhang<sup>2</sup>

Krystal Walker<sup>2</sup>

Adriano Brandelli<sup>1\*</sup>

Daniel Figeys<sup>2,3\*</sup>

<sup>1</sup> Laboratório de Bioquímica e Microbiologia Aplicada, Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, 91510-970 Porto Alegre, Brazil

<sup>2</sup> Ottawa Institute of Systems Biology and Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada

<sup>3</sup> Canadian Institute for Advanced Research, Toronto, Canada

\* **Correspondence:** Prof. D. Figeys ([dfigeys@uottawa.ca](mailto:dfigeys@uottawa.ca)); Prof. A. Brandelli ([abrand@ufrgs.br](mailto:abrand@ufrgs.br))

**Abbreviations:**

Arginine deiminase pathway (ADI)

False discovery rate (FDR)

Fructo-oligosacchrides (FOS)

Galacto-oligosaccharides (GOS)

Glutathione (GSH)

Human gastrointestinal tract (GIT)

Label-free quantification (LFQ)

Lactic acid bacteria (LAB)

Synthetic medium (SM)

Variable importance in projection (VIP)

## Abstract

The variation in the bioavailability of oxygen constitutes the environmental conditions found by bacteria in their passage through the host gastro-intestinal tract. Given the importance of oxygen in the defense mechanism of bacteria, it is important to understand how bacteria respond to this stress at a metabolic level. The probiotic strain *Enterococcus durans* LAB18S was cultivated under aerobic and anaerobic conditions using prebiotic oligosaccharides as carbon source. The whole cell proteome and secretome were analyzed through label-free quantitative proteomics approach. The results showed that the LAB18S isolate when grown with fructo-oligosacchrides (FOS) showed a higher number of differentially expressed proteins compared to samples with galacto-oligosaccharides (GOS) or glucose. Clinically important enzymes for the treatment of cancer, L-asparaginase and arginine deiminase, were overexpressed when the isolate was cultured in FOS. In addition, the absence of oxygen induced the strain to produce proteins related to cell multiplication, cell wall integrity and resistance, and H<sub>2</sub>O<sub>2</sub> detoxification. This study showed that *E. durans* LAB18S growing on FOS was stimulated to produce clinically important biomolecules, including proteins that have been investigated as potential antineoplastic agents.

**Significance:** The probiotic strain *E. durans* LAB18S produce clinically relevant enzymes for the treatment of cancer when cultivated in symbiosis with fructo-oligosacchrides (FOS). In addition, proteins associated with cellular multiplication, cell wall integrity and resistance, and H<sub>2</sub>O<sub>2</sub> detoxification were induced under anaerobic growth. These characteristics could be relevant to support maintenance of intestinal health.

**Key words:** *Enterococcus*; prebiotics; probiotics; proteomics

## 1. Introduction

The consumption of probiotics has increased in the last years and consequently more detailed studies regarding their molecular properties are needed. Probiotics are viable microorganisms with beneficial effects on human and animal health<sup>[1]</sup>. *Enterococcus* spp. are lactic acid bacteria (LAB) naturally found in the human gastrointestinal tract (GIT), and several *Enterococcus* strains, including *Enterococcus durans*, have been related as potential probiotics<sup>[2]</sup>. However, some enterococci are associated with resistance to multiple drugs, as well as having the ability to acquire and transfer genes and virulence factors<sup>[3]</sup>. Therefore, the use of these versatile microorganisms as probiotics generates a concern, leading to the need for in-depth studies to distinguish safe strains and to select them as efficient probiotics.

Enterococci are facultative anaerobes and can produce different stress proteins in response to oxygen<sup>[4]</sup>. Many of these proteins are enzymes such as catalase, NADH peroxidase, NADH oxidase, superoxide dismutase and glutathione reductase<sup>[5]</sup>. The understanding of *Enterococcus* response to oxidative stress can provide important information for survival in the host environment, a desirable characteristic for probiotic isolates. In this regard, several *Enterococcus* spp. from food origin shown antioxidant activity associated with both cellular extracts and culture supernatants<sup>[6]</sup>.

Non-digestible prebiotic ingredients in combination with probiotic bacteria promote a beneficial synergistic effect on the host. Currently, fructooligosaccharides (FOS) (and inulin), galactooligosaccharides (GOS) and lactulose are the most used and accepted prebiotics by the European Union. These carbohydrates have been extensively studied and demonstrate that symbiosis with probiotic strains generates effective results in some illnesses such as inflammatory bowel disease, irritable bowel syndrome, possible inhibitory

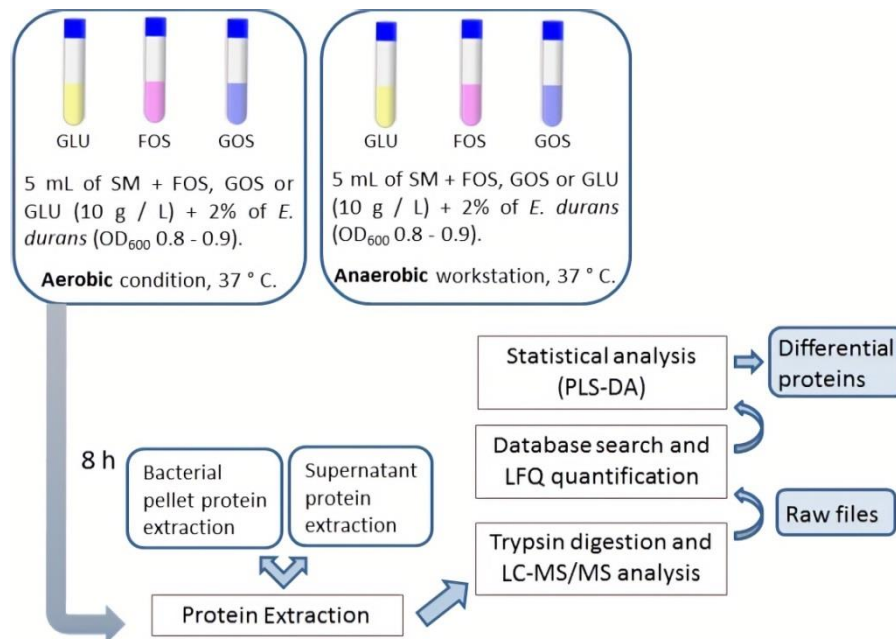
mechanisms in colon carcinogenesis<sup>[7, 8, 9]</sup>. Modulation of gene expression by prebiotics has been investigated for *Bifidobacterium* species through transcriptome approach<sup>[10]</sup> and proteomic analysis<sup>[11]</sup>. Such -omic strategies have been widely used in order to obtain molecular basis of aerobic adaptation and/or respiration conditions of probiotics<sup>[12]</sup>. However, their applications on *Enterococcus* spp. are scarce and have been focused on strains of clinical interest<sup>[13]</sup>.

*Enterococcus durans* LAB18S was previously characterized as a strain with probiotic properties, including non-hemolytic activity<sup>[14]</sup> and absence of virulence factors and resistance genes<sup>[15]</sup> as criteria considered by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) as safe aspects for selection of probiotic isolates<sup>[16]</sup>. It was isolated from a Brazilian soft cheese, belonging to the collection of the Laboratory of Applied Microbiology and Biochemistry (Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil). In a preliminary study it was observed that LAB18S showed a differential proteomic profile when growing on FOS as carbon source (unpublished results). Thus, in this study, *E. durans* LAB18S was cultivated on prebiotic oligosaccharides FOS and GOS in presence or absence of oxygen. The objective was to evaluate the differential expression of proteins in particular those linked to a potential probiotic effect in symbiosis with carbon sources considered as prebiotic and the influence of oxygen in protein expression.

## 2. Experimental Section

### 2.1. Growth of probiotic bacteria

*E. durans* was cultured under aerobic conditions in Luria-Bertani (LB) broth for 24 h at 37 °C to cell reactivation. In order to minimize the detection of proteins from the culture media in mass spectrometry analysis the probiotic isolate was grown in a synthetic medium (SM)<sup>[17]</sup>. The prebiotic substrates (FOS and GOS) and glucose (used as control) were added to SM to reach a final concentration of 10 g L<sup>-1</sup> and *E. durans* cells from 24 h LB culture were inoculated (2%, v/v) with an initial O.D. at 600 nm between 0.8 and 0.9. The incubation was performed at 37 °C for 8 h (mid-log phase) under aerobic or anaerobic conditions until the protein extraction were performed and three samples were analyzed for each experimental group. The experimental workflow is depicted in Figure 1.



**Figure 1.** Experimental workflow. FOS, GOS and GLU were added to SM with the probiotic bacteria, *E. durans*. The growth was performed under aerobic and anaerobic conditions, respectively. The proteins were extracted from bacterial pellet and supernatant followed by proteomic analysis. Partial least squares-discriminant analysis (PLS-DA) was used to identify differentially abundant proteins in response to each factor.

## 2.2. Protein extraction, trypsin digestion and LC-MS/MS analysis

Proteins were extracted from the bacterial pellets and culture supernatants (secretome) of both aerobic and anaerobic culture samples. Protein extraction from bacterial pellets<sup>[18]</sup> and from culture supernatant<sup>[19]</sup> were performed for a total of 36 samples. Briefly, the secretome was extracted from the supernatant of the culture samples that were centrifuged at 14,000 *g* for 20 min at 4 °C. Supernatants were carefully collected and filtered through 0.22 μm membranes into new tubes. The precipitation was performed using 20% (w/v) trichloroacetic acid (TCA) and centrifuged at 16,000 *g* and 4 °C for 20 min. Next, the supernatants were discarded and the pellets were washed twice with ice-cold acetone. The bacterial pellets were lysed in 200 μL of lysis buffer (8 M urea, 4% sodium dodecyl sulfate in 50 mM Tris-HCl buffer at pH 8.0 and Roche cOmplete Mini tablet were added for every 10 mL of lysis buffer) using three cycles of ultrasonication (30 s each with 1 min interval on ice) with a Q125 Sonicator (Qsonica, LLC) and an amplitude of 25%. The protein lysates were precipitated with acetic acid, acetone and ethanol buffer at -20 °C overnight. The pellets were washed three times with ice-cold acetone and centrifuged at 16,000 *g* for 25 min.

For trypsin digestion, the precipitated proteins were dissolved in 50 mM ammonium bicarbonate (pH 8) containing 6 M urea. Trypsin digestion and desalting was performed following the procedures<sup>[16]</sup>. Tryptic peptides were dissolved in 0.1% formic acid, and 4 μg of protein was loaded for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis with an Agilent 1100 Capillary LC system (Agilent Technologies, San Jose, CA) and a Q Exactive mass spectrometer (Thermo Electron, Waltham, MA).

### 2.3. Proteomics data processing

Peptides were identified and quantified using MaxQuant version 1.6.3.4 in a single run against the *Enterococcus durans* Uniprot database (UP000014199; downloaded 2019/04/11). Label-free quantification (LFQ) intensity of each protein group was log10 transformed and filtered with the criteria that the protein should be identified by  $\geq 1$  unique peptides in  $\geq 50\%$  of the samples (Q50). The output was then uploaded into MetaboAnalyst (version 4.0, <http://www.metaboanalyst.ca/>) for further analysis. The protein groups were analyzed by hierarchical clustering and principal component analysis (PCA). Missing values were estimated with the KNN algorithm.

Partial least-squares discriminant analyses (PLS-DA) was performed in MetaboAnalyst for discriminating proteins differentially abundant in response to each prebiotic substrate and between aerobic and anaerobic growth. Cross-validation with  $R^2$  and  $Q^2$  were used to evaluate the performance of the PLS-DA models. Identification of the differential proteins in response to each oligosaccharide and growth condition was achieved using the variable importance in projection (VIP); a VIP score higher than one was considered as an important feature for group discrimination. LFQ intensities of the proteins with  $\geq 1.0$  VIPs in all PLS-DA models were combined and used for hierarchical clustering analysis. Each cluster represents a group of proteins with a similar expression pattern in response to different substrates and growth conditions.



## 2.4. Statistical analysis

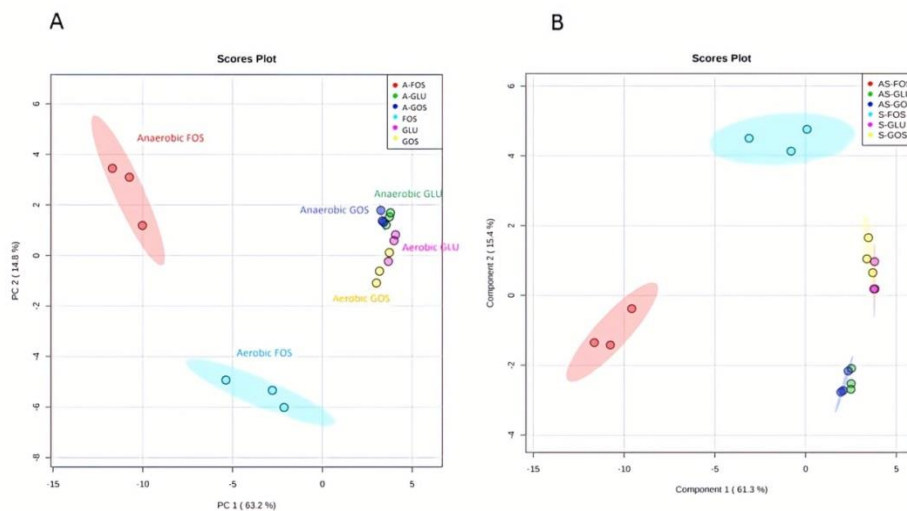
The statistical comparisons among the 6 groups (GLU, FOS and GOS, under aerobic or anaerobic conditions) were conducted by one-way analysis of variance (ANOVA) in MetaboAnalyst v. 4.0. The  $p < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Differentially cellular protein expressions in response to different carbon sources

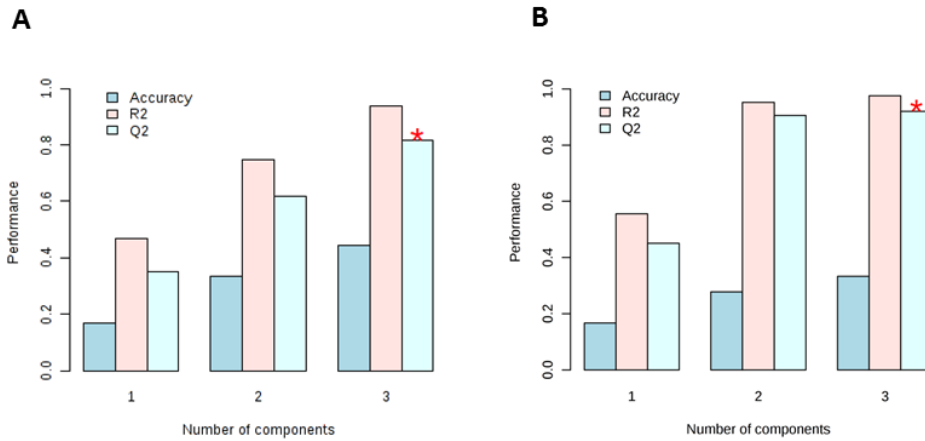
*E. durans* LAB18S was cultivated on prebiotic oligosaccharides under aerobic and anaerobic conditions and the protein extracts obtained from cell pellets (proteome) were subjected to proteomic analysis. The evaluation of bacterial proteome identified a total of 11,394 peptides and 1,192 protein groups with a false discovery rate (FDR) threshold of 1%. To obtain an accurate assessment of the effects of oligosaccharides and oxygen, data filtering criteria were used to identify 1,192 protein groups present in more than 50% of the samples.

PCA using the log transformed LFQ intensity of the protein groups showed the effect of prebiotics and oxygen on the *E. durans* proteome (Figure 2A). The probiotic bacteria growing with FOS were clearly separated from GOS and GLU groups under both aerobic and anaerobic conditions. These results indicate that the use of FOS had a distinct effect on the functional activities of *E. durans* during *in vitro* cultivation. In addition, it was possible to observe a separation of the GOS groups regarding the influence of oxygen in cultures grown under either aerobiosis or anaerobiosis.

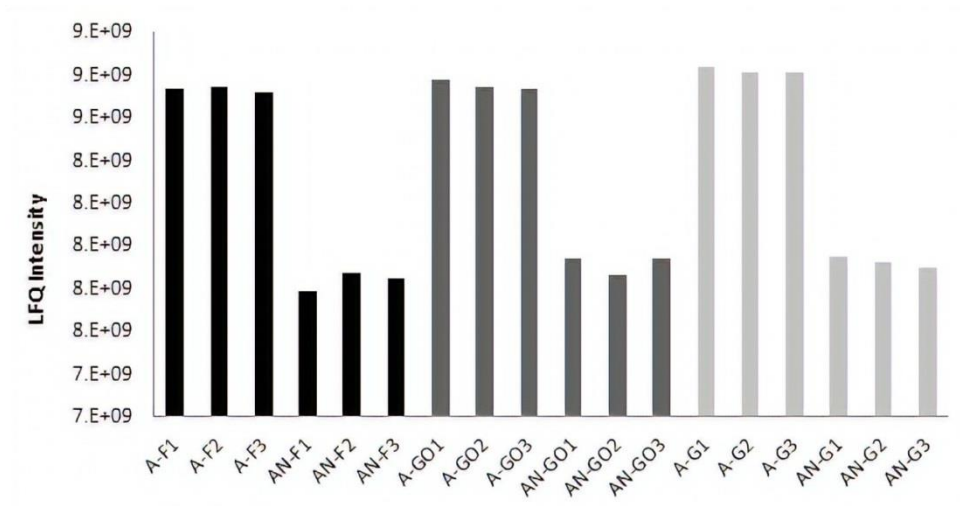


**Figure 2. A.** Principal Component Analysis (PCA) and **B.** Partial Least Squares - Discriminant Analysis (PLS-DA) in bacterial pellet samples based on the LFQ intensities of the protein groups showed tendencies of protein changes with the use of different carbon sources and the presence or absence of oxygen.

PLS-DA (Figure 2B) was used to identify differentially expressed proteins related with growth on different carbon sources (FOS, GOS and GLU) in the presence or absence of oxygen. Thus, 187 differentially abundant proteins were found in the proteome with the VIP >1.0 in the first component of PLS-DA (supplementary Table S1). Cross-validation showed high performance for PLS-DA models ( $R^2 > 0.93$ ,  $Q^2 > 0.81$ ; supplementary Figure S1A). Through the ANOVA test, the protein with the lowest  $P$  value in the whole cell proteome was NADH peroxidase ( $P = 1.0215 \times 10^{-14}$ ). NADH peroxidase was the most differentially expressed protein in pellet samples with FOS in both presence and absence of oxygen. Anaerobic conditions showed a decrease in the amount of this enzyme when compared with oxygen treatments (Figure 3). However, NADH peroxidase was not detected in protein samples secreted by *E. durans* under anaerobic conditions, but in the presence of oxygen, this enzyme appears to be secreted by the cell. In addition, the NADH oxidase enzyme was only stimulated in the presence of oxygen ( $P < 0.05$ ).

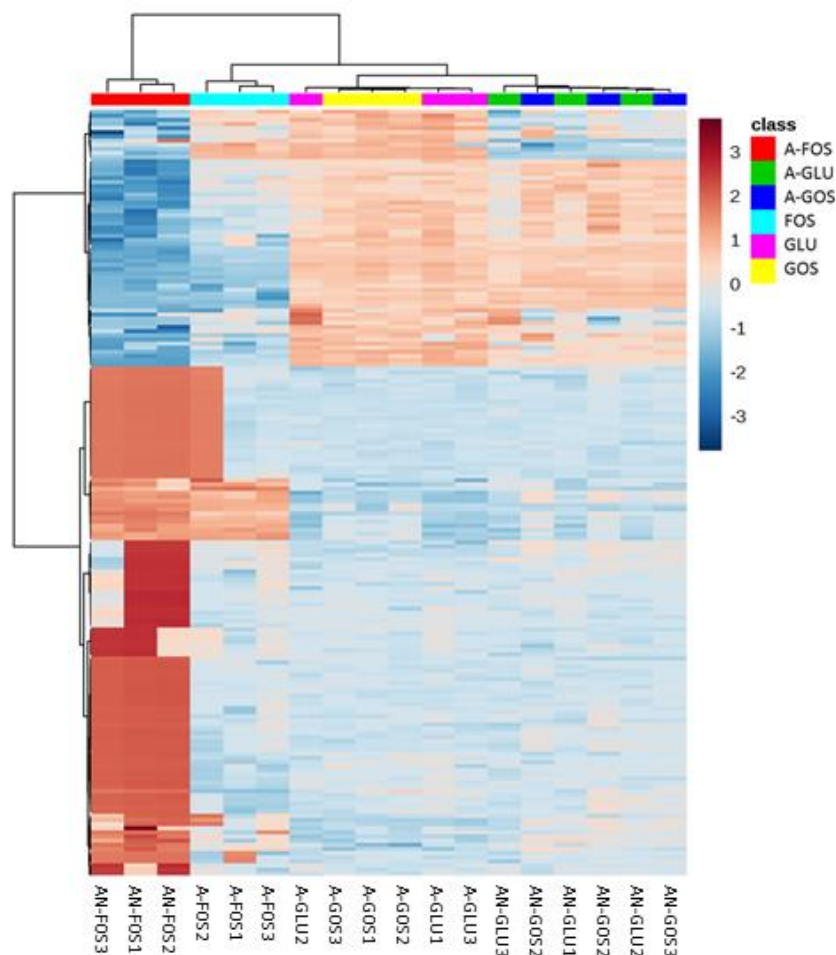


**Figure S1.** Cross validation with  $R^2$  and  $Q^2$  were used to evaluate the performance of the PLS-DA models in *E. durans* LAB 18S proteome (A) and secretome (B).



**Figure 3.** Variation of LFQ Intensity of NADH peroxidase enzyme in cellular protein expression according to the different cultivation conditions: aerobic FOS (F1, F2 and F3), anaerobic FOS (F1A, F2A and F3A), aerobic GOS (GO1, GO2 and GO3), anaerobic GOS (GO1A, GO2A and GO3A), aerobic glucose (G1, G2 and G3) and anaerobic glucose (G1A, G2A and G3A).

Hierarchical clustering analysis revealed distinct proteomic patterns in response to different oligosaccharide treatments (supplementary Figure S2). There are 187 differentially expressed proteins from the total protein sample represented in Figure S2. FOS showed the greatest effect on the proteome, especially under anaerobic conditions. The presence of FOS under anaerobiosis stimulated the expression of cellular components related to protein translation, proteins related to carbohydrate and nucleotide metabolism, and others.

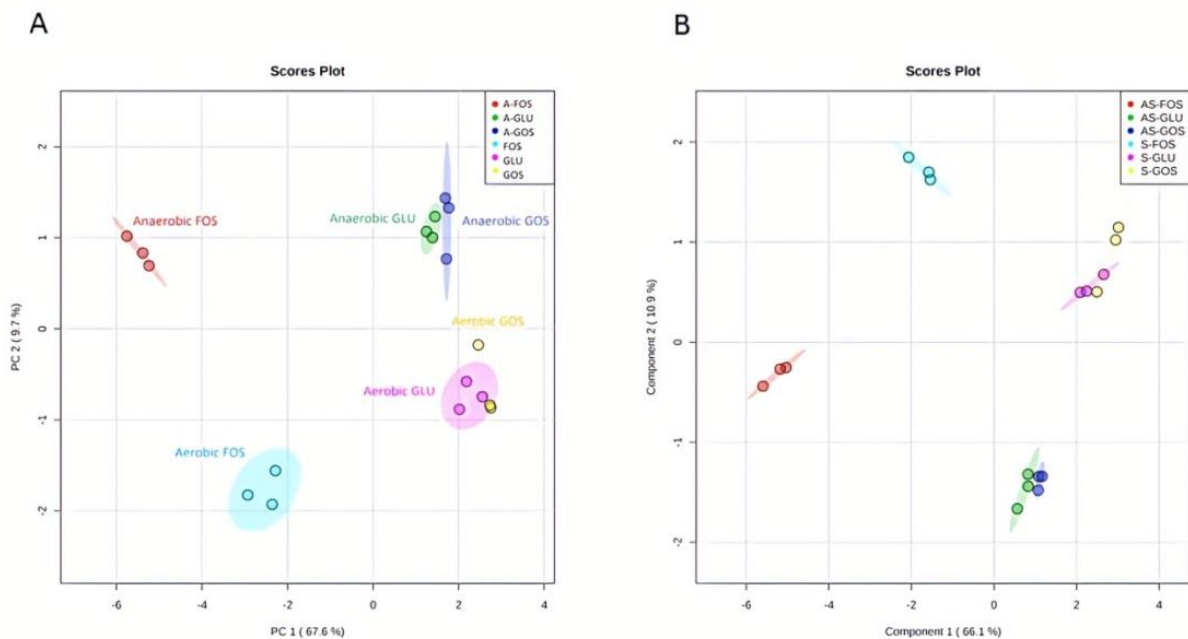


**Figure S2.** Hierarchical clustering of *E. durans* LAB18S secretome concentration values, with samples in rows and proteins in columns.

### 3.2. Differential protein secretion in response to carbon sources

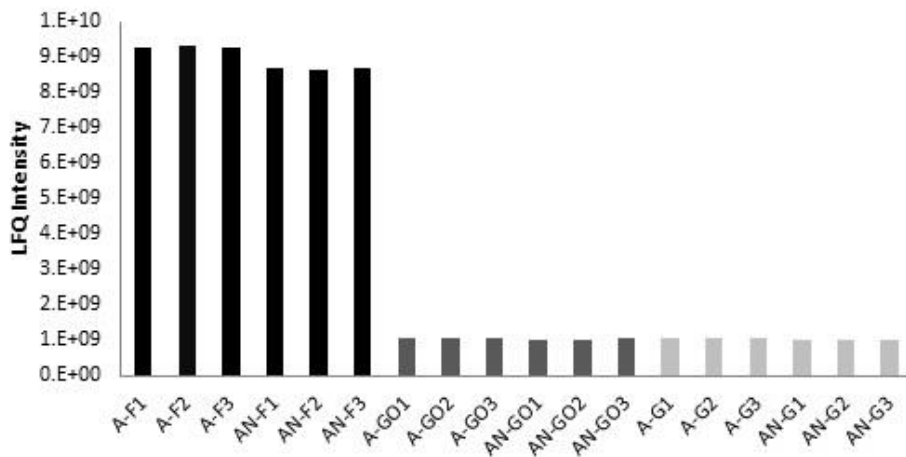
*E. durans* LAB18S was grown under the same conditions as in whole cell proteome analysis described above and the culture supernatants (secretome) were subjected to proteomic analysis. In secretome samples, 4,122 peptides and 295 protein groups were identified with a FDR) threshold of 1%.

PCA showed the effect of prebiotics and oxygen on the *E. durans* secretome (Figure 4A). The results presented by the secretome under these conditions were similar to the proteome results, indicating that the proteins secreted by *E. durans* LAB18S may be a close reflection of intracellular proteins.



**Figure 4.** **A.** Principal Component Analysis (PCA) and **B.** Partial Least Squares - Discriminant Analysis (PLS-DA) in secretome samples based on the LFQ intensities of the protein groups with the use of different carbon sources and the presence or absence of oxygen.

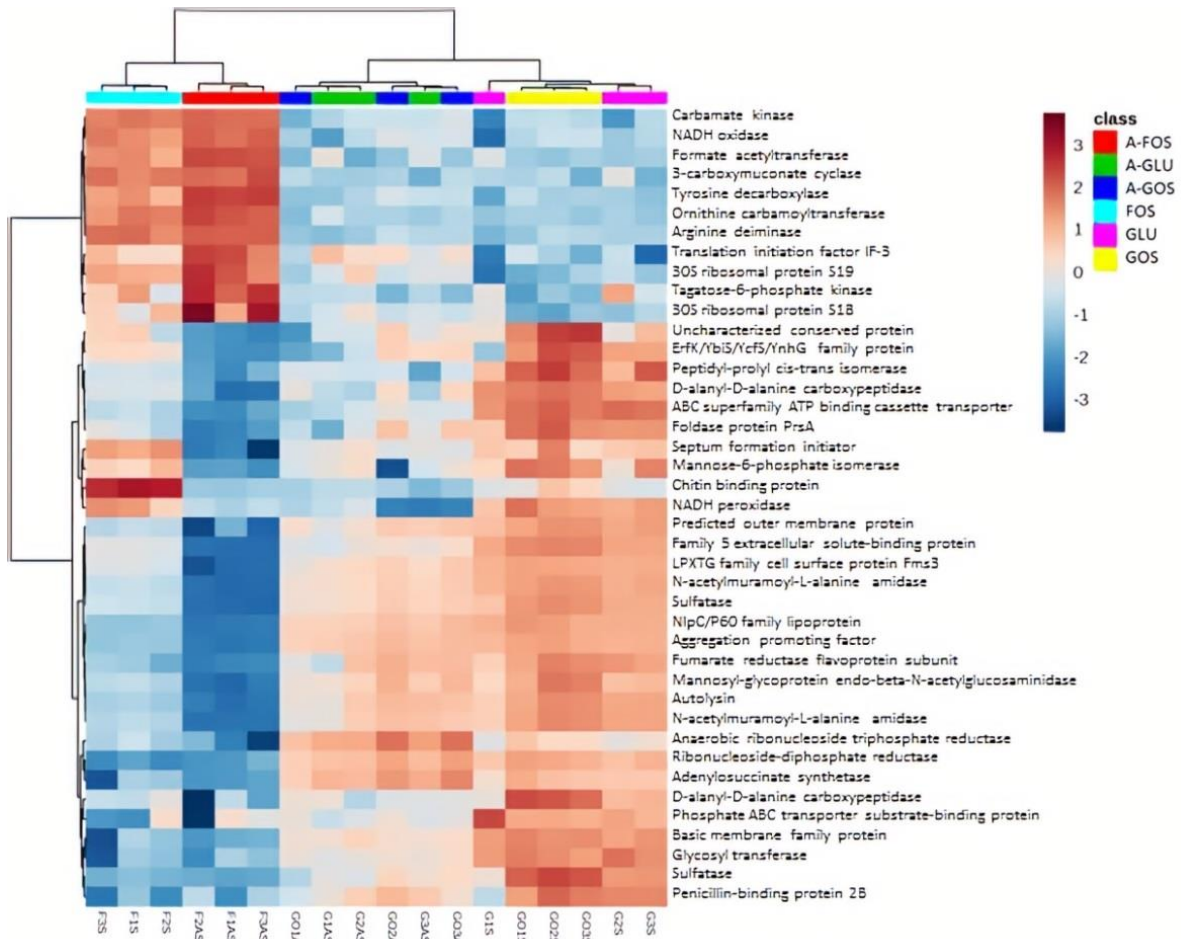
PLS-DA (Figure 4B) of the secretome samples showed 41 differentially abundant proteins with the VIP >1.0 in the first component of PLS-DA (Supplementary Table S2). Cross-validation showed high performance for PLS-DA models ( $R^2 >0.97$  and  $Q^2 >0.92$ ; supplementary Figure S1B). Using ANOVA test, the protein with the lowest  $P$  value in the whole secretome was NlpC/P60 family lipoprotein, which was significantly increased in groups symbiosis with FOS ( $P = 1.4222 \text{ E}^{-13}$ ; Figure 5).



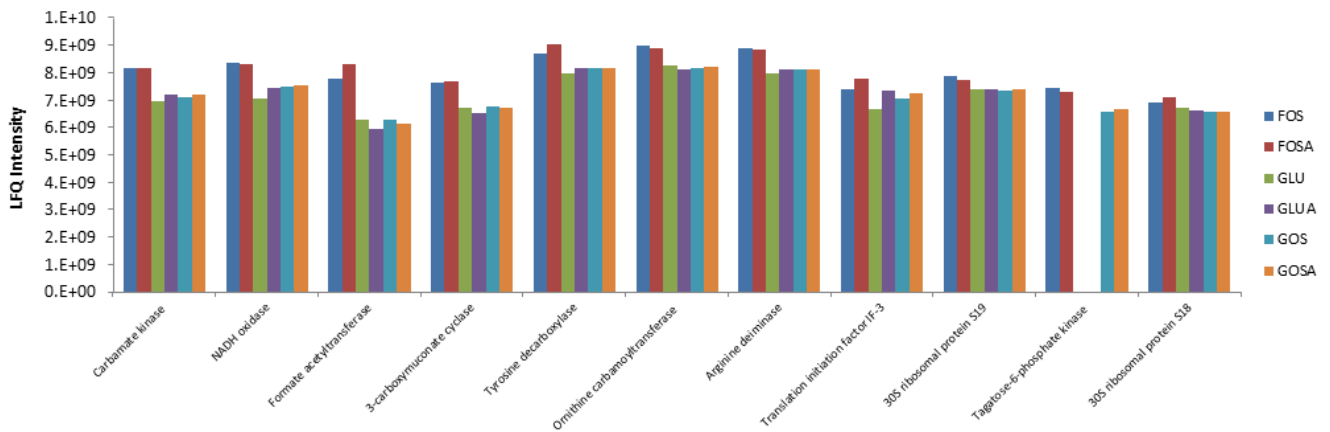
**Figure 5.** Variation of LFQ Intensity of NlpC/P60 family lipoprotein in secretome expression according to the different cultivation conditions: aerobic FOS (F1, F2 and F3), anaerobic FOS (F1A, F2A and F3A), aerobic GOS (GO1, GO2 and GO3), anaerobic GOS (GO1A, GO2A and GO3A), aerobic glucose (G1, G2 and G3) and anaerobic glucose (G1A, G2A and G3A).

Hierarchical clustering analysis revealed 42 differential proteins from the secretome samples presented in Figure 6. Relative to the secretome, FOS had a different effect on the proteome when compared with glucose and GOS treatments. In the first clusters, FOS showed a greater effect on the proteome in both aerobic and anaerobic conditions (supplementary Figure S3). In addition, the effect of FOS on expression of four proteins, namely septum formation initiator, mannose-6-phosphate isomerase, chitin binding protein

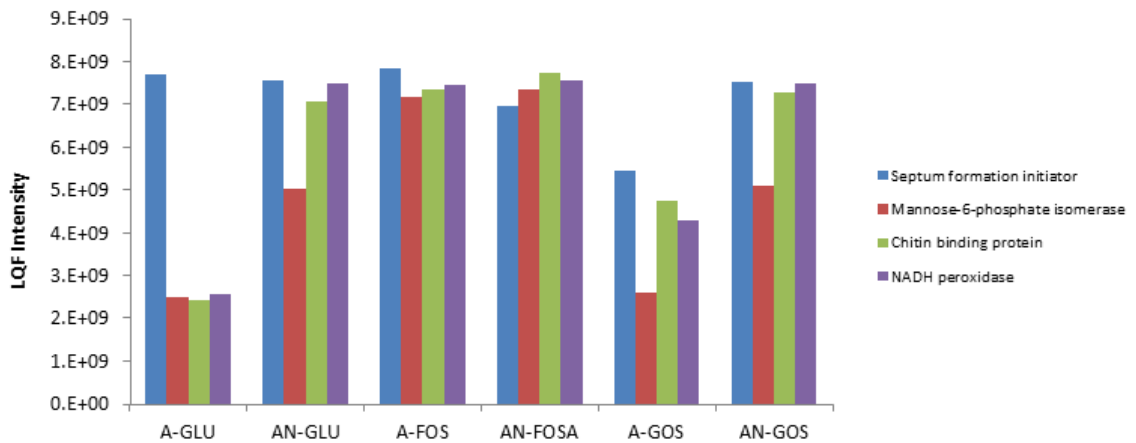
and NADH peroxidase, was higher under aerobic as compared to the anaerobic conditions (supplementary Figure S4).



**Figure 6.** Hierarchical clustering of the 42 differentially abundant secreted proteins of *E. durans* LAB18S, with samples in rows and proteins in columns.



**Figure S3.** Overexpressed proteins of *E. durans* LAB18S secretome related to FOS utilization.

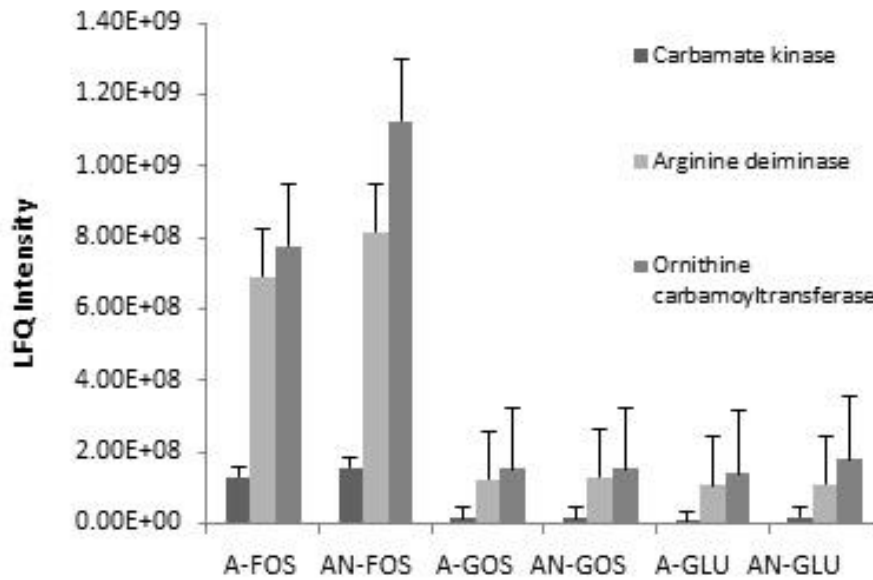


**Figure S4.** Overexpressed proteins of *E. durans* LAB18S secretome related to the presence of oxygen.

Some proteins deserve more attention due to their relevance for bacterial survival, production of bioactive molecules, and desirable characteristics of probiotic samples (Table 1). Enzymes of therapeutic importance, such as L-asparaginase and arginine deiminase, were upregulated in FOS samples. L-asparaginase was detected in total proteins sample under anaerobiosis, while the secretome with either presence or absence of oxygen showed greater abundance of arginine deiminase. There was an increase in the arginine deiminase pathway with FOS utilization, in which the proteins carbamate kinase, arginine deiminase and ornithine carbamoyltransferase are associated (Figure 7). In addition, proteins related to cell



wall resistance were upregulated under aerobic conditions in GOS and glucose samples. The effect of glucose and GOS on the proteome was similar in different samples and an increase of proteins associated to the glycolytic pathway was observed as well.



**Figure 7.** Proteins related to the arginine deiminase pathway and their expression in FOS, GOS and glucose (GLU).

**Table 1.** Differentially expressed proteins in secretome of *E. durans* LAB 18S related to probiotic characteristics in FOS, GOS and Glucose treatments.

Protein description	Gene symbol	<i>p</i> -value	Biological Process	Expression						Reference
				A-FOS	AN-FOS	A-GOS	AN-GOS	A-GLU	AN-GLU	
Chitin-binding protein	<i>gbpA_3</i>	3.12 E <sup>-05</sup>	mucin adhesion	HIGH	LOW	LOW	LOW	LOW	LOW	[20]
NADH oxidase	<i>nox_3</i>	4.20 E <sup>-05</sup>	oxidative resistance	HIGH	HIGH	LOW	LOW	LOW	LOW	[21]
NADH peroxidase	<i>npr_2</i>	1.73 E <sup>-04</sup>	oxidative resistance	HIGH	LOW	HIGH	LOW	HIGH	LOW	[22]
NlpC/P60 family lipoprotein	NCTC8129_008 18	1.42 E <sup>-09</sup>	cell wall maintenance and survival	LOW	LOW	HIGH	HIGH	HIGH	HIGH	[19]
Arginine deiminase	<i>arcA</i>	2.30 E <sup>-05</sup>	potential anticancer agent	HIGH	HIGH	LOW	LOW	LOW	LOW	[23]
ErfK/YbiS/YcfS/YnhG family protein	<i>ErfK/YbiS/YcfS/Y nhG</i>	0.003824	cell wall biosynthesis	HIGH	LOW	HIGH	LOW	HIGH	LOW	[24]
D-alanyl-D-alanine carboxypeptidase	<i>dacC</i>	3.65 E <sup>-05</sup>	cell wall integrity	LOW	LOW	HIGH	LOW	HIGH	LOW	[25]

#### 4. Discussion

The cellular proteome and secretome of *E. durans* LAB18S cultivated on prebiotic oligosaccharides FOS and GOS under aerobic and anaerobic conditions were investigated. This study shows that *E. durans* was able to ferment both prebiotics (FOS and GOS) and FOS was an effective prebiotic in the stimulation of the fermentative capacity of this isolate under anaerobic conditions. The main structural difference between milk-derived prebiotic GOS and vegetable origin FOS is the presence of residues of galactose and fructose, respectively. Thus, differences between treatments could be expected, since the chemical structure of FOS (ketose units) differs from GOS and GLU that resemble each other (aldose units), and bacteria must contain specific transporters and enzymes that allow the metabolism of these carbohydrates. Fructose uptake in enterococci has been associated to a fructose-specific phosphotransferase system (PTS) encoded in the fructose operon and more than one mannose/fructose/sorbose PTS<sup>[26]</sup>. In contrast, the presence of specific galactose transporters in *Enterococcus* is unclear. Evidence from some *Lactococcus* strains indicate two galactose-specific systems, a permease and a PTS, even if they lack the lactose utilization plasmid, suggesting that a lactose-independent galactose PTS exists. Although the PTS galactose transporter was never identified, the identities of two low-affinity galactose PTS were recently revealed in *L. lactis* MG1363 through transcriptome analyses and knock-out mutants<sup>[27]</sup>.

Many proteins were differentially expressed by *E. durans* under anaerobiosis, in particular those related to cell division and H<sub>2</sub>O<sub>2</sub> detoxification. Interestingly, the effect of prebiotics on *Lactobacillus rhamnosus* protein profile showed that cell damage caused by the extraction procedure was avoided by more than 80% when the bacterium was grown in

FOS<sup>[28]</sup>. The chemopreventive effects of yacon roots, an abundant source of FOS in colon cancer of male rats has been reported. A reduction in cell proliferation, number and quantity of pre-neoplastic lesions and invasive adenocarcinomas was observed in the group that received 1% yacon powder<sup>[29]</sup>. Therefore, FOS may have the ability to modulate the human intestinal microbiome, increase uptake of glucose in peripheral tissues, stimulate insulin secretion in the pancreas, and modulate cell pathways related to lipid homeostasis<sup>[30]</sup>.

Probiotic microorganisms have many health benefits, including the scavenging of reactive oxygen species (ROS) and repression of oxidative stress in the host<sup>[31, 32]</sup>. *Enterococcus* spp. produce different stress proteins depending on growth conditions. Many of these proteins are induced in response to oxygen, such as catalase, NADH peroxidase, NADH oxidase, superoxide dismutase and glutathione (GSH) reductase<sup>[5]</sup>. In this study, NADH peroxidase was the most differentially expressed protein in pellet samples with FOS in both presence and absence of oxygen. In addition, the expression of NADH oxidase was only stimulated in the presence of oxygen. Oxidative stress can provide information on the survival of *E. durans* in the host. The most conserved mechanism of oxidative resistance in LAB results from the functions of NADH oxidase and NADH peroxidase<sup>[22]</sup>. Oxygen is the main electron acceptor of NADH oxidase. Oxidation of NADH to NAD<sup>+</sup> via NADH oxidase produces H<sub>2</sub>O<sub>2</sub>, which is reduced to water by NADH peroxidase. NADH oxidase may have alternative electron acceptors under anaerobic conditions, as demonstrated for the H<sub>2</sub>O<sub>2</sub>-forming NADH oxidase of *Thermus thermophilus*, which is capable of using several other electron acceptors<sup>[33]</sup>. In a previous study <sup>[34]</sup> *Lactobacillus* strains were investigated in relation to their antioxidant capacity with supplementation with different prebiotics and found higher levels of activity in the sample supplemented with FOS.

The most abundant protein identified in *E. durans* supernatant was a peptidase from NlpC/P60 family. The members of this family are able to hydrolyze the D- $\gamma$ -glutamyl-meso-diaminopimelate linkage in peptidoglycan<sup>[35, 36]</sup>. Inactivation of these peptidases in Gram-positive bacteria resulted in defective cell division. In a previous study<sup>[19]</sup> was demonstrated the mechanisms of protection of intestinal bacteria through infection of the *Caenorhabditis elegans* model with *Salmonella* Typhimurium. *E. faecium* NlpC/p60 was the most expressed protein in the supernatant and was sufficient to protect *C. elegans* and mice from enteric pathogens. The mechanism of action consists of the NlpC/p60 hydrolase activity that forms peptidoglycan fragments that activate the host immune system by increasing the integrity of the epithelial barrier and imprisoning the pathogens in the intestinal lumen, promoting tolerance to infection. In this study, we can observe that the NlpC/p60 protein is present in abundance only in the supernatant, and that both GOS and glucose induced the production of this protein in a more pronounced way.

In this study, the action of FOS seems to have affected the expression of both total proteins and proteins secreted by *E. durans*. However, two proteins of biological importance in cancer treatments were observed in these samples, arginine deiminase (*arcA* gene) and L-asparaginase (*ansA* gene). L-asparaginase is an enzyme that show efficacy in treatments of some types of leukemia and lymphomas<sup>[37]</sup>. One-third of the global requirements for the treatment of leukemia and anti-lymphoma agents are attributed to this enzyme<sup>[38]</sup>. Tumor cells require large amounts of the amino acid asparagine, more specifically lymphatic tumor cells. Intravenous administration of L-asparaginase depletes extracellular asparagine by limiting rapid growth and even killing tumor cells, while normal cells are able to produce all the asparagine they need internally<sup>[39]</sup>. This enzyme is produced in fungi, plants and bacteria and is commercially prepared from bacterial sources, such as *Escherichia coli* and *Erwinia*

*caratovora*. The production of asparaginases has been described in diverse bacterial species, such as *Bacillus licheniformis*<sup>[40]</sup>, *Pseudomonas fluorescens*<sup>[41]</sup>, and *Lactobacillus reuteri*<sup>[42]</sup>.

Arginine deiminase is an important arginine-degrading enzyme that converts peptidyl arginine to peptidyl citrulline through a reaction called ‘citrullination’. Protein citrullination mediated by these enzymes modulates the function or interactions of target proteins thus regulating cellular processes<sup>[43]</sup>. Our results showed that this enzyme was differentially expressed only in the secretome of *E. durans* cultivated on FOS, regardless of the presence or absence of oxygen. One of the major physiological functions of the arginine deiminase pathway (ADI) in bacteria seems to be related with the supply of ATP under anaerobic conditions. The ADI pathway is induced by arginine in *E. faecalis* isolates, regardless of the aeration state<sup>[44]</sup>. The *ansA* and *arcA* genes were found in three probiotic lactobacilli isolated from human infant feces<sup>[45]</sup>. ADI has been investigated as a potential anticancer agent and associated as an inhibitor of cell proliferation in some tumors such as advanced melanoma<sup>[20]</sup>, small cell lung cancer<sup>[46]</sup> and colorectal cancer (CRC)<sup>[8]</sup>.

CRC is the third most diagnosed type of cancer and one of the leading causes of death in the Western world. Although the family history of colorectal cancer is an important factor, most of them (around 80%) occur sporadically and are associated with epigenetic factors, such as lifestyle and diet<sup>[47, 48]</sup>. A recent study showed that CRC cell lines did not have the ability to proliferate in the absence of arginine and the growth of these cells *in vivo* was decreased by administration of an arginine-free diet<sup>[49]</sup>. In addition, their results indicated a greater sensitivity of CRC cells using the treatment with this enzyme. In another study, arginine deiminases are downregulated both in tumors and in colon cancer cell lines. Overexpression of ADI disrupted the G1 phase cell division cycle in conjunction with increased citrullination in CRC cells<sup>[8]</sup>.

Among the oligosaccharides tested, the isolate *E. durans* LAB18S growing on FOS showed the most modulation to the bacterial proteome as well as secretome as compared to GOS and glucose. In this study, we also showed that the cultivation under anaerobiosis produced more proteins related to cell multiplication, cell wall integrity and resistance and H<sub>2</sub>O<sub>2</sub> detoxification, desirable characteristics for a probiotic strain. In addition, two enzymes of clinical importance for the treatment of cancer, L-asparaginase and arginine deiminase, were overexpressed when the strain was cultivated in FOS. Thus, this study further demonstrated that *E. durans* LAB18S in symbiosis with FOS was stimulated to produce biomolecules of clinical importance, including proteins that has been investigated as a potential anticancer agents.

## **Acknowledgments**

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) [grant 306936/2017-8] and the Emerging Leaders in the Americas Program (ELAP, Canada).

## **Conflicts of interest**

Authors declare no conflicts of interest.

## **5. References**

- [1] N. I. Agudelo-Higuita, M. M. Huycke, Enterococci: From Commensals to Leading Causes of Drug Resistant Infection, Vol. 1 (Eds: M. S. Gilmore, D. B. Clewell, Y. Ike, N. Shankar), Massachusetts Eye and Ear Infirmary, Boston, USA **2014**, Ch. 2.
- [2] H. Hanchi, W. Mottawea, K. Sebei, R. Hammami, *Front. Microbiol.* 2018, **9**, 1791.

- [3] C. M. A. P. Franz, A. B. Muscholl-Silberhorn, N. M. K. Yousif, M. Vancanneyt, J. Swings, W. H. Holzapfel, *Appl. Environ. Microbiol.* 2001, *67*, 4385.
- [4] K. Fisher, C. Phillips, *Microbiology* 2009, *155*, 1749.
- [5] C. A. Portela, K. F. Smart, S. Tumanov, G. M. Cook, S. G. Villas-Bôas, *J. Bacteriol.* 2014, *196*, 2012.
- [6] S. Pieniz, R. Andrezza, B. C. Okeke, F. A. C. Oliveira, A. Brandelli, *Braz. J. Biol.* 2015, *75*, 923.
- [7] S. Kolida, G. R. Gibson, *Annu. Rev. Food Sci. Technol.* 2011, *2*, 373.
- [8] R. Funayama, H. Taniguchi, M. Mizuma, F. Fujishima, M. Kobayashi, S. Ohnuma, M. Unno, K. Nakayama, *Cancer Sci.* 2017, *108*, 713.
- [9] D. Liang, N. Longgui, X. Guoqiang, *Med. J.* 2019, *98*, 16068.
- [10] L. M. Andersen, R. Barrangou, M. A. Hachen, S. J. Lahtinen, Y. J. Goh, B. Svensson, T. R. Klaenhammer, *BMC Genomics* **2013**, *14*, 312.
- [11] J. Zhao, P. C. K. Cheung, *J. Agric. Food Chem.* **2013**, *61*, 4360.
- [12] S. Boronat, A. Domènech, E. Hidalgo, *Antioxid. Redox Signal.* **2017**, *26*, 329.
- [13] T. Wagner, B. Joshi, J. Janice, F. Askarian, N. Skalko-Basnet, O. C. Hagestad, A. Mekhlif, S. N. Wai, K. Hegstad, M. Johannensen, *J. Proteomics* 2018, *187*, 28.
- [14] S. Pieniz, R. Andrezza, T. Anghinoni, F. Camargo, A. Brandelli, *Food Control* 2014, *37*, 251.
- [15] S. Pieniz, T. M. Moura, A. P. Cassenego, R. Andrezza, A. P. Frazzon, F. A. O. Oliveira, A. Brandelli, *Food Control* 2015, *51*, 49.
- [16] D. Zielinska, D. Kolozyn-Krajewska, *Biomed Res. Int.* 2018, *2018*, 5063185.
- [17] M. Rossi, C. Corradini, A. Amaretti, M. Nicolini, A. Pompei, S. Zanoni, D. Matteuzzi, *Appl. Environ. Microbiol.* **2005**, *71*, 6150.
- [18] X. Zhang, Z. Ning, J. Mayne, J. I. Moore, J. Li, J. Butcher, S. A. Deeke, R. Chen, C. K. Chiang, M. Wen, D. Mack, A. Stintzi, D. Figeys, *Microbiome* **2016**, *4*, 31.
- [19] S. A. Deeke, A. E. Starr, Z. Ning, S. Ahmadi, X. Zhang, J. Mayne, C. K. Chiang, R. Singleton, E. I. Benchimol, D. R. Mack, A. Stintzi, D. Figeys, *Am. J. Gastroenterol.* 2018, *113*, 713.
- [20] B. Sanchez, C. Gonzalez-Tejedo, P. Ruas-Madiedo, M. C. Urdaci, A. Margolles, *Appl. Environ. Microbiol.* **2011**, *77*, 1123.
- [21] X. C. Shi, Y. N. Zou, Y. Chen, C. Zheng, B. B. Li, J. H. Xu, X. N. Shen, H. J. Ying, *Biotechnol. Biofuels* 2016, *9*, 103.
- [22] K. J. Rangan, V. A. Pedicord, Y. C. Wang, B. Kim, Y. Lu, S. Shaham, D. Mucida, H. C. Hang, *Science* **2016**, *353*, 1434.
- [23] P. A. Ott, F. S. Hodi, C. Robert, *Clin. Cancer Res.* 2013, *19*, 5300.
- [24] S. Y. Jang, J. Heo, M. R. Park, M. H. Song, J. N. Kim, S. H. Jo, D. Y. Jeong, H. K. Lee, Y. Kim, S. Oh, *J. Microbiol. Biotechnol.* 2017, *27*, 1266.
- [25] K. Peters, S. Kannan, V. A. Rao, J. Biboy, D. Vollmer, S. W. Erickson, R. J. Lewis, K. D. Young, W. Vollmer, *MBio.* 2016, *7*, 00819.
- [26] Z. Peng, M. A. Ehrmann, A. Waldhuber, C. Niemeuer, T. Miethke, J. S. Frick, T. Xiong, R. F. Vogel, *Res. Microbiol.* **2017**, *168*, 558.
- [27] A. Solopova, H. Bachmann, B. Teusink, J. Kok, O. P. Kuipers, *Front. Microbiol.* **2018**, *9*, 1803.
- [28] D. Murtini, N. P. Aryantini, I. N. Sujaya, T. Urashima, K. Fukuda, *Biosci. Microbiota Food Health* 2016, *35*, 41.



- [29] N. A. De Moura, B. F. R. Caetano, K. Sivieri, L. H. Urbano, C. Cabello, M. A. Rodrigues, L. F. Barbisan, *Food Chem. Toxicol.* **2012**, *50*, 2902.
- [30] B. F. R. Caetano, N. A. De Moura, A. P. S. Almeida, M. C. Dias, K. Sivieri, L. F. Barbisan, *Nutrients* **2016**, *8*, 436.
- [31] D. Martarelli, M. C. Verdenelli, S. Scuri, M. Cocchioni, S. Silvi, C. Cecchini, P. Pompei, *Curr. Microbiol.* 2011, *62*, 1689.
- [32] H. S. Ejtahed, J. Mohtadi-Nia, A. Homayouni-Rad, M. Niafar, M. Asghari-Jafarabadi, V. Mofid, *Nutrition* 2012, *28*, 539.
- [33] H. J. Park, C. O. Reiser, S. Kondruweit, H. Erdmann, R. D. Schmid, M. Sprinzl, *Eur. J. Biochem.* 1992, *205*, 881.
- [34] E. Kaambo, C. Africa, R. Chambuso, J. S. Passmore, *Front. Public Health* **2018**, *6*, 78.
- [35] G. Parthasarathy, S. Lun, H. Guo, N. C. Ammerman, D. E. Geiman, W. R. Bishai, *PLoS One* 2012, *7*, 43429.
- [36] C. Staley, G. M. Dunny, M. J. Sadowsky, *Adv. Appl. Microbiol.* 2014, *87*, 147.
- [37] X. Lu, L. Li, R. Wu, X. Feng, Z. Li, H. Yang, C. Wang, H. Guo, A. Galkin, O. Herzberg, P. S. Mariano, B. M. Martin, D. Dunaway-Mariano, *Biochemistry* 2006, *45*, 1162.
- [38] S. C. Warangkar, C. N. Khobragade, *J. Cell Tissue Res.* 2009, *9*, 1963.
- [39] U. K. Narta, S. S. Kanwar, W. Azmi, *Crit. Rev. Oncol. Hematol.* 2007, *61*, 208.
- [40] A. P. Sudhir, B. R. Dave, A. S. Prajapati, K. Panchal, D. Patel, R. B. Subramanian, *Appl. Biochem. Biotechnol.* 2014, *174*, 2504.
- [41] R. K. Sinha, H. R. Singh, S. K. Jha, *Int. J. Pharm. Sci.* 2015, *7*, 135.
- [42] S. A. Susan, S. Iyappan, K. L. Vijaya, K. N. Rajnish, *J. Biotechnol.* 2017, *7*, 348.
- [43] J. E. Jones, C. P. Causey, B. Knuckley, J. L. Slack-Noyes, P. R. Thompson, *Curr. Opin. Drug. Discov. Devel.* 2009, *12*, 616.
- [44] B. Barcelona-Andres, A. Marina, V. Rubio, *J. Bacteriol.* **2002**, *184*, 6289.
- [45] M. N. Amer, N. M. Mansour, A. I. El-Diwany, I. E. Dawoud, F. M. Rashad, *Ann. Microbiol.* **2013**, *63*, 1121.
- [46] A. E. Walts, J. M. Mirocha, T. Leong, A. M. Marchevsky, *Am. J. Clin. Pathol.* 2016, *145*, 244.
- [47] K. W. Jaspersen, T. M. Tuohy, D. W. Neklason, R. W. Burt, *Gastroenterology* **2010**, *138*, 2044.
- [48] R. L. Siegel, K. D. Miller, A. Jemal, *CA Cancer J. Clin.* **2016**, *66*, 7.
- [49] C. Alexandrou, S. S. Al-Aqbi, J. A. Higgins, W. Boyle, A. Karmokar, C. Andreadi, J. L. Luo, D. A. Moore, M. Viskaduraki, M. Blades, G. I. Murray, L. M. Howells, A. Thomas, K. Brown, P. N. Cheng, A. Rufini, *Sci. Rep.* **2018**, *8*, 12096.

**Table S1.** PLS-DA values used to identify differentially expressed proteins related to the use of different carbon sources (FOS, GOS and GLU) and the presence or absence of oxygen in *E. durans* bacterial pellet with the VIP threshold > 1.0 in the first component of PLS-DA.

Protein Group	Comp. 1	Comp. 2	Comp. 3
trA0A367CII2A0A367CII2_9ENTE ABC-type uncharacterized transport system ATPase component OSEnterococcus durans OX53345 GNybbL_1 PE4 SV1	42.309	54.313	50.768
trA0A377KL98A0A377KL98_9ENTE Adenylosuccinate lyase OSEnterococcus durans OX53345 GNpurB PE3 SV1	37.981	31.857	30.712
trA0A377L0K4A0A377L0K4_9ENTE Mannose-6-phosphate isomerase OSEnterococcus durans OX53345 GNNCTC8129_00093 PE4 SV1	37.255	48.912	46.496
trA0A377KH40A0A377KH40_9ENTE NADH peroxidase OSEnterococcus durans OX53345 GNnpr_2 PE4 SV1	31.964	42.435	40.541
trA0A377KNK5A0A377KNK5_9ENTE Xanthine phosphoribosyltransferase OSEnterococcus durans OX53345 GNxpt PE3 SV1	29.318	23.957	23.553
trA0A377KPU7A0A377KPU7_9ENTE Formamidopyrimidine-DNA glycosylase OSEnterococcus durans OX53345 GNmutM PE3 SV1	27.843	22.255	19.868
trA0A377KMB8A0A377KMB8_9ENTE Methylenetetrahydrofolate--tRNA-uracil-5--methyltransferase TrmFO OSEnterococcus durans OX53345 GNgidA_2 PE3 SV1	27.618	22.977	21.499
trA0A367CC09A0A367CC09_9ENTE RNA-binding protein YhbY OSEnterococcus durans OX53345 GNyhbY PE4 SV1	27.552	2.184	19.506
trA0A377KMX2A0A377KMX2_9ENTE PTS system fructose-specific transporter subunit IABC OSEnterococcus durans OX53345 GNfruA_4 PE4 SV1	27.105	2.614	2.334
trA0A367CEI1A0A367CEI1_9ENTE Arginine repressor OSEnterococcus durans OX53345 GNargR_2 PE3 SV1	26.608	21.739	20.193
trA0A377KH2A0A377KH2_9ENTE FMN-binding domain-containing protein OSEnterococcus durans OX53345 GNNCTC8129_00960 PE4 SV1	25.667	2.056	22.108
trA0A377KGW5A0A377KGW5_9ENTE Beta-propeller domains of methanol dehydrogenase type OSEnterococcus durans OX53345 GNNCTC8129_00310 PE4 SV1	25.438	20.842	18.608
trA0A377KIN7A0A377KIN7_9ENTE Energy-coupling factor transporter ATP-binding protein EcfA OSEnterococcus durans OX53345 GNcbiO PE3 SV1	25.254	20.622	19.293
trA0A377KG31A0A377KG31_9ENTE ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNecsA_1 PE4 SV1	25.179	21.852	20.056
trA0A377KHW3A0A377KHW3_9ENTE Pyridine nucleotide-disulfide family oxidoreductase OSEnterococcus durans OX53345 GNyjID PE4 SV1	25.122	22.091	2.122
trA0A377KLT8A0A377KLT8_9ENTE ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNthiQ PE4 SV1	25.057	2.019	18.244
trA0A27SRV6A0A27SRV6_9ENTE Holo-acyl-carrier-protein synthase OSEnterococcus durans OX53345 GNacpS PE3 SV1	25.019	20.012	18.568
trA0A377KH19A0A377KH19_9ENTE Cysteine synthase B OSEnterococcus durans OX53345 GNmccA PE4 SV1	24.713	20.374	18.546
trA0A27SL24A0A27SL24_9ENTE O-methyltransferase OSEnterococcus durans OX53345 GNEA71_00765 PE4 SV1	24.514	1.972	17.856
trA0A377KH48A0A377KH48_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNNCTC8129_00514 PE4 SV1	23.971	19.462	18.437
trA0A377KKF0A0A377KKF0_9ENTE Adenylosuccinate synthetase OSEnterococcus durans OX53345 GNpurA PE3 SV1	23.833	21.244	19.403
trA0A377KIG2A0A377KIG2_9ENTE Ribonuclease HIII OSEnterococcus durans OX53345 GNrnHC PE3 SV1	23.796	1.892	1.706
trA0A377KLZ2A0A377KLZ2_9ENTE ATP-dependent protease ATP-binding subunit ClpX OSEnterococcus durans OX53345 GNclpX_1 PE4 SV1	23.534	19.079	17.718
trA0A27SSA2A0A27SSA2_9ENTE Deoxynucleoside kinase OSEnterococcus durans OX53345 GNNCTC8129_01240 PE4 SV1	23.431	19.078	17.766
trA0A377KKH4A0A377KKH4_9ENTE Lipoprotein OSEnterococcus durans OX53345 GNNCTC8129_01671 PE4 SV1	23.215	18.957	17.412
trA0A377KGF6A0A377KGF6_9ENTE Acyl-ACP thioesterase OSEnterococcus durans OX53345 GNfat PE4 SV1	23.139	18.399	17.155
trA0A377KJ46A0A377KJ46_9ENTE Calcineurin-like phosphoesterase OSEnterococcus durans OX53345 GNNCTC8129_01148 PE4 SV1	22.929	18.533	17.639
trA0A27SQZ7A0A27SQZ7_9ENTE GntR family transcriptional regulator OSEnterococcus durans OX53345 GNCUM72_12880 PE4 SV1	22.839	18.829	18.077
trA0A367CJW2A0A367CJW2_9ENTE CobB/CobQ-like glutamine amidotransferase OSEnterococcus durans OX53345 GNEA71_00567 PE4 SV1	22.771	18.487	18.344
trA0A27SKW5A0A27SKW5_9ENTE Glucitol/sorbitol phosphotransferase system enzyme IIA OSEnterococcus durans OX53345 GNEA71_00544 PE4 SV1	22.718	19.516	18.084
trA0A377KHJ0A0A377KHJ0_9ENTE Esterase OSEnterococcus durans OX53345 GNfrmB PE4 SV1	22.495	18.335	19.634
trA0A377KM79A0A377KM79_9ENTE Penicillin-binding protein 4 OSEnterococcus durans OX53345 GNftsl PE4 SV1	22.485	17.921	18.084

trA0A377KJG8A0A377KJG8_9ENTE Arginine repressor OSEnterococcus durans OX53345 GNargR_1 PE3 SV1	22.462	1.785	16.087
trA0A367CEE6A0A367CEE6_9ENTE Leucine-isoleucine-valine-threonine-and alanine-binding protein OSEnterococcus durans OX53345 GNbraC PE4 SV1	22.328	18.066	16.571
trA0A377KM39A0A377KM39_9ENTE ATP-dependent Zn protease OSEnterococcus durans OX53345 GNEA71_01399 PE4 SV1	22.315	17.767	1.64
trA0A367CHF2A0A367CHF2_9ENTE HAD superfamily hydrolase OSEnterococcus durans OX53345 GNyhaX_2 PE4 SV1	22.312	17.891	16.712
trA0A377KP59A0A377KP59_9ENTE Peptidase propeptide and YPEB domain-containing protein OSEnterococcus durans OX53345 GNypmB PE4 SV1	22.168	18.165	16.526
trA0A2A7SN77A0A2A7SN77_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNEA71_01388 PE4 SV1	21.918	17.672	18.081
trA0A377KIK8A0A377KIK8_9ENTE L-asparaginase OSEnterococcus durans OX53345 GNansA PE4 SV1	21.819	17.246	16.819
trA0A367CAI3A0A367CAI3_9ENTE SPFH domain / Band 7 family protein OSEnterococcus durans OX53345 GNEA71_03016 PE4 SV1	21.713	17.227	15.383
trA0A377KH71A0A377KH71_9ENTE Primosomal protein N Replication factor Y -superfamily II helicase OSEnterococcus durans OX53345 GNNCTC8129_00309 PE4 SV1	21.656	17.717	16.265
trA0A377KJX7A0A377KJX7_9ENTE M16C subfamily protease OSEnterococcus durans OX53345 GNNCTC8129_00853 PE4 SV1	21.493	1.711	15.396
trA0A377KGY3A0A377KGY3_9ENTE Copper homeostasis protein CutC OSEnterococcus durans OX53345 GNcutC PE3 SV1	21.466	17.493	16.202
trA0A377KGQ3A0A377KGQ3_9ENTE V-type ATP synthase subunit D OSEnterococcus durans OX53345 GNntpd PE3 SV1	21.449	17.282	18.452
trA0A377KM26A0A377KM26_9ENTE Metallo-beta-lactamase superfamily protein OSEnterococcus durans OX53345 GNNCTC8129_02258 PE4 SV1	21.113	1.743	16.657
trA0A377KI9A0A377KI9_9ENTE Hydrolase OSEnterococcus durans OX53345 GNyfnB_1 PE4 SV1	21.054	16.638	16.167
trA0A377KNB1A0A377KNB1_9ENTE Uncharacterized conserved protein OSEnterococcus durans OX53345 GNNCTC8129_02084 PE4 SV1	21.017	16.613	18.014
trA0A377KP98A0A377KP98_9ENTE ABC transporter permease protein Putative OSEnterococcus durans OX53345 GNNCTC8129_02451 PE4 SV1	20.966	17.692	16.487
trA0A377KH8A0A377KH8_9ENTE Ribonucleoside-diphosphate reductase OSEnterococcus durans OX53345 GNnrde2 PE3 SV1	20.929	20.979	19.539
trA0A2A7SP53A0A2A7SP53_9ENTE Putative pyruvate phosphate dikinase regulatory protein OSEnterococcus durans OX53345 GNyqfL PE3 SV1	20.678	16.564	17.853
trA0A2A7SSE8A0A2A7SSE8_9ENTE Family 2 glycosyl transferase OSEnterococcus durans OX53345 GNNCTC8129_01312 PE4 SV1	20.471	16.569	16.032
trA0A2A7SRU9A0A2A7SRU9_9ENTE Chromosome partitioning protein ParB OSEnterococcus durans OX53345 GNparB PE3 SV1	20.461	16.203	16.255
trA0A377KJ50A0A377KJ50_9ENTE Amino acid permease OSEnterococcus durans OX53345 GNgadC_2 PE4 SV1	20.392	17.007	16.197
trA0A2A7SLI8A0A2A7SLI8_9ENTE YfaA OSEnterococcus durans OX53345 GNNCTC8129_02122 PE4 SV1	20.379	16.112	18.111
trA0A377KLR6A0A377KLR6_9ENTE HD domain-containing protein OSEnterococcus durans OX53345 GNyfbR PE4 SV1	20.131	16.414	15.383
trA0A377KJ47A0A377KJ47_9ENTE Ribosomal large subunit pseudouridine synthase D OSEnterococcus durans OX53345 GNrluD_1 PE4 SV1	20.062	16.533	21.674
trA0A2A7SQG5A0A2A7SQG5_9ENTE 3-deoxy-7-phosphoheptulonate synthase OSEnterococcus durans OX53345 GNaroF_2 PE4 SV1	20.023	24.907	24.485
trA0A377KI18A0A377KI18_9ENTE DNA repair protein Rada OSEnterococcus durans OX53345 GNradA PE3 SV1	19.884	15.772	20.768
trA0A377KP43A0A377KP43_9ENTE Formate--tetrahydrofolate ligase OSEnterococcus durans OX53345 GNfhs1 PE3 SV1	19.813	16.532	17.618
trA0A377KFW9A0A377KFW9_9ENTE Xaa-Pro dipeptidase OSEnterococcus durans OX53345 GNpqpQ_1 PE4 SV1	19.629	1.552	1.48
trA0A377KIJ7A0A377KIJ7_9ENTE Oxidoreductase OSEnterococcus durans OX53345 GNqorA PE4 SV1	19.413	15.647	17.331
trA0A377KH98A0A377KH98_9ENTE Ribosomal RNA small subunit methyltransferase A OSEnterococcus durans OX53345 GNksgA PE3 SV1	19.213	15.184	19.129
trA0A377KKK8A0A377KKK8_9ENTE HAD-superfamily hydrolase OSEnterococcus durans OX53345 GNNCTC8129_01712 PE4 SV1	19.106	15.161	14.016
trA0A377KLB0A0A377KLB0_9ENTE Phosphoribosylaminoimidazole-succinocarboxamide synthase OSEnterococcus durans OX53345 GNpurC PE3 SV1	18.825	26.091	24.621
trA0A377KHD8A0A377KHD8_9ENTE HAD superfamily hydrolase OSEnterococcus durans OX53345 GNNCTC8129_00370 PE4 SV1	18.737	1.486	1.481
trA0A377L1B3A0A377L1B3_9ENTE Exodeoxyribonuclease OSEnterococcus durans OX53345 GNexoA PE4 SV1	18.723	14.817	14.093
trA0A377KGM7A0A377KGM7_9ENTE ABC transporter ATP-binding protein/permease OSEnterococcus durans OX53345 GNNCTC8129_00454 PE4 SV1	18.715	14.853	14.348

trA0A2A7SP93A0A2A7SP93_9ENTE UPF0356 protein CUM72_10905 OSEnterococcus durans OX53345 GNCUM72_10905 PE3 SV1	18.709	17.011	15.295
trA0A377KL51A0A377KL51_9ENTE Deoxyguanosine kinase OSEnterococcus durans OX53345 GNEA71_00673 PE4 SV1	18.604	14.702	14.312
trA0A377KGR0A0A377KGR0_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNNCTC8129_00363 PE4 SV1	18.429	14.564	1.506
trA0A2A7SQY4A0A2A7SQY4_9ENTE PTS sugar transporter subunit IIB OSEnterococcus durans OX53345 GNlicB_2 PE4 SV1	18.375	21.015	19.327
trA0A377KMD0A0A377KMD0_9ENTE Formate acetyltransferase OSEnterococcus durans OX53345 GNpflB PE4 SV1	18.272	15.999	15.334
trA0A377KJS7A0A377KJS7_9ENTE Transcriptional repressor of the arabinose operon OSEnterococcus durans OX53345 GNaraR_1 PE4 SV1	18.124	14.328	12.993
trA0A377KKA5A0A377KKA5_9ENTE Ribosomal RNA small subunit methyltransferase H OSEnterococcus durans OX53345 GNmraW PE3 SV1	17.935	14.285	1.518
trA0A377KPG3A0A377KPG3_9ENTE Carboxymuconolactone decarboxylase family protein OSEnterococcus durans OX53345 GNNCTC8129_02894 PE4 SV1	17.862	14.255	1.41
trA0A377KQJ3A0A377KQJ3_9ENTE Cupin superfamily protein OSEnterococcus durans OX53345 GNNCTC8129_02893 PE4 SV1	17.795	14.255	16.319
trA0A377KGH9A0A377KGH9_9ENTE Phosphate import ATP-binding protein PstB OSEnterococcus durans OX53345 GNpstB2 PE3 SV1	17.754	14.038	14.077
trA0A377KHH0A0A377KHH0_9ENTE Leucine--tRNA ligase OSEnterococcus durans OX53345 GNleuS PE3 SV1	17.726	1.42	12.988
trA0A377KH21A0A377KH21_9ENTE Prephenate dehydratase OSEnterococcus durans OX53345 GNpheA PE4 SV1	17.456	15.226	13.594
trA0A377KH15A0A377KH15_9ENTE Small ribosomal subunit biogenesis GTPase RsgA OSEnterococcus durans OX53345 GNrsgA PE3 SV1	17.405	14.116	12.877
trA0A377KGH1A0A377KGH1_9ENTE Class V aminotransferase OSEnterococcus durans OX53345 GNiscS_1 PE3 SV1	17.298	13.791	14.545
trA0A377KHL0A0A377KHL0_9ENTE Sun protein OSEnterococcus durans OX53345 GNrsmB PE3 SV1	17.252	13.756	13.106
trA0A377KJL8A0A377KJL8_9ENTE D-ribose pyranase OSEnterococcus durans OX53345 GNrbsD PE3 SV1	16.882	27.466	24.791
trA0A377KGY4A0A377KGY4_9ENTE L-lactate oxidase OSEnterococcus durans OX53345 GNNCTC8129_00575 PE3 SV1	16.842	14.511	12.954
trA0A2A7SRA3A0A2A7SRA3_9ENTE Amino acid ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNartM_1 PE4 SV1	16.825	13.306	12.171
trA0A377KL00A0A377KL00_9ENTE Cell division protein DivIB OSEnterococcus durans OX53345 GNftsQ PE3 SV1	16.725	13.505	13.962
trA0A377KL42A0A377KL42_9ENTE ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNtagH PE4 SV1	16.374	13.004	12.566
trA0A377KIK5A0A377KIK5_9ENTE Peptidyl-tRNA hydrolase OSEnterococcus durans OX53345 GNpth PE3 SV1	16.283	12.896	12.078
trA0A377KM97A0A377KM97_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNNCTC8129_01702 PE4 SV1	16.072	13.269	11.883
trA0A377KP21A0A377KP21_9ENTE Lactose phosphotransferase system repressor OSEnterococcus durans OX53345 GNlacR PE4 SV1	15.951	13.009	11.638
trA0A377KHZ0A0A377KHZ0_9ENTE Pheromone cAD1 lipoprotein OSEnterococcus durans OX53345 GNNCTC8129_00959 PE4 SV1	15.867	13.164	14.857
trA0A377KN58A0A377KN58_9ENTE Short-chain alcohol dehydrogenase of uncharacterized specificity OSEnterococcus durans OX53345 GNNCTC8129_02892 PE3 SV1	15.831	1.253	13.486
trA0A2A7SRG7A0A2A7SRG7_9ENTE ABC superfamily ATP binding cassette transporter binding protein OSEnterococcus durans OX53345 GNtmpC_1 PE4 SV1	15.757	12.834	13.355
trA0A377L1H3A0A377L1H3_9ENTE Spermidine/putrescine import ATP-binding protein PotA OSEnterococcus durans OX53345 GNpotA PE3 SV1	15.747	12.488	14.585
trA0A377KKQ0A0A377KKQ0_9ENTE DNA helicase OSEnterococcus durans OX53345 GNhelD_3 PE4 SV1	15.673	12.426	13.044
trA0A377KK35A0A377KK35_9ENTE HAD superfamily hydrolase OSEnterococcus durans OX53345 GNywpJ_1 PE4 SV1	15.424	12.217	13.183
trA0A377KHK3A0A377KHK3_9ENTE Adhesion lipoprotein OSEnterococcus durans OX53345 GNadcA_2 PE3 SV1	15.209	12.485	12.423
trA0A377KMN3A0A377KMN3_9ENTE Diacylglycerol kinase OSEnterococcus durans OX53345 GNdagK_2 PE4 SV1	15.208	12.019	13.502
trA0A2A7SLH7A0A2A7SLH7_9ENTE N5-carboxyaminoimidazole ribonucleotide synthase OSEnterococcus durans OX53345 GNpurK PE3 SV1	15.081	15.294	17.964
trA0A377KIP0A0A377KIP0_9ENTE Tyrosine decarboxylase OSEnterococcus durans OX53345 GNddc_2 PE4 SV1	15.068	1.201	11.558
trA0A377KKS2A0A377KKS2_9ENTE Sensor histidine kinase OSEnterococcus durans OX53345 GNarlS PE4 SV1	14.868	11.789	12.555
trA0A377KKA3A0A377KKA3_9ENTE Inosine-5-monophosphate dehydrogenase OSEnterococcus durans OX53345 GNguaB_1 PE3 SV1	14.853	11.794	14.024
trA0A377KP08A0A377KP08_9ENTE NADH oxidase OSEnterococcus durans OX53345 GNnox_3 PE4 SV1	14.742	11.703	13.923

trA0A377KHL5A0A377KHL5_9ENTE B3/4 domain-containing protein OSEnterococcus durans OX53345 GNNCTC8129_00680 PE4 SV1	14.556	11.528	13.197
trA0A377KIW1A0A377KIW1_9ENTE Purine nucleoside phosphorylase DeoD-type OSEnterococcus durans OX53345 GNdeoD PE3 SV1	14.479	12.035	13.971
trA0A377KKJ2A0A377KKJ2_9ENTE Sigma-54 factor interaction domain-containing protein OSEnterococcus durans OX53345 GNluxO_1 PE4 SV1	14.446	11.986	11.983
trA0A377L0G6A0A377L0G6_9ENTE Rrf2 family protein OSEnterococcus durans OX53345 GNywnA_1 PE4 SV1	14.396	12.832	14.597
trA0A377KGV4A0A377KGV4_9ENTE Phosphate import ATP-binding protein PstB OSEnterococcus durans OX53345 GNpstB1 PE3 SV1	14.335	1.133	12.549
trA0A377KKM0A0A377KKM0_9ENTE DEAD/DEAH box helicase OSEnterococcus durans OX53345 GNcshA_2 PE4 SV1	14.227	11.403	1.088
trA0A377KMG3A0A377KMG3_9ENTE Aminotransferase AlaT OSEnterococcus durans OX53345 GNalaT PE4 SV1	14.224	11.243	13.915
trA0A377KNR4A0A377KNR4_9ENTE HD domain-containing protein OSEnterococcus durans OX53345 GNNCTC8129_03096 PE4 SV1	14.211	11.457	10.355
trA0A2A7SL88A0A2A7SL88_9ENTE Acyl carrier protein OSEnterococcus durans OX53345 GNacpA PE3 SV1	14.177	1.133	14.197
trA0A377KGC6A0A377KGC6_9ENTE Uncharacterized protein conserved in bacteria OSEnterococcus durans OX53345 GNNCTC8129_00221 PE4 SV1	14.105	11.286	12.989
trA0A377KKX2A0A377KKX2_9ENTE Malonyl CoA-acyl carrier protein transacylase OSEnterococcus durans OX53345 GNfabD_2 PE3 SV1	14.025	11.096	10.573
trA0A377KMZ1A0A377KMZ1_9ENTE GTPase domain-containing protein OSEnterococcus durans OX53345 GNNCTC8129_01933 PE4 SV1	13.883	1.098	13.191
trA0A2A7SRM4A0A2A7SRM4_9ENTE Acyl carrier protein OSEnterococcus durans OX53345 GNacpP PE3 SV1	13.774	11.833	10.703
trA0A377KKR3A0A377KKR3_9ENTE Threonine--tRNA ligase OSEnterococcus durans OX53345 GNthrS PE3 SV1	13.488	11.041	10.734
trA0A377KLJ1A0A377KLJ1_9ENTE Acetyl-CoA acetyltransferase OSEnterococcus durans OX53345 GNthIA PE4 SV1	13.156	10.545	0.98791
trA0A377KKV4A0A377KKV4_9ENTE UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase OSEnterococcus durans OX53345 GNmurF PE3 SV1	12.876	10.536	0.99132
trA0A377KLU1A0A377KLU1_9ENTE ABC transporter ATP-binding protein/permease OSEnterococcus durans OX53345 GNyheH_2 PE4 SV1	12.846	10.162	10.019
trA0A367CCB7A0A367CCB7_9ENTE Flavodoxin OSEnterococcus durans OX53345 GNmioC PE4 SV1	12.808	10.172	0.94719
trA0A248V9B1A0A248V9B1_9ENTE Queuine tRNA-ribosyltransferase OSEnterococcus durans OX53345 GNtgt PE3 SV1	12.789	10.567	0.9587
trA0A377KL63A0A377KL63_9ENTE Choline ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNproV_2 PE4 SV1	12.648	10.117	0.92858
trA0A377KKA9A0A377KKA9_9ENTE Phosphoglycerate mutase family protein OSEnterococcus durans OX53345 GNcobC_2 PE3 SV1	12.637	10.786	0.97314
trA0A377KMW0A0A377KMW0_9ENTE TrmA family RNA methyltransferase OSEnterococcus durans OX53345 GNrlmCD_2 PE3 SV1	12.623	10.335	10.101
trA0A377KM63A0A377KM63_9ENTE Cyclopropane-fatty-acyl-phospholipid synthase OSEnterococcus durans OX53345 GNcfa PE4 SV1	12.295	0.99343	0.96636
trA0A377L0H3A0A377L0H3_9ENTE S-hydroxymethylglutathione dehydrogenase OSEnterococcus durans OX53345 GNfrmA PE3 SV1	12.206	1.273	11.659
trA0A367CCU1A0A367CCU1_9ENTE C4-dicarboxylate anaerobic carrier OSEnterococcus durans OX53345 GNEA71_01026 PE4 SV1	12.136	15.777	14.296
trA0A377KLB2A0A377KLB2_9ENTE Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OSEnterococcus durans OX53345 GNaccA PE3 SV1	12.116	10.893	10.457
trA0A377KKD3A0A377KKD3_9ENTE Aminopeptidase OSEnterococcus durans OX53345 GNpepS PE4 SV1	11.985	0.96988	0.89871
trA0A377KKX4A0A377KKX4_9ENTE Xaa-Pro dipeptidase OSEnterococcus durans OX53345 GNpepQ_2 PE3 SV1	11.915	0.95351	0.89983
trA0A377KJU9A0A377KJU9_9ENTE Beta-lactamase class C-like protein OSEnterococcus durans OX53345 GNampH PE4 SV1	11.909	0.95517	11.261
trA0A377L328A0A377L328_9ENTE Aspartate carbamoyltransferase OSEnterococcus durans OX53345 GNpyrB PE3 SV1	11.873	22.046	20.294
trA0A377KGD6A0A377KGD6_9ENTE Aminopeptidase C OSEnterococcus durans OX53345 GNpepC PE4 SV1	11.837	10.786	12.028
trA0A377KGE4A0A377KGE4_9ENTE Valine--tRNA ligase OSEnterococcus durans OX53345 GNvalS PE3 SV1	11.818	0.94538	0.86929
trA0A2A7SNZ0A0A2A7SNZ0_9ENTE Lactoylglutathione lyase OSEnterococcus durans OX53345 NGloA_2 PE4 SV1	11.489	10.473	10.864
trA0A377KLI3A0A377KLI3_9ENTE Nitroreductase family protein OSEnterococcus durans OX53345 GNNCTC8129_02296 PE4 SV1	11.436	1.29	13.156
trA0A377KMP9A0A377KMP9_9ENTE Tagatose-6-phosphate kinase OSEnterococcus durans OX53345 GNlacC_1 PE3 SV1	11.409	11.097	10.026
trA0A377KN72A0A377KN72_9ENTE UPF0340 protein NCTC8129_02462 OSEnterococcus durans OX53345 GNNCTC8129_02462 PE3 SV1	11.152	1.097	10.857

trA0A377KLL5A0A377KLL5_9ENTE Histidine--tRNA ligase OSEnterococcus durans OX53345 GNhisS PE3 SV1	11.076	0.88406	0.82332
trA0A377KLS3A0A377KLS3_9ENTE Putative ribose-phosphate pyrophosphokinase OSEnterococcus durans OX53345 GNprs2 PE3 SV1	11.031	0.87298	0.78002
trA0A377KMN2A0A377KMN2_9ENTE Phosphoglycerate mutase family protein OSEnterococcus durans OX53345 GNrpmA_3 PE4 SV1	10.984	0.86822	0.77518
trA0A377KMT4A0A377KMT4_9ENTE Glucose 1-dehydrogenase Gdh OSEnterococcus durans OX53345 GNgdh PE4 SV1	10.971	0.86801	0.86673
trA0A2A7SQA6A0A2A7SQA6_9ENTE Ribonucleoside-diphosphate reductase subunit beta OSEnterococcus durans OX53345 GNnrdF_1 PE3 SV1	10.968	11.792	13.246
trA0A377KLX1A0A377KLX1_9ENTE N5-carboxyaminoimidazole ribonucleotide mutase OSEnterococcus durans OX53345 GNpurE PE3 SV1	10.959	0.8687	0.81066
trA0A377KJF6A0A377KJF6_9ENTE N-acetylmuramoyl-L-alanine amidase OSEnterococcus durans OX53345 GNNCTC8129_00658 PE4 SV1	10.902	0.87143	0.82311
trA0A377KH6A0A377KH60_9ENTE Oxidoreductase OSEnterococcus durans OX53345 GNNCTC8129_00674 PE4 SV1	10.884	2.376	27.679
trA0A377KJ5A0A377KJ5_9ENTE Serine hydroxymethyltransferase OSEnterococcus durans OX53345 GNglyA PE3 SV1	10.882	0.97806	0.9873
trA0A377KJY4A0A377KJY4_9ENTE Oxidoreductase OSEnterococcus durans OX53345 GNiolS PE4 SV1	10.881	0.85997	0.78823
trA0A377KH64A0A377KH64_9ENTE Peptide ABC transporter permease/transmembrane protein OSEnterococcus durans OX53345 GNNCTC8129_00659 PE4 SV1	10.818	0.90297	0.81408
trA0A377KMU4A0A377KMU4_9ENTE DNA polymerase III PolC-type OSEnterococcus durans OX53345 GNpolC_2 PE3 SV1	10.747	0.85414	11.087
trA0A377KI13A0A377KI13_9ENTE Beta sliding clamp OSEnterococcus durans OX53345 GNdnaB_1 PE3 SV1	10.689	0.8632	0.7993
trA0A377KG96A0A377KG96_9ENTE PTS system cellobiose-specific transporter subunit IIB OSEnterococcus durans OX53345 GNlicB_1 PE4 SV1	10.684	19.557	26.336
trA0A377K55A0A377K55_9ENTE ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNyheS_3 PE4 SV1	10.488	0.91094	0.81328
trA0A377KPP3A0A377KPP3_9ENTE Endopeptidase PepO OSEnterococcus durans OX53345 GNpepO PE4 SV1	10.464	0.82925	0.80346
trA0A377KN82A0A377KN82_9ENTE ATP-dependent Clp protease ATP-binding subunit ClpX OSEnterococcus durans OX53345 GNclpX_2 PE3 SV1	10.369	0.85793	0.78344
trA0A377KGJ1A0A377KGJ1_9ENTE Ribonuclease J OSEnterococcus durans OX53345 GNrnjB PE3 SV1	10.284	0.90325	0.8685
trA0A377KH54A0A377KH54_9ENTE Aminotransferase OSEnterococcus durans OX53345 GNpatA PE3 SV1	10.161	0.8268	1.069
trA0A377KMF9A0A377KMF9_9ENTE Oxidoreductase OSEnterococcus durans OX53345 GNNCTC8129_01766 PE4 SV1	10.093	11.737	11.816
trA0A377KPS9A0A377KPS9_9ENTE Acetolactate synthase OSEnterococcus durans OX53345 GNbudB PE3 SV1	10.087	0.80119	15.834
trA0A377KMZ9A0A377KMZ9_9ENTE Dephospho-CoA kinase OSEnterococcus durans OX53345 GNcoaE PE3 SV1	2.587	21.309	19.182
trA0A2A7SR92A0A2A7SR92_9ENTE Putative RNA-binding protein containing a PIN domain OSEnterococcus durans OX53345 GNEA71_02868 PE4 SV1	2.581	20.978	18.845
trA0A377KNM6A0A377KNM6_9ENTE RNA methyltransferase OSEnterococcus durans OX53345 GNNCTC8129_03070 PE3 SV1	2.476	21.242	22.092
trA0A2A7SS74A0A2A7SS74_9ENTE Gluconate 5-dehydrogenase OSEnterococcus durans OX53345 GNgn0_2 PE4 SV1	2.458	19.541	18.582
trA0A2A7SLB4A0A2A7SLB4_9ENTE Probable nicotinate-nucleotide adenyltransferase OSEnterococcus durans OX53345 GNnadD PE3 SV1	2.448	20.035	18.813
trA0A2A7SSW2A0A2A7SSW2_9ENTE RNA polymerase factor sigma-54 OSEnterococcus durans OX53345 GNEA71_02132 PE4 SV1	2.429	1.965	18.006
trA0A2A7SME0A0A2A7SME0_9ENTE Transcriptional repressor NrdR OSEnterococcus durans OX53345 GNnrdR PE3 SV1	2.428	1.961	18.349
trA0A377KMY0A0A377KMY0_9ENTE Zinc metalloprotease OSEnterococcus durans OX53345 GNrseP PE3 SV1	2.382	1.938	18.105
trA0A377KMA5A0A377KMA5_9ENTE Fur family transcriptional regulator OSEnterococcus durans OX53345 GNperR PE3 SV1	2.111	16.727	14.998
trA0A377KJG0A0A377KJG0_9ENTE Ribokinase OSEnterococcus durans OX53345 GNrbsK PE3 SV1	1.783	2.167	20.437
trA0A377KLX9A0A377KLX9_9ENTE Phosphoribosylglycinamide formyltransferase OSEnterococcus durans OX53345 GNpurN PE3 SV1	1.761	20.994	20.687
trA0A377KJB0A0A377KJB0_9ENTE Oligoendopeptidase F plasmid OSEnterococcus durans OX53345 GNpepF1_2 PE3 SV1	1.753	15.776	16.479
trA0A377KHF0A0A377KHF0_9ENTE PTS system lactose/cellobiose-specific IIA component OSEnterococcus durans OX53345 GNlacF_1 PE4 SV1	1.657	18.916	17.511
trA0A377KKQ8A0A377KKQ8_9ENTE Holliday junction ATP-dependent DNA helicase RuvA OSEnterococcus durans OX53345 GNruvA PE3 SV1	1.611	12.809	13.932
trA0A377KLW7A0A377KLW7_9ENTE Lipase/acylhydrolase OSEnterococcus durans OX53345 GNNCTC8129_02440 PE4 SV1	1.602	12.737	14.437

trA0A377KLC1A0A377KLC1_9ENTE ATP-dependent DNA helicase RecQ OSEnterococcus durans OX53345 GNrecQ_3 PE4 SV1	1.495	23.614	2.166
trA0A377KLZ9A0A377KLZ9_9ENTE Pyruvate carboxylase OSEnterococcus durans OX53345 GNcfiB PE4 SV1	1.401	11.445	10.402
trA0A377KG22A0A377KG22_9ENTE V-type ATP synthase subunit I OSEnterococcus durans OX53345 GNNCTC8129_00256 PE3 SV1	1.368	11.141	10.026
trA0A377KNT7A0A377KNT7_9ENTE Oxidoreductase zinc-binding protein OSEnterococcus durans OX53345 GNNCTC8129_03104 PE4 SV1	1.344	10.842	0.99757
trA0A377KMOV3A0A377KMOV3_9ENTE Arginine--tRNA ligase OSEnterococcus durans OX53345 GNargS PE3 SV1	1.313	10.434	0.93213
trA0A377KNG7A0A377KNG7_9ENTE Probable manganese-dependent inorganic pyrophosphatase OSEnterococcus durans OX53345 GNppaC PE3 SV1	1.296	10.558	10.161
trA0A377KMM5A0A377KMM5_9ENTE Glucose-6-phosphate 1-dehydrogenase OSEnterococcus durans OX53345 GNzwf PE3 SV1	1.232	0.9845	0.88015
trA0A377KN79A0A377KN79_9ENTE Cation transporter E1-E2 family ATPase OSEnterococcus durans OX53345 GNctpF PE4 SV1	1.103	0.97205	0.87323
trA0A377KI54A0A377KI54_9ENTE ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNYufO PE4 SV1	1.032	0.90021	13.699
trA0A377KHNOA0A377KHNO_9ENTE Deoxyuridine 5-triphosphate nucleotidohydrolase OSEnterococcus durans OX53345 GNdut PE3 SV1	2.32	1.858	17.155
trA0A377KLM6A0A377KLM6_9ENTE Enoyl-acyl-carrier-protein reductase NADH OSEnterococcus durans OX53345 GNfabI PE3 SV1	1.1	0.90572	0.97381
trA0A2A7SLE6A0A2A7SLE6_9ENTE Redox-sensing transcriptional repressor Rex OSEnterococcus durans OX53345 GNrex1 PE3 SV1	1.09	0.88892	0.79365
trA0A377KI95A0A377KI95_9ENTE Bacterial membrane protein YfhO OSEnterococcus durans OX53345 GNYfhO PE4 SV1	0.98761	0.81095	0.7243
trA0A377KMR6A0A377KMR6_9ENTE Signal peptide peptidase SppA OSEnterococcus durans OX53345 GNsppA PE4 SV1	0.98581	0.77911	0.75338
trA0A377KN69A0A377KN69_9ENTE Sulfatase OSEnterococcus durans OX53345 GNltaS1_2 PE4 SV1	0.98503	0.78045	0.73804
trA0A377L19A0A377L19_9ENTE dTDP-4-dehydrorhamnose 3 5-epimerase OSEnterococcus durans OX53345 GNrfbC PE3 SV1	0.98104	0.99454	10.804
trA0A2A7SR71A0A2A7SR71_9ENTE DNA protection during starvation protein 1 OSEnterococcus durans OX53345 GNdps PE3 SV1	0.9785	11.023	10.359
trA0A377KLX2A0A377KLX2_9ENTE Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta OSEnterococcus durans OX53345 GNaccD_2 PE3 SV1	0.97442	0.78266	0.83471
trA0A2A7SKC4A0A2A7SKC4_9ENTE PTS system mannose/fructose/sorbose-specific IIAB component OSEnterococcus durans OX53345 GNmanX_2 PE4 SV1	0.97082	0.81778	0.86994
trA0A377KND0A0A377KND0_9ENTE Glucose-6-phosphate isomerase OSEnterococcus durans OX53345 GNpgi PE3 SV1	0.96894	0.80489	0.88149
trA0A377KNF0A0A377KNF0_9ENTE Putative phage-encoded protein-like protein OSEnterococcus durans OX53345 GNNCTC8129_02877 PE4 SV1	0.95985	0.92662	0.8296
trA0A377KKZ5A0A377KKZ5_9ENTE Hydrolase OSEnterococcus durans OX53345 GNNCTC8129_02098 PE4 SV1	0.95115	0.75619	0.67577
trA0A377KLN2A0A377KLN2_9ENTE Glycoside hydrolase OSEnterococcus durans OX53345 GNwecA PE4 SV1	0.94926	12.642	13.318
trA0A2A7SLI0A0A2A7SLI0_9ENTE HAD superfamily hydrolase OSEnterococcus durans OX53345 GNEA71_00918 PE4 SV1	0.9441	0.75001	0.66957
trA0A377KJ39A0A377KJ39_9ENTE Dipeptidase PepV OSEnterococcus durans OX53345 GNpepV_2 PE4 SV1	0.94256	0.74538	0.68502
trA0A2A7SRV3A0A2A7SRV3_9ENTE Preprotein translocase subunit YajC OSEnterococcus durans OX53345 GNYajC PE4 SV1	0.93945	0.78933	0.71896
trA0A377KNP8A0A377KNP8_9ENTE Glycine--tRNA ligase alpha subunit OSEnterococcus durans OX53345 NGlyQ PE3 SV1	0.9388	10.153	0.94756
trA0A2A7SLJ7A0A2A7SLJ7_9ENTE ATP-dependent 6-phosphofructokinase OSEnterococcus durans OX53345 GNpfkA PE3 SV1	0.93219	0.79871	0.77361
trA0A2A7SKG6A0A2A7SKG6_9ENTE PTS sugar transporter subunit IIB OSEnterococcus durans OX53345 GNlicB_3 PE4 SV1	0.93127	29.469	27.175
trA0A367CFB0A0A367CFB0_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNEA71_02076 PE4 SV1	0.92813	0.74994	0.98035
trA0A377KGC7A0A377KGC7_9ENTE Ribonuclease J OSEnterococcus durans OX53345 GNrnjA PE3 SV1	0.92559	0.89107	0.81518
trA0A377KJ82A0A377KJ82_9ENTE Aspartate--tRNA ligase OSEnterococcus durans OX53345 GNaspS PE3 SV1	0.92461	0.8262	0.77976
trA0A377KH35A0A377KH35_9ENTE Shikimate kinase OSEnterococcus durans OX53345 GNaroK PE3 SV1	0.9211	0.76727	0.76188
trA0A377KHH9A0A377KHH9_9ENTE Phosphopentomutase OSEnterococcus durans OX53345 GNdeoB PE3 SV1	0.9208	0.94962	0.97745
trA0A377K04A0A377K04_9ENTE Glycerol-3-phosphate dehydrogenase OSEnterococcus durans OX53345 GNglpO PE4 SV1	0.91653	25.265	35.077
trA0A377KME6A0A377KME6_9ENTE Dihydroorotase OSEnterococcus durans OX53345 GNpyrC PE3 SV1	0.91567	2.117	20.061
trA0A377KHH9A0A377KHH9_9ENTE Autolysin OSEnterococcus durans OX53345 GNNCTC8129_00758 PE4 SV1	0.90438	0.83218	1.406

trAOA377KM47A0A377KM47_9ENTE Hydroxymethylglutaryl-CoA synthase OSEnterococcus durans OX53345 GNpksG PE4 SV1	0.8972	0.83889	0.78247
trAOA377KK39A0A377KK39_9ENTE Methionine synthase II Cobalamin-independent OSEnterococcus durans OX53345 GNmetE_1 PE3 SV1	0.8962	0.78772	1.064
trAOA377KIW4A0A377KIW4_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNNCTC8129_01354 PE4 SV1	0.89442	0.71491	0.64173
trAOA377K9A0A377K9_9ENTE 3-oxoacyl-acyl-carrier-protein synthase 2 OSEnterococcus durans OX53345 GNfabF PE3 SV1	0.89203	0.70498	0.83045
trAOA377KHM9A0A377KHM9_9ENTE Pyrroline-5-carboxylate reductase OSEnterococcus durans OX53345 GNproC PE3 SV1	0.89108	0.70639	0.72496
trAOA377KHH3A0A377KHH3_9ENTE NlpC/P60 family lipoprotein OSEnterococcus durans OX53345 GNNCTC8129_00818 PE4 SV1	0.8885	11.014	13.027
trAOA367CCK2A0A367CCK2_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNEA71_01136 PE4 SV1	0.88829	0.91436	12.306
trAOA377KLR0A0A377KLR0_9ENTE Response regulator OSEnterococcus durans OX53345 GNgraR PE4 SV1	0.88425	0.71377	0.75068
trAOA377K1A0A377K1_9ENTE OsmC/Ohr family protein OSEnterococcus durans OX53345 GNohrB_2 PE4 SV1	0.88379	0.91188	0.83327
trAOA248V7T4A0A248V7T4_9ENTE Peptide methionine sulfoxide reductase MsrB OSEnterococcus durans OX53345 GNmsrB PE3 SV1	0.88306	12.425	11.331
trAOA248V6U6A0A248V6U6_9ENTE 50S ribosomal protein L7/L12 OSEnterococcus durans OX53345 GNrplL PE3 SV1	0.88065	0.81635	0.75194
trAOA377KI30A0A377KI30_9ENTE Deoxyribose-phosphate aldolase OSEnterococcus durans OX53345 GNdeoC2 PE3 SV1	0.8782	0.96046	10.765
trAOA377KP8A0A377KP8_9ENTE RNA binding protein S1 OSEnterococcus durans OX53345 GNyhgf PE4 SV1	0.87813	0.82753	0.75016
trAOA377KI8A0A377KI8_9ENTE 5-methylthioadenosine/S-adenosylhomocysteine nucleosidase OSEnterococcus durans OX53345 GNmntN PE3 SV1	0.87654	0.82512	0.83243
trAOA377KMR0A0A377KMR0_9ENTE UPF0176 protein NCTC8129_02639 OSEnterococcus durans OX53345 GNNCTC8129_02639 PE3 SV1	0.87395	0.7385	0.78966
trAOA377KG16A0A377KG16_9ENTE V-type ATPase subunit C OSEnterococcus durans OX53345 GNntpC PE4 SV1	0.87365	0.69042	0.62
trAOA377KMNSA0A377KMNS_9ENTE Bifunctional disulfide isomerase/thiol-disulfide oxidase OSEnterococcus durans OX53345 GNNCTC8129_02721 PE4 SV1	0.87353	0.89918	0.9152
trAOA2A7SSR8A0A2A7SSR8_9ENTE Thioredoxin OSEnterococcus durans OX53345 GNtrxA_1 PE4 SV1	0.87061	0.68816	0.72637
trAOA377KN18A0A377KN18_9ENTE Asparagine synthetase B OSEnterococcus durans OX53345 GNasnB PE4 SV1	0.87014	0.80683	0.95562
trAOA377KL54A0A377KL54_9ENTE CoA-binding domain-containing protein OSEnterococcus durans OX53345 GNyccU PE4 SV1	0.86998	0.70318	0.63369
trAOA377L116A0A377L116_9ENTE Primosomal protein N OSEnterococcus durans OX53345 GNNCTC8129_01743 PE4 SV1	0.86944	11.978	10.942
trAOA2A7SS09A0A2A7SS09_9ENTE TetR family dihydroxyacetone kinase regulator OSEnterococcus durans OX53345 GNdhaS PE4 SV1	0.868	0.68886	0.6779
trAOA377KMY7A0A377KMY7_9ENTE Diacylglycerol kinase catalytic subunit OSEnterococcus durans OX53345 GNdagK_1 PE4 SV1	0.86748	0.73375	0.69897
trAOA2A7SQE9A0A2A7SQE9_9ENTE 50S ribosomal protein L33 OSEnterococcus durans OX53345 GNrpmG PE3 SV1	0.86453	0.78445	0.70032
trAOA377KPS3A0A377KPS3_9ENTE UDP-N-acetylmuramate--L-alanine ligase OSEnterococcus durans OX53345 GNmurC PE3 SV1	0.86369	0.893	0.8258
trAOA2A7SQD7A0A2A7SQD7_9ENTE NAD kinase OSEnterococcus durans OX53345 GNppnK PE3 SV1	0.8616	0.68484	0.6951
trAOA377KNI3A0A377KNI3_9ENTE tRNA guanine-N1--methyltransferase OSEnterococcus durans OX53345 GNtrmD PE3 SV1	0.86126	0.68138	0.61672
trAOA377KHY3A0A377KHY3_9ENTE U32 family peptidase OSEnterococcus durans OX53345 GNyhbU_1 PE4 SV1	0.86017	0.77625	0.693
trAOA377KCC2A0A377KCC2_9ENTE DNA gyrase subunit A OSEnterococcus durans OX53345 GNgyrA PE3 SV1	0.85994	0.79013	0.73243
trAOA377KQB7A0A377KQB7_9ENTE UDP-glucose 4-epimerase OSEnterococcus durans OX53345 GNgalE_2 PE4 SV1	0.85522	0.9554	0.93823
trAOA2A7SQF5A0A2A7SQF5_9ENTE Putative tRNA cytidine34-2-O-methyltransferase OSEnterococcus durans OX53345 GNtrmL PE3 SV1	0.85306	0.71112	0.6839
trAOA377KFF9A0A377KFF9_9ENTE 2 3-bisphosphoglycerate-dependent phosphoglycerate mutase OSEnterococcus durans OX53345 GNgpmA_1 PE3 SV1	0.85013	0.67204	0.609
trAOA377KX0A0A377KX0_9ENTE ABC transporter substrate binding protein OSEnterococcus durans OX53345 GNNCTC8129_02408 PE4 SV1	0.84063	0.89496	0.8744
trAOA377KNE8A0A377KNE8_9ENTE Transcription termination/antitermination protein NusA OSEnterococcus durans OX53345 GNnusA PE3 SV1	0.83939	0.80287	0.91224
trAOA2A7SNE8A0A2A7SNE8_9ENTE Arginine deiminase pathway transcriptional regulator Crp family protein OSEnterococcus durans OX53345 GNntcA PE4 SV1	0.83748	0.66721	0.63627
trAOA377KIM5A0A377KIM5_9ENTE Phosphomevalonate kinase OSEnterococcus durans OX53345 GNNCTC8129_01237 PE4 SV1	0.8369	0.71233	0.636



trAOA377KL96A0A377KL96_9ENTE GNAT family acetyltransferase OSEnterococcus durans OX53345 GNbltD PE4 SV1	0.83085	0.67883	0.69522
trAOA2A7SPM8A0A2A7SPM8_9ENTE Nucleoid-associated protein EA71_02227 OSEnterococcus durans OX53345 GNEA71_02227 PE3 SV1	0.82701	0.6604	0.5908
trAOA2A7SMP9A0A2A7SMP9_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNCUM72_03200 PE4 SV1	0.8212	0.69734	0.78947
trAOA377KH89A0A377KH89_9ENTE 2 5-diketo-D-gluconate reductase OSEnterococcus durans OX53345 GNdkgB_2 PE4 SV1	0.81983	15.338	16.954
trAOA377KI93A0A377KI93_9ENTE Putative type IV conjugative transfer system protein TraL OSEnterococcus durans OX53345 GNtraL PE4 SV1	0.81976	0.66206	0.59779
trAOA367CIG0A0A367CIG0_9ENTE ABC superfamily ATP binding cassette transporter ABC protein OSEnterococcus durans OX53345 GNsufC PE4 SV1	0.81824	10.694	10.496
trAOA377KPK1A0A377KPK1_9ENTE Stress response protein OSEnterococcus durans OX53345 GNgapA_3 PE4 SV1	0.80414	0.82423	10.086
trAOA2A7SQ46A0A2A7SQ46_9ENTE Nicotinate phosphoribosyltransferase OSEnterococcus durans OX53345 GNEA71_02432 PE3 SV1	0.8028	0.74427	0.66464
trAOA377KIQ1A0A377KIQ1_9ENTE LysM domain-containing protein OSEnterococcus durans OX53345 GNNCTC8129_01502 PE4 SV1	0.80269	0.6584	0.59339
trAOA377KKQ6A0A377KKQ6_9ENTE HAD superfamily hydrolase OSEnterococcus durans OX53345 GNyidA_3 PE4 SV1	0.80128	0.63876	0.67762
trAOA2A7SM37A0A2A7SM37_9ENTE ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNglNq_2 PE4 SV1	0.79082	0.65063	0.98579
trAOA2A7SN15A0A2A7SN15_9ENTE Protein of hypothetical function OSEnterococcus durans OX53345 GNEA71_01569 PE4 SV1	0.78877	0.63935	0.64397
trAOA377KLJ0A0A377KLJ0_9ENTE Phosphoesterase OSEnterococcus durans OX53345 GNNCTC8129_02312 PE3 SV1	0.78694	0.686	0.72721
trAOA377KLU7A0A377KLU7_9ENTE Orotate phosphoribosyltransferase OSEnterococcus durans OX53345 GNpyrE PE3 SV1	0.78435	17.928	16.063
trAOA377KID6A0A377KID6_9ENTE Glutathione biosynthesis bifunctional protein GshAB OSEnterococcus durans OX53345 GNgshAB PE3 SV1	0.78143	0.6232	0.55782
trAOA248V5I4A0A248V5I4_9ENTE GatB/YqeY domain-containing protein OSEnterococcus durans OX53345 GNyqeY PE4 SV1	0.77515	0.62062	0.56283
trAOA377KKI2A0A377KKI2_9ENTE dTDP-4-dehydrorhamnose reductase OSEnterococcus durans OX53345 GNrmID PE3 SV1	0.77321	0.79278	0.72597
trAOA377KKN5A0A377KKN5_9ENTE 2-hydroxy-3-oxopropionate reductase OSEnterococcus durans OX53345 GNgarR PE4 SV1	0.77241	0.64109	0.57418
trAOA377KH26A0A377KH26_9ENTE Bifunctional folylpolyglutamate synthase/ dihydrofolate synthase family protein OSEnterococcus durans OX53345 GNfgs PE3 SV1	0.76918	0.60885	0.54765
trAOA377KKN0A0A377KKN0_9ENTE 3-oxoacyl-ACP reductase OSEnterococcus durans OX53345 GNfabG PE4 SV1	0.76836	0.60863	0.58577
trAOA377KMW4A0A377KMW4_9ENTE tRNA N6-adenosine threonylcarbamoyltransferase OSEnterococcus durans OX53345 GNgcP PE3 SV1	0.76816	0.60784	0.56588
trAOA2A7SPP7A0A2A7SPP7_9ENTE Fructose-16-bisphosphate aldolase class II OSEnterococcus durans OX53345 GNfba_2 PE3 SV1	0.76145	0.69328	0.62038
trAOA377KM44A0A377KM44_9ENTE Dihydroorotate dehydrogenase OSEnterococcus durans OX53345 GNpyrDA PE3 SV1	0.761	0.91115	0.95645
trAOA377KM84A0A377KM84_9ENTE Cytidylate kinase OSEnterococcus durans OX53345 GNcmk PE3 SV1	0.755	0.93178	0.92113
trAOA377KME0A0A377KME0_9ENTE DNA polymerase I OSEnterococcus durans OX53345 GNpolA PE3 SV1	0.75347	0.61842	0.59665
trAOA377KHG6A0A377KHG6_9ENTE Hypoxanthine phosphoribosyltransferase OSEnterococcus durans OX53345 GNhpt PE3 SV1	0.75334	0.61947	0.62618
trAOA2A7SMV8A0A2A7SMV8_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNEA71_01264 PE4 SV1	0.74766	0.92632	0.85586
trAOA377KR58A0A377KR58_9ENTE Glycine--tRNA ligase beta subunit OSEnterococcus durans OX53345 GNglyS PE3 SV1	0.7475	0.72424	0.67564
trAOA377L368A0A377L368_9ENTE GMP reductase OSEnterococcus durans OX53345 GNguaC PE3 SV1	0.74681	0.74824	0.67031
trAOA2A7SRJ2A0A2A7SRJ2_9ENTE DNA gyrase subunit B OSEnterococcus durans OX53345 GNgyrB PE3 SV1	0.74672	0.84301	0.75688
trAOA367CBU0A0A367CBU0_9ENTE LuxR family DNA-binding response regulator OSEnterococcus durans OX53345 GNvraR PE4 SV1	0.74476	0.59523	0.56804
trAOA367CBN9A0A367CBN9_9ENTE Cell division protein SepF OSEnterococcus durans OX53345 GNsepF PE3 SV1	0.74411	0.68289	0.8687
trAOA377KLI8A0A377KLI8_9ENTE DNA repair protein RecN OSEnterococcus durans OX53345 GNrecN PE3 SV1	0.74309	0.59385	0.54272
trAOA377KGH5A0A377KGH5_9ENTE DHH family protein OSEnterococcus durans OX53345 GNnra_1 PE4 SV1	0.74143	11.689	10.465
trAOA377KJE4A0A377KJE4_9ENTE Purine nucleoside phosphorylase OSEnterococcus durans OX53345 GNpunA PE3 SV1	0.73917	0.79657	0.96255

trA0A2A7SPW4A0A2A7SPW4_9ENTE CsbD-like protein OSEnterococcus durans OX53345 GNEA71_02356 PE3 SV1	0.73718	0.68916	0.6543
trA0A2A7SR29A0A2A7SR29_9ENTE Pantothenate kinase OSEnterococcus durans OX53345 GNcoaA PE3 SV1	0.73095	0.58788	0.57324
trA0A248V7D7A0A248V7D7_9ENTE Lysine--tRNA ligase OSEnterococcus durans OX53345 GNlysS PE3 SV1	0.72829	0.61382	0.56222
trA0A377KFT2A0A377KFT2_9ENTE Catabolite control protein A OSEnterococcus durans OX53345 GNccpA_1 PE4 SV1	0.72425	0.70579	0.64073
trA0A377KMS1A0A377KMS1_9ENTE Glutamyl-tRNA <sup>Gln</sup> amidotransferase subunit A OSEnterococcus durans OX53345 GNgatA PE3 SV1	0.72141	0.6079	0.6913
trA0A2A7SKA6A0A2A7SKA6_9ENTE Mur ligase middle domain-containing protein OSEnterococcus durans OX53345 GNEA71_00568 PE4 SV1	0.72063	0.5739	0.51848
trA0A377KLP1A0A377KLP1_9ENTE Ribonuclease Z OSEnterococcus durans OX53345 GNrnz PE3 SV1	0.71866	0.67052	0.61005
trA0A377KKQ7A0A377KKQ7_9ENTE Biotin carboxyl carrier protein of acetyl-CoA carboxylase OSEnterococcus durans OX53345 GNaccB PE4 SV1	0.71131	0.58594	0.89426
trA0A2A7SPH2A0A2A7SPH2_9ENTE Cell division protein ZapA OSEnterococcus durans OX53345 GNzapA PE4 SV1	0.71116	0.57695	0.61331
trA0A2A7SKL9A0A2A7SKL9_9ENTE HIT family protein OSEnterococcus durans OX53345 GNhit PE4 SV1	0.71107	0.56395	0.65744
trA0A377KLZ1A0A377KLZ1_9ENTE Phosphomethylpyrimidine kinase OSEnterococcus durans OX53345 GNpdxK PE4 SV1	0.70285	0.86256	0.90635
trA0A377KFS1A0A377KFS1_9ENTE ATPases with chaperone activity ATP-binding subunit OSEnterococcus durans OX53345 GNclpC_1 PE4 SV1	0.70211	0.57234	0.51443
trA0A2A7SIX2A0A2A7SIX2_9ENTE Uracil phosphoribosyltransferase OSEnterococcus durans OX53345 GNupp PE3 SV1	0.69875	0.59465	0.55966
trA0A377KIROA0A377KIRO_9ENTE PTS system N-acetylglucosamine-specific EIICBA component OSEnterococcus durans OX53345 GNptsG_1 PE4 SV1	0.69846	0.63759	0.6245
trA0A377KG20A0A377KG20_9ENTE V-type ATP synthase alpha chain OSEnterococcus durans OX53345 GNntpA PE3 SV1	0.69734	0.55521	0.49956
trA0A2A7SQ30A0A2A7SQ30_9ENTE ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNmacB_1 PE4 SV1	0.69489	0.66862	0.65942
trA0A2A7SPE3A0A2A7SPE3_9ENTE Phosphoglycerate kinase OSEnterococcus durans OX53345 GNpgk PE3 SV1	0.69401	0.62709	0.61781
trA0A377KPT7A0A377KPT7_9ENTE Peptidase T OSEnterococcus durans OX53345 GNpepT PE3 SV1	0.69308	0.56407	0.50739
trA0A377KGY0A0A377KGY0_9ENTE Phosphoglycerate mutase family protein OSEnterococcus durans OX53345 GNcobC_1 PE4 SV1	0.69011	0.57396	0.59109
trA0A377KHI6A0A377KHI6_9ENTE Protein phosphatase 2C OSEnterococcus durans OX53345 GNstp_3 PE4 SV1	0.68693	0.55478	0.54498
trA0A2A7SQ04A0A2A7SQ04_9ENTE Peptidyl-prolyl cis-trans isomerase OSEnterococcus durans OX53345 GNppi PE3 SV1	0.68273	0.60249	0.54709
trA0A377KNJ5A0A377KNJ5_9ENTE Flavin reductase OSEnterococcus durans OX53345 GNazr PE4 SV1	0.6808	0.91864	11.038
trA0A377KMB2A0A377KMB2_9ENTE General stress protein Gls33 OSEnterococcus durans OX53345 GNglS33 PE4 SV1	0.67747	0.54614	0.97485
trA0A377KI04A0A377KI04_9ENTE ATP-dependent Clp protease ATP-binding protein ClpC OSEnterococcus durans OX53345 GNclpC_2 PE3 SV1	0.67568	0.53397	0.50042
trA0A2A7SLH9A0A2A7SLH9_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNCUM72_12610 PE4 SV1	0.67551	0.53687	0.4866
trA0A377KKG5A0A377KKG5_9ENTE Methionine aminopeptidase OSEnterococcus durans OX53345 GNmap PE3 SV1	0.67304	0.61141	0.55513
trA0A377L556A0A377L556_9ENTE UTP--glucose-1-phosphate uridylyltransferase OSEnterococcus durans OX53345 GNgalU PE3 SV1	0.67228	0.53954	0.4953
trA0A377KNL3A0A377KNL3_9ENTE Copper-translocating P-type ATPase OSEnterococcus durans OX53345 GNcopB PE3 SV1	0.67047	0.70901	0.6413
trA0A2A7SLK8A0A2A7SLK8_9ENTE DNA topoisomerase 4 subunit B OSEnterococcus durans OX53345 GNparE PE3 SV1	0.66935	0.53538	0.5112
trA0A377KM75A0A377KM75_9ENTE Glutamate racemase OSEnterococcus durans OX53345 GNmurI PE3 SV1	0.66915	0.53358	0.55296
trA0A377KKQ1A0A377KKQ1_9ENTE Low molecular weight protein tyrosine phosphatase OSEnterococcus durans OX53345 GNyfkj PE3 SV1	0.66531	0.52775	0.47171
trA0A2A7SKN6A0A2A7SKN6_9ENTE Pyrrolidone-carboxylate peptidase OSEnterococcus durans OX53345 GNpcp PE3 SV1	0.66448	0.54383	0.54566
trA0A377KKE9A0A377KKE9_9ENTE Phosphopantothenoylecysteine decarboxylase OSEnterococcus durans OX53345 GNcoaBC PE4 SV1	0.66259	0.57046	0.58653
trA0A377KLY8A0A377KLY8_9ENTE Dihydroliipoamide acetyltransferase component of pyruvate dehydrogenase complex OSEnterococcus durans OX53345 GNpdhC PE3 SV1	0.66221	1.046	0.93798
trA0A2A7SLC7A0A2A7SLC7_9ENTE Trk system potassium uptake protein TrkA OSEnterococcus durans OX53345 GNktrA PE4 SV1	0.6609	0.75783	0.87578
trA0A377KI48A0A377KI48_9ENTE Aspartate-semialdehyde dehydrogenase OSEnterococcus durans OX53345 GNasd PE3 SV1	0.65942	0.9179	10.743

trAOA377KLJ9A0A377KLJ9_ENTE UDP-N-acetylglucosamine--N-acetylmuramyl-pentapeptide pyrophosphoryl-undecaprenol N-acetylglucosamine transferase OSEnterococcus durans OX53345 GNmurG_2 PE3 SV1	0.65613	0.52174	0.61825
trAOA377KMY6A0A377KMY6_ENTE Biotin carboxylase OSEnterococcus durans OX53345 GNaccC PE4 SV1	0.65271	0.59396	0.55046
trAOA377MS03A0A377MS03_ENTE Pyridine nucleotide-disulfide oxidoreductase OSEnterococcus durans OX53345 GNpdhD_3 PE3 SV1	0.64137	10.902	11.379
trAOA248V5T9A0A248V5T9_ENTE Thioredoxin reductase OSEnterococcus durans OX53345 GNtrxB PE3 SV1	0.63932	0.56644	0.57786
trAOA377KK29A0A377KK29_ENTE Phosphopantothenate--cysteine ligase OSEnterococcus durans OX53345 GNNCTC8129_01769 PE4 SV1	0.63921	11.073	0.99579
trAOA377KNK6A0A377KNK6_ENTE N-acetylglucosamine-6-phosphate deacetylase OSEnterococcus durans OX53345 GNnagA PE3 SV1	0.63448	0.50219	0.45022
trAOA2S7MFM9A0A2S7MFM9_ENTE Pyruvate kinase OSEnterococcus durans OX53345 GNpyk PE3 SV1	0.63414	0.6762	0.65788
trAOA377KFT5A0A377KFT5_ENTE HPr kinase/phosphorylase OSEnterococcus durans OX53345 GNhprK PE3 SV1	0.63301	0.74407	0.70144
trAOA377KP54A0A377KP54_ENTE Carbamoyl-phosphate synthase small chain OSEnterococcus durans OX53345 GNcarA PE3 SV1	0.62765	18.727	17.671
trAOA377KFX3A0A377KFX3_ENTE UDP-N-acetylglucosamine 1-carboxyvinyltransferase OSEnterococcus durans OX53345 GNmurA2 PE3 SV1	0.62355	0.57413	0.60187
trAOA377KH46A0A377KH46_ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNNCTC8129_00394 PE3 SV1	0.62043	0.64952	0.71052
trAOA2A7SQR5A0A2A7SQR5_ENTE S-ribosylhomocysteine lyase OSEnterococcus durans OX53345 GNluxS PE3 SV1	0.61997	0.56181	0.52906
trAOA377KIP2A0A377KIP2_ENTE 50S ribosomal protein L20 OSEnterococcus durans OX53345 GNrpIT1 PE3 SV1	0.61972	0.72488	0.64823
trAOA377KH06A0A377KH06_ENTE Primosomal protein N OSEnterococcus durans OX53345 GNNCTC8129_00238 PE4 SV1	0.61819	0.82619	0.74538
trAOA377KQ13A0A377KQ13_ENTE Phosphoglucosamine mutase OSEnterococcus durans OX53345 GNglmM PE3 SV1	0.6179	0.48832	0.4755
trAOA2A7SR68A0A2A7SR68_ENTE Ribose-phosphate pyrophosphokinase OSEnterococcus durans OX53345 GNprsA4 PE3 SV1	0.61759	0.67079	0.60129
trAOA377KMC7A0A377KMC7_ENTE Fumarate reductase flavoprotein subunit OSEnterococcus durans OX53345 GNfccA PE4 SV1	0.61575	13.158	12.461
trAOA377KR02A0A377KR02_ENTE Asparagine--tRNA ligase OSEnterococcus durans OX53345 GNasnS PE3 SV1	0.61137	0.52256	0.46667
trAOA2A7SRW3A0A2A7SRW3_ENTE Holliday junction ATP-dependent DNA helicase RuvB OSEnterococcus durans OX53345 GNruvB PE3 SV1	0.61126	0.49202	0.50211
trAOA377KP04A0A377KP04_ENTE 6-phospho-beta-galactosidase OSEnterococcus durans OX53345 GNlacG_2 PE3 SV1	0.60636	19.186	22.043
trAOA377KH9A0A377KH9_ENTE Serine/threonine-protein kinase OSEnterococcus durans OX53345 GNprkC PE4 SV1	0.60378	0.6265	0.59243
trAOA2A7SLW7A0A2A7SLW7_ENTE Bifunctional protein PyrR OSEnterococcus durans OX53345 GNpyrR PE3 SV1	0.60058	11.715	10.473
trAOA377KJ7A0A377KJ7_ENTE Polysaccharide deacetylase OSEnterococcus durans OX53345 GNNCTC8129_01271 PE4 SV1	0.59944	0.5534	0.55125
trAOA377KLT2A0A377KLT2_ENTE Superoxide dismutase OSEnterococcus durans OX53345 GNsodA PE3 SV1	0.59897	0.47956	0.53972
trAOA377KK85A0A377KK85_ENTE M42 glutamyl aminopeptidase OSEnterococcus durans OX53345 GNysdC PE3 SV1	0.59708	0.47391	0.43159
trAOA2A7SLE1A0A2A7SLE1_ENTE Elongation factor Tu OSEnterococcus durans OX53345 GNtuf PE3 SV1	0.59532	0.74921	0.66925
trAOA377KMF5A0A377KMF5_ENTE Nucleoside 2-deoxyribosyltransferase OSEnterococcus durans OX53345 GNEA71_01539 PE4 SV1	0.59352	0.50532	0.73737
trAOA377KG21A0A377KG21_ENTE V-type ATPase subunit E OSEnterococcus durans OX53345 GNNCTC8129_00254 PE4 SV1	0.5892	0.47127	0.50218
trAOA377KL65A0A377KL65_ENTE Transcription elongation factor GreA OSEnterococcus durans OX53345 GNgreA PE3 SV1	0.58827	0.49246	0.63825
trAOA377KNM2A0A377KNM2_ENTE ErpQ protein OSEnterococcus durans OX53345 GNerpQ PE4 SV1	0.58733	0.96962	0.9964
trAOA2A7SRF1A0A2A7SRF1_ENTE L-lactate dehydrogenase OSEnterococcus durans OX53345 GNldh PE3 SV1	0.58537	0.5018	0.45059
trAOA2A7SM63A0A2A7SM63_ENTE GTPase Obg OSEnterococcus durans OX53345 GNobg PE3 SV1	0.58397	0.78332	0.81104
trAOA2A7SMK4A0A2A7SMK4_ENTE Aspartyl/glutamyl-tRNAAsn/Gln amidotransferase subunit C OSEnterococcus durans OX53345 GNgatC_4 PE3 SV1	0.58354	0.46554	0.44802
trAOA377KNG2A0A377KNG2_ENTE Glutathione reductase OSEnterococcus durans OX53345 GNpdhD_1 PE4 SV1	0.58352	0.57274	0.55912
trAOA377KJY5A0A377KJY5_ENTE Bifunctional protein GlmU OSEnterococcus durans OX53345 GNglmU PE3 SV1	0.57661	0.5855	0.52588
trAOA377KLK6A0A377KLK6_ENTE GTP diphosphokinase OSEnterococcus durans OX53345 GNrelA_3 PE3 SV1	0.57538	0.51712	0.73881

trAOA377KKB0A0A377KKB0_9ENTE PTS system mannose/fructose/sorbose transporter subunit IID OSEnterococcus durans OX53345 GNmanZ_3 PE4 SV1	0.57446	10.432	11.535
trAOA2A7SQL6A0A2A7SQL6_9ENTE CBS protein OSEnterococcus durans OX53345 GNEA71_02483 PE4 SV1	0.56667	0.45232	0.40382
trAOA367CEA1A0A367CEA1_9ENTE Chaperone protein ClpB OSEnterococcus durans OX53345 GNclpB PE3 SV1	0.56571	0.47804	0.44135
trAOA2A7SS91A0A2A7SS91_9ENTE Recombinase family protein OSEnterococcus durans OX53345 GNhin_3 PE4 SV1	0.55429	0.6665	0.83693
trAOA367CDS8A0A367CDS8_9ENTE Primosomal protein Dnal OSEnterococcus durans OX53345 GNdnaL PE4 SV1	0.55384	0.44905	0.43069
trAOA377KHK2A0A377KHK2_9ENTE S4 RNA-binding domain-containing protein OSEnterococcus durans OX53345 GNNCTC8129_00807 PE4 SV1	0.54816	0.6576	0.58726
trAOA377KIT8A0A377KIT8_9ENTE DNA mismatch repair protein MutL OSEnterococcus durans OX53345 GNmutL PE3 SV1	0.54687	0.49977	0.50177
trAOA377KN56A0A377KN56_9ENTE Proline--tRNA ligase OSEnterococcus durans OX53345 GNproS PE3 SV1	0.54191	0.52428	0.4866
trAOA2A7SMU4A0A2A7SMU4_9ENTE Carbamoyl phosphate synthase-like protein OSEnterococcus durans OX53345 GNEA71_01655 PE4 SV1	0.54102	0.72503	0.65272
trAOA377KLC8A0A377KLC8_9ENTE Phosphoribosylformylglycinamide synthase subunit PurQ OSEnterococcus durans OX53345 GNpurQ PE3 SV1	0.54089	10.805	10.365
trAOA377KJY0A0A377KJY0_9ENTE Dihydroxyacetone kinase family protein OSEnterococcus durans OX53345 GNdhaL PE4 SV1	0.54028	10.619	0.94905
trAOA377KIS7A0A377KIS7_9ENTE Glutamyl aminopeptidase OSEnterococcus durans OX53345 GNpepA_1 PE3 SV1	0.53118	0.56245	0.51751
trAOA377KK67A0A377KK67_9ENTE Protein DltD OSEnterococcus durans OX53345 GNdltD PE3 SV1	0.53072	0.71858	10.461
trAOA377KH1A0A377KH1_9ENTE Probable transcriptional regulatory protein NCTC8129_00814 OSEnterococcus durans OX53345 GNNCTC8129_00814 PE3 SV1	0.52447	0.49453	11.839
trAOA377KJM0A0A377KJM0_9ENTE FeS assembly protein SufB OSEnterococcus durans OX53345 GNNCTC8129_01606 PE4 SV1	0.5199	0.69648	0.65001
trAOA377KIX2A0A377KIX2_9ENTE 50S ribosomal protein L25 OSEnterococcus durans OX53345 GNrplY PE3 SV1	0.50914	0.42381	0.44342
trAOA377KG89A0A377KG89_9ENTE Phosphate ABC transporter substrate-binding protein OSEnterococcus durans OX53345 GNpst_1 PE4 SV1	0.50806	0.42821	0.38426
trAOA377KNV6A0A377KNV6_9ENTE ATP-dependent DNA helicase OSEnterococcus durans OX53345 GNpca_2 PE3 SV1	0.50743	0.41469	0.42992
trAOA377KKR7A0A377KKR7_9ENTE 6-phosphogluconate dehydrogenase decarboxylating OSEnterococcus durans OX53345 GNngd PE3 SV1	0.50417	0.41633	0.41034
trAOA377KN68A0A377KN68_9ENTE Glutamate dehydrogenase OSEnterococcus durans OX53345 GNgdhA PE3 SV1	0.50316	0.97945	0.93893
trAOA377KGT6A0A377KGT6_9ENTE NH3-dependent NAD synthetase OSEnterococcus durans OX53345 GNnadE PE3 SV1	0.50124	0.7724	0.73182
trAOA2A7SN94A0A2A7SN94_9ENTE Cytosolic protein YlxR OSEnterococcus durans OX53345 GNYlxR PE4 SV1	0.49964	0.65555	0.59416
trAOA377KKM5A0A377KKM5_9ENTE Family 5 extracellular solute-binding protein OSEnterococcus durans OX53345 GNappa PE4 SV1	0.49914	0.39531	0.35926
trAOA2A7SRB8A0A2A7SRB8_9ENTE Single-stranded DNA-binding protein OSEnterococcus durans OX53345 GNsb PE3 SV1	0.49908	0.49951	0.44635
trAOA2A7SPD0A0A2A7SPD0_9ENTE GNAT family acetyltransferase OSEnterococcus durans OX53345 GNEA71_02120 PE4 SV1	0.49722	0.39398	0.35236
trAOA2A7SSA5A0A2A7SSA5_9ENTE 50S ribosomal protein L16 OSEnterococcus durans OX53345 GNrplP PE3 SV1	0.49667	0.48953	0.45286
trAOA2A7SPT7A0A2A7SPT7_9ENTE Glycine betaine transport ATP-binding protein OpuAA OSEnterococcus durans OX53345 GNproV_1 PE4 SV1	0.49506	0.5519	0.58827
trAOA377KLP0A0A377KLP0_9ENTE Predicted membrane protein OSEnterococcus durans OX53345 GNNCTC8129_02325 PE4 SV1	0.4923	0.43169	0.81114
trAOA377KMN9A0A377KMN9_9ENTE Aspartyl/glutamyl-tRNA <sup>Asn</sup> /Gln amidotransferase subunit B OSEnterococcus durans OX53345 GNgatB_3 PE3 SV1	0.48848	0.44323	0.44031
trAOA2A7SP52A0A2A7SP52_9ENTE UPF0154 protein CUM72_11575 OSEnterococcus durans OX53345 GNYneF PE3 SV1	0.48811	0.5361	0.68734
trAOA377KHG5A0A377KHG5_9ENTE S1 RNA binding domain-containing protein OSEnterococcus durans OX53345 GNYugl_2 PE4 SV1	0.48761	0.38909	0.35027
trAOA377KMQ4A0A377KMQ4_9ENTE Putative gluconeogenesis factor OSEnterococcus durans OX53345 GNNCTC8129_02726 PE3 SV1	0.48584	0.38545	0.36375
trAOA377KLU5A0A377KLU5_9ENTE Phenylalanine--tRNA ligase beta subunit OSEnterococcus durans OX53345 GNpheT_3 PE3 SV1	0.48398	0.44712	0.46075
trAOA377KH63A0A377KH63_9ENTE Deoxynucleoside kinase OSEnterococcus durans OX53345 GNduka PE4 SV1	0.48144	0.39397	0.43596
trAOA377KHP0A0A377KHP0_9ENTE NmrA-like family protein OSEnterococcus durans OX53345 GNqorB PE4 SV1	0.47166	0.87306	0.82615
trAOA377KHY4A0A377KHY4_9ENTE Integral membrane protein OSEnterococcus durans OX53345 GNNCTC8129_00999 PE4 SV1	0.47118	0.59503	0.53944

trAOA2A7SRA9A0A2A7SRA9_9ENTE Membrane protein insertase YidC OSEnterococcus durans OX53345 GNmisCA PE3 SV1	0.47036	0.42089	0.37621
trAOA377KIJ5A0A377KIJ5_9ENTE D-alanine--D-alanine ligase OSEnterococcus durans OX53345 GNddl PE3 SV1	0.46708	0.36912	0.34059
trAOA377KQ46A0A377KQ46_9ENTE UvrABC system protein A OSEnterococcus durans OX53345 GNuvrA PE3 SV1	0.46707	0.38618	0.37139
trAOA248V856A0A248V856_9ENTE ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNoppF PE3 SV1	0.46545	0.37471	0.33562
trAOA377KKE3A0A377KKE3_9ENTE Isoleucine--tRNA ligase OSEnterococcus durans OX53345 GNileS PE3 SV1	0.46538	0.53106	0.48635
trAOA377KKV0A0A377KKV0_9ENTE Regulatory protein RecX OSEnterococcus durans OX53345 GNrecX PE3 SV1	0.46534	0.36952	0.78022
trAOA377KJR9A0A377KJR9_9ENTE ATP-dependent zinc metalloprotease FtsH OSEnterococcus durans OX53345 GNhflB PE3 SV1	0.46519	0.41784	0.38461
trAOA248V551A0A248V551_9ENTE Triosephosphate isomerase OSEnterococcus durans OX53345 GNtpiA PE3 SV1	0.46446	0.41112	0.37632
trAOA377KN93A0A377KN93_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNNCTC8129_02064 PE4 SV1	0.46426	0.64544	0.57626
trAOA377KNF1A0A377KNF1_9ENTE Short chain dehydrogenase/reductase family oxidoreductase OSEnterococcus durans OX53345 GNfad PE4 SV1	0.46242	0.36679	0.74436
trAOA377KJ53A0A377KJ53_9ENTE 3-5 exoribonuclease yhaM OSEnterococcus durans OX53345 GNyhaM PE4 SV1	0.45927	0.83971	11.119
trAOA377KJZ9A0A377KJZ9_9ENTE Snf2 family protein OSEnterococcus durans OX53345 GNNCTC8129_01489 PE4 SV1	0.45676	0.36183	0.93634
trAOA377KPZ1A0A377KPZ1_9ENTE DNA ligase OSEnterococcus durans OX53345 GNligA PE3 SV1	0.45374	0.3706	0.48037
trAOA377KKI0A0A377KKI0_9ENTE D-aminoacyl-tRNA deacylase OSEnterococcus durans OX53345 GNdtd PE3 SV1	0.44854	0.35467	0.35799
trAOA2A7SRP7A0A2A7SRP7_9ENTE 30S ribosomal protein S7 OSEnterococcus durans OX53345 GNrpsG PE3 SV1	0.44545	0.43608	0.41424
trAOA248VA19A0A248VA19_9ENTE GTP-sensing transcriptional pleiotropic repressor CodY OSEnterococcus durans OX53345 GNcodY PE3 SV1	0.44417	0.50931	0.6899
trAOA248V9B3A0A248V9B3_9ENTE Exodeoxyribonuclease 7 small subunit OSEnterococcus durans OX53345 GNxseB PE3 SV1	0.4428	0.36993	0.64467
trAOA377KHM4A0A377KHM4_9ENTE Methionine--tRNA ligase OSEnterococcus durans OX53345 GNmetG_1 PE3 SV1	0.44082	0.74853	0.75206
trAOA377KMZ7A0A377KMZ7_9ENTE LysR family transcriptional regulator OSEnterococcus durans OX53345 GNcynR_2 PE3 SV1	0.43886	0.3506	0.39341
trAOA377KKK9A0A377KKK9_9ENTE D-isomer specific 2-hydroxyacid dehydrogenase family protein OSEnterococcus durans OX53345 GNghrB PE3 SV1	0.43869	0.50828	0.59625
trAOA377KL69A0A377KL69_9ENTE Oxidoreductase aldo/keto reductase OSEnterococcus durans OX53345 GNyvgN PE4 SV1	0.43557	0.53599	0.49317
trAOA2A7SLK1A0A2A7SLK1_9ENTE 50S ribosomal protein L32 OSEnterococcus durans OX53345 GNrpmF PE3 SV1	0.43207	0.69913	0.62929
trAOA2A7SL91A0A2A7SL91_9ENTE Glucose-1-phosphate thymidyltransferase OSEnterococcus durans OX53345 GNrmlA1 PE3 SV1	0.42886	0.65831	0.59464
trAOA377KLH9A0A377KLH9_9ENTE Phenylalanine--tRNA ligase alpha subunit OSEnterococcus durans OX53345 GNpheS_2 PE3 SV1	0.42879	0.54648	0.48941
trAOA377KL39A0A377KL39_9ENTE Glycerol kinase OSEnterococcus durans OX53345 GNglpK PE3 SV1	0.4274	33.307	36.033
trAOA2A7SR09A0A2A7SR09_9ENTE Veg family protein OSEnterococcus durans OX53345 GNCUM72_12545 PE4 SV1	0.42714	0.95713	0.93834
trAOA377KM81A0A377KM81_9ENTE Stress response protein OSEnterococcus durans OX53345 NGgapA_2 PE4 SV1	0.42589	0.58592	0.54081
trAOA377KJH0A0A377KJH0_9ENTE Mannose-6-phosphate isomerase OSEnterococcus durans OX53345 NGmuF PE3 SV1	0.42541	0.39243	0.40556
trAOA2A7SQG0A0A2A7SQG0_9ENTE ATP-dependent Clp protease proteolytic subunit OSEnterococcus durans OX53345 GNclpP PE3 SV1	0.42481	0.33687	0.36482
trAOA2A7SMW4A0A2A7SMW4_9ENTE Asp23/Gls24 family envelope stress response protein OSEnterococcus durans OX53345 GNCUM72_00245 PE4 SV1	0.42378	0.63643	0.58111
trAOA2S7MJP1A0A2S7MJP1_9ENTE Alpha-ketoacid dehydrogenase subunit beta OSEnterococcus durans OX53345 GNpdhB PE4 SV1	0.42275	11.607	10.715
trAOA377KGM4A0A377KGM4_9ENTE TatD family hydrolase OSEnterococcus durans OX53345 GNycfH PE4 SV1	0.42075	0.33326	0.38019
trAOA377KQ36A0A377KQ36_9ENTE Pyruvate-flavodoxin oxidoreductase OSEnterococcus durans OX53345 GNporA PE3 SV1	0.41859	0.8992	0.94928
trAOA248V5F5A0A248V5F5_9ENTE Membrane protein insertase YidC OSEnterococcus durans OX53345 GNyidC PE3 SV1	0.41851	0.42323	0.39076
trAOA377KG32A0A377KG32_9ENTE Enolase OSEnterococcus durans OX53345 GNeno PE3 SV1	0.41828	0.49857	0.45102
trAOA377KLT4A0A377KLT4_9ENTE Dihydroorotate dehydrogenase OSEnterococcus durans OX53345 GNpyrDB PE3 SV1	0.41679	20.041	17.949

trA0A248VAA1A0A248VAA1_9ENTE Dihydrolipoyl dehydrogenase OSEnterococcus durans OX53345 GNpdhD_2 PE3 SV1	0.41572	0.76644	0.93054
trA0A377KJF0A0A377KJF0_9ENTE Class V aminotransferase OSEnterococcus durans OX53345 GNiscS_2 PE3 SV1	0.4143	0.53584	0.49041
trA0A377KNL6A0A377KNL6_9ENTE Amidophosphoribosyltransferase OSEnterococcus durans OX53345 GNpurF PE3 SV1	0.41426	14.305	14.419
trA0A2A7SRC3A0A2A7SRC3_9ENTE Sporulation initiation inhibitor protein Soj OSEnterococcus durans OX53345 GNsoj_4 PE4 SV1	0.41376	0.4096	0.40257
trA0A377KJL2A0A377KJL2_9ENTE Universal stress protein family OSEnterococcus durans OX53345 GNNCTC8129_01342 PE4 SV1	0.41336	0.49204	0.50243
trA0A377KIB5A0A377KIB5_9ENTE Guanylate kinase OSEnterococcus durans OX53345 GNgmK_1 PE3 SV1	0.41303	0.49457	0.55283
trA0A377KJ40A0A377KJ40_9ENTE UPF0210 protein NCTC8129_01399 OSEnterococcus durans OX53345 GNNCTC8129_01399 PE3 SV1	0.41198	0.38876	0.35251
trA0A377KN07A0A377KN07_9ENTE Amino acid ABC transporter amino acid-binding/permease OSEnterococcus durans OX53345 GNartQ_2 PE3 SV1	0.40886	0.3283	0.46575
trA0A2A7SLZ7A0A2A7SLZ7_9ENTE DNA-binding response regulator OSEnterococcus durans OX53345 GNarIR PE4 SV1	0.40816	0.33423	0.30262
trA0A377KL11A0A377KL11_9ENTE Dihydroxyacetone kinase family protein OSEnterococcus durans OX53345 GNdhak PE4 SV1	0.4081	13.156	12.303
trA0A2A7SP43A0A2A7SP43_9ENTE Rhodanese family protein OSEnterococcus durans OX53345 GNYibN PE4 SV1	0.40706	0.44006	0.42125
trA0A2A7SQF1A0A2A7SQF1_9ENTE Transcription termination factor Rho OSEnterococcus durans OX53345 GNrho PE3 SV1	0.40521	0.32272	0.28892
trA0A2A7SP06A0A2A7SP06_9ENTE Glyceraldehyde-3-phosphate dehydrogenase OSEnterococcus durans OX53345 GNgap PE3 SV1	0.40484	0.34613	0.30905
trA0A377KLK9A0A377KLK9_9ENTE Short chain dehydrogenase/reductase family oxidoreductase OSEnterococcus durans OX53345 GNNCTC8129_02327 PE3 SV1	0.40422	0.5054	0.45463
trA0A2A7SRB3A0A2A7SRB3_9ENTE DNA-binding response regulator OSEnterococcus durans OX53345 GNsrrA_1 PE4 SV1	0.40374	0.63625	0.57125
trA0A377KLV0A0A377KLV0_9ENTE Orotidine 5-phosphate decarboxylase OSEnterococcus durans OX53345 GNpyrF PE3 SV1	0.40315	17.039	15.582
trA0A377KQ24A0A377KQ24_9ENTE Nucleotide-binding protein NCTC8129_02727 OSEnterococcus durans OX53345 GNyvcJ PE3 SV1	0.40145	0.31728	0.40753
trA0A367CGA0A0A367CGA0_9ENTE V-type ATP synthase subunit G OSEnterococcus durans OX53345 GNEA71_02160 PE4 SV1	0.39867	0.34413	0.54777
trA0A377KLOA0A377KLO_9ENTE Gfo/ldh/MocA family oxidoreductase OSEnterococcus durans OX53345 GNafR PE4 SV1	0.39807	0.74828	0.71986
trA0A377KMN6A0A377KMN6_9ENTE Elongation factor 4 OSEnterococcus durans OX53345 GNlepA PE3 SV1	0.39746	0.35851	0.32798
trA0A377KGZ3A0A377KGZ3_9ENTE 3-phosphoshikimate 1-carboxyvinyltransferase OSEnterococcus durans OX53345 GNaroA PE3 SV1	0.39637	0.66953	0.63182
trA0A377KIQ6A0A377KIQ6_9ENTE Serine--tRNA ligase OSEnterococcus durans OX53345 GNserS2 PE3 SV1	0.39563	0.31278	0.32861
trA0A2A7SL95A0A2A7SL95_9ENTE DUF1797 domain-containing protein OSEnterococcus durans OX53345 GNYkuJ PE4 SV1	0.39389	0.53326	0.64897
trA0A367CCE3A0A367CCE3_9ENTE Alkaline shock protein OSEnterococcus durans OX53345 GNasp PE4 SV1	0.39241	0.31027	0.28761
trA0A377KL37A0A377KL37_9ENTE ATP-dependent protease ATPase subunit HslU OSEnterococcus durans OX53345 GNhslU PE3 SV1	0.39083	0.352	0.41438
trA0A377KPI0A0A377KPI0_9ENTE Organic hydroperoxide resistance family protein OSEnterococcus durans OX53345 GNohrB_3 PE4 SV1	0.38815	13.651	12.512
trA0A367CEB0A0A367CEB0_9ENTE Flotillin OSEnterococcus durans OX53345 GNYuaG PE4 SV1	0.38791	0.30801	0.3893
trA0A2A7SQB9A0A2A7SQB9_9ENTE Myosin-cross-reactive antigen-like protein OSEnterococcus durans OX53345 GNsph PE4 SV1	0.3868	0.76199	0.68285
trA0A377KK17A0A377KK17_9ENTE Succinate-semialdehyde dehydrogenase OSEnterococcus durans OX53345 GNgabD PE4 SV1	0.38459	0.7737	10.642
trA0A377KM73A0A377KM73_9ENTE General stress protein A OSEnterococcus durans OX53345 GNgsPA_1 PE4 SV1	0.38372	0.30454	0.3552
trA0A377KJEOA0A377KJEO_9ENTE Tellurite resistance protein OSEnterococcus durans OX53345 GNNCTC8129_01531 PE3 SV1	0.3796	0.34586	0.38072
trA0A377KIV1A0A377KIV1_9ENTE Universal stress protein OSEnterococcus durans OX53345 GNNCTC8129_01316 PE3 SV1	0.37899	0.31833	0.41454
trA0A2A7SPY2A0A2A7SPY2_9ENTE Phosphate-specific transport system accessory protein PhoU OSEnterococcus durans OX53345 GNphoU PE3 SV1	0.37423	0.29589	0.26419
trA0A377KLA9A0A377KLA9_9ENTE Phosphoribosylamine--glycine ligase OSEnterococcus durans OX53345 GNpurD PE3 SV1	0.37356	12.239	11.999
trA0A377KL34A0A377KL34_9ENTE DegV family protein OSEnterococcus durans OX53345 GNNCTC8129_02120 PE4 SV1	0.37279	0.30067	0.33768

trAOA377KJ12A0A377KJ12_9ENTE DAK2 domain fusion protein YloV OSEnterococcus durans OX53345 GNyloV PE4 SV1	0.37213	0.4897	0.49624
trAOA377KIZ8A0A377KIZ8_9ENTE Cation transporter E1-E2 family ATPase OSEnterococcus durans OX53345 GNNCTC8129_01223 PE4 SV1	0.37026	0.3145	0.3429
trAOA377KIX6A0A377KIX6_9ENTE Serine protease do-like htrA OSEnterococcus durans OX53345 GNhtrA_1 PE4 SV1	0.36883	0.29395	0.29266
trAOA2A7SRU2A0A2A7SRU2_9ENTE 50S ribosomal protein L18 OSEnterococcus durans OX53345 GNrplR PE3 SV1	0.36806	0.46224	0.46005
trAOA2A7SQD3A0A2A7SQD3_9ENTE MerR family transcriptional regulator OSEnterococcus durans OX53345 GNglrR PE4 SV1	0.36773	0.63856	0.64989
trAOA2A7SPN6A0A2A7SPN6_9ENTE UDP-N-acetylglucosamine 1-carboxyvinyltransferase OSEnterococcus durans OX53345 GNmurA1 PE3 SV1	0.36747	0.30307	0.42339
trAOA377KMP4A0A377KMP4_9ENTE UvrABC system protein B OSEnterococcus durans OX53345 GNuvrB PE3 SV1	0.36706	0.29745	0.32223
trAOA248V540A0A248V540_9ENTE Signal recognition particle protein OSEnterococcus durans OX53345 GNffh PE3 SV1	0.36392	0.38774	0.44462
trAOA377KKJ6A0A377KKJ6_9ENTE Bifunctional protein Fold OSEnterococcus durans OX53345 GNfoID PE3 SV1	0.36181	0.30891	0.78509
trAOA377KFY6A0A377KFY6_9ENTE ATP synthase subunit b OSEnterococcus durans OX53345 GNatpF PE3 SV1	0.35775	0.43764	0.39764
trAOA2A7SL50A0A2A7SL50_9ENTE DUF1827 domain protein OSEnterococcus durans OX53345 GNCUM72_08520 PE4 SV1	0.35745	0.30287	0.3551
trAOA2A7SLQ2A0A2A7SLQ2_9ENTE Glutamine-binding periplasmic protein OSEnterococcus durans OX53345 GNpeb1A PE4 SV1	0.35694	0.28531	0.50771
trAOA367CCJ7A0A367CCJ7_9ENTE lojap-like ribosome-associated protein OSEnterococcus durans OX53345 GNybeB PE4 SV1	0.35599	0.62772	0.62092
trAOA2A7SR85A0A2A7SR85_9ENTE 30S ribosomal protein S13 OSEnterococcus durans OX53345 GNrpsM PE3 SV1	0.35523	0.32167	0.30829
trAOA377KJG7A0A377KJG7_9ENTE 3-carboxymuconate cyclase OSEnterococcus durans OX53345 GNNCTC8129_01543 PE4 SV1	0.35517	11.084	10.484
trAOA248V826A0A248V826_9ENTE Tyrosine--tRNA ligase OSEnterococcus durans OX53345 GNtyrS1 PE3 SV1	0.35167	0.65082	0.58119
trAOA377KNM9A0A377KNM9_9ENTE Pyruvate dehydrogenase E1 component subunit alpha OSEnterococcus durans OX53345 GNpdhA PE4 SV1	0.35093	0.87949	0.8112
trAOA377KMY3A0A377KMY3_9ENTE Galactose-1-phosphate uridylyltransferase OSEnterococcus durans OX53345 GNgalT PE3 SV1	0.35048	0.29722	0.29894
trAOA377KHJ9A0A377KHJ9_9ENTE Oligoendopeptidase F OSEnterococcus durans OX53345 GNpepF1_1 PE3 SV1	0.35041	0.51207	0.48464
trAOA377KLE7A0A377KLE7_9ENTE Lipoate--protein ligase OSEnterococcus durans OX53345 GNlplJ_1 PE4 SV1	0.34814	0.3956	0.39022
trAOA377KNX3A0A377KNX3_9ENTE Probable GTP-binding protein EngB OSEnterococcus durans OX53345 GNysxC PE3 SV1	0.34735	0.31774	0.29648
trAOA377KGY1A0A377KGY1_9ENTE ATP synthase gamma chain OSEnterococcus durans OX53345 GNatpG PE3 SV1	0.34657	0.33489	0.3414
trAOA2A7SQ09A0A2A7SQ09_9ENTE ATP synthase subunit delta OSEnterococcus durans OX53345 GNatpH PE3 SV1	0.34629	0.43669	0.4609
trAOA2A7SR20A0A2A7SR20_9ENTE Ribonuclease Y OSEnterococcus durans OX53345 GNrny PE3 SV1	0.3448	0.27423	0.27783
trAOA377KI81A0A377KI81_9ENTE V-type ATP synthase beta chain OSEnterococcus durans OX53345 GNntpB PE3 SV1	0.3411	0.31026	0.28245
trAOA2A7SPW8A0A2A7SPW8_9ENTE 50S ribosomal protein L31 type B OSEnterococcus durans OX53345 GNrpmE2 PE3 SV1	0.33951	0.56965	0.53037
trAOA248VC59A0A248VC59_9ENTE Glyceraldehyde-3-phosphate dehydrogenase OSEnterococcus durans OX53345 GNgapA_1 PE3 SV1	0.33868	0.85709	0.81729
trAOA2S7MGW0A0A2S7MGW0_9ENTE UDP-N-acetylenolpyruvoylglucosamine reductase OSEnterococcus durans OX53345 GNmurB PE3 SV1	0.33741	0.53317	0.48088
trAOA377KIE7A0A377KIE7_9ENTE Cysteine--tRNA ligase OSEnterococcus durans OX53345 GNcysS PE3 SV1	0.33615	0.36092	0.63134
trAOA377KKL3A0A377KKL3_9ENTE Iron-sulfur cluster-binding protein OSEnterococcus durans OX53345 GNqueG PE4 SV1	0.33601	0.35482	0.34824
trAOA2A7SLI1A0A2A7SLI1_9ENTE Probable transcriptional regulatory protein CUM72_13235 OSEnterococcus durans OX53345 GNCUM72_13235 PE3 SV1	0.33492	0.34415	0.32649
trAOA377KMV4A0A377KMV4_9ENTE Cysteine synthase OSEnterococcus durans OX53345 GNcysK PE3 SV1	0.334	0.35269	0.33595
trAOA2A7SMU5A0A2A7SMU5_9ENTE Adenine phosphoribosyltransferase OSEnterococcus durans OX53345 GNapt PE3 SV1	0.33302	0.4171	0.40155
trAOA2A7SRW7A0A2A7SRW7_9ENTE 50S ribosomal protein L9 OSEnterococcus durans OX53345 GNrplI PE3 SV1	0.33197	0.29908	0.27489
trAOA2A7SNT0A0A2A7SNT0_9ENTE TetR family transcriptional regulator OSEnterococcus durans OX53345 GNCUM72_06825 PE4 SV1	0.33073	0.28937	0.36016
trAOA377KKV9A0A377KKV9_9ENTE S-adenosylmethioninetRNA ribosyltransferase-isomerase OSEnterococcus durans OX53345 GNqueA PE3 SV1	0.32985	0.34792	0.60555

trA0A247SP18A0A247SP18_9ENTE Arginine deiminase OSEnterococcus durans OX53345 GNarcA PE3 SV1	0.32926	13.907	12.737
trA0A377KQL2A0A377KQL2_9ENTE HAD superfamily hydrolase OSEnterococcus durans OX53345 GNywpJ_2 PE4 SV1	0.32836	0.3851	0.34898
trA0A248V5M5A0A248V5M5_9ENTE Thioredoxin OSEnterococcus durans OX53345 GNtrxA PE3 SV1	0.32529	0.78778	0.81054
trA0A377KMG2A0A377KMG2_9ENTE GNAT family acetyltransferase OSEnterococcus durans OX53345 GNNCTC8129_02610 PE4 SV1	0.3217	0.43559	0.38936
trA0A377KMB5A0A377KMB5_9ENTE Metal-dependent carboxypeptidase OSEnterococcus durans OX53345 GNypwA PE3 SV1	0.32089	0.25378	0.34297
trA0A377KMT6A0A377KMT6_9ENTE Glutamine--fructose-6-phosphate aminotransferase isomerizing OSEnterococcus durans OX53345 GNglmS_2 PE3 SV1	0.31979	0.93743	15.202
trA0A377KJS1A0A377KJS1_9ENTE UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--L-lysine ligase OSEnterococcus durans OX53345 GNmurE PE3 SV1	0.31918	0.35523	0.33839
trA0A377KHV3A0A377KHV3_9ENTE DNA-directed RNA polymerase subunit beta OSEnterococcus durans OX53345 GNrpoB PE3 SV1	0.31901	0.25337	0.22645
trA0A377KJD6A0A377KJD6_9ENTE Methyltransferase OSEnterococcus durans OX53345 GNrsmC PE4 SV1	0.31872	0.31602	0.51979
trA0A377KJZ3A0A377KJZ3_9ENTE Cysteine desulfurase OSEnterococcus durans OX53345 GNcsd PE3 SV1	0.31808	0.84096	0.96019
trA0A377KKG4A0A377KKG4_9ENTE NAD-dependent epimerase/dehydratase OSEnterococcus durans OX53345 GNgalE_1 PE4 SV1	0.31727	0.31795	0.5411
trA0A377KGN0A0A377KGN0_9ENTE Cell division protein FtsK OSEnterococcus durans OX53345 GNspolIIE PE3 SV1	0.31663	0.28402	0.2536
trA0A377KLS0A0A377KLS0_9ENTE Endolytic murein transglycosylase OSEnterococcus durans OX53345 GNYceG PE3 SV1	0.31563	0.39598	0.35416
trA0A247SR75A0A247SR75_9ENTE 30S ribosomal protein S8 OSEnterococcus durans OX53345 GNrpsH PE3 SV1	0.31498	0.4569	0.42645
trA0A247SPA1A0A247SPA1_9ENTE Ribosome hibernation promoting factor OSEnterococcus durans OX53345 GNhpf PE3 SV1	0.31258	0.84001	0.75907
trA0A377KKU3A0A377KKU3_9ENTE Alanine racemase OSEnterococcus durans OX53345 GNalr PE3 SV1	0.30991	0.28482	0.59797
trA0A377KKF8A0A377KKF8_9ENTE Glycosyl transferase family protein OSEnterococcus durans OX53345 GNkfoC PE4 SV1	0.30606	0.58464	0.6075
trA0A377KKL2A0A377KKL2_9ENTE Pyridoxal phosphate homeostasis protein OSEnterococcus durans OX53345 GNNCTC8129_01852 PE3 SV1	0.30574	0.25147	0.2704
trA0A377KM41A0A377KM41_9ENTE Zinc-type alcohol dehydrogenase-like protein OSEnterococcus durans OX53345 GNNCTC8129_01669 PE3 SV1	0.3043	0.36224	0.42043
trA0A247SRI1A0A247SRI1_9ENTE DNA-directed RNA polymerase subunit omega OSEnterococcus durans OX53345 GNrpoZ PE3 SV1	0.30349	0.41552	0.46253
trA0A377KIJ1A0A377KIJ1_9ENTE Ribose-5-phosphate isomerase A OSEnterococcus durans OX53345 GNrpiA PE3 SV1	0.30287	0.48323	0.44434
trA0A247SR88A0A247SR88_9ENTE PilT domain-containing protein OSEnterococcus durans OX53345 GNEA71_02874 PE4 SV1	0.30043	0.38184	0.3459
trA0A377L4A1A0A377L4A1_9ENTE Nucleic acid-binding protein OSEnterococcus durans OX53345 GNNCTC8129_01993 PE4 SV1	0.2994	0.4656	0.43125
trA0A377KN94A0A377KN94_9ENTE Glucokinase OSEnterococcus durans OX53345 GNglcK PE4 SV1	0.29908	0.41936	0.37475
trA0A247SRV0A0A247SRV0_9ENTE DNA-directed RNA polymerase subunit alpha OSEnterococcus durans OX53345 GNrpoA PE3 SV1	0.29828	0.23605	0.24101
trA0A377KH62A0A377KH62_9ENTE ABC transporter ATP-binding protein/permease OSEnterococcus durans OX53345 GNmacB_2 PE3 SV1	0.29655	0.30801	0.37596
trA0A377KGD8A0A377KGD8_9ENTE Septation ring formation regulator EzrA OSEnterococcus durans OX53345 GNzrA PE3 SV1	0.294	0.34412	0.30729
trA0A377KII4A0A377KII4_9ENTE Putative pre-16S rRNA nuclease OSEnterococcus durans OX53345 GNyrrK PE3 SV1	0.2935	0.3471	0.33923
trA0A377KJ38A0A377KJ38_9ENTE L-serine dehydratase iron-sulfur-dependent subunit alpha OSEnterococcus durans OX53345 GNsdhA_1 PE4 SV1	0.2932	0.23206	0.33482
trA0A247SL04A0A247SL04_9ENTE Iron dependent repressor DNA binding domain protein OSEnterococcus durans OX53345 GNideR PE4 SV1	0.29279	0.43008	0.43185
trA0A377KLL9A0A377KLL9_9ENTE Oxidoreductase aldo/keto reductase family protein OSEnterococcus durans OX53345 GNydhF PE4 SV1	0.29214	0.81442	13.369
trA0A377KHN9A0A377KHN9_9ENTE 3-dehydroquinate synthase OSEnterococcus durans OX53345 GNaroB PE3 SV1	0.29095	11.244	10.225
trA0A247SK26A0A247SK26_9ENTE Aggregation promoting factor OSEnterococcus durans OX53345 GNNCTC8129_01509 PE4 SV1	0.29029	14.094	13.393
trA0A247SNB7A0A247SNB7_9ENTE Antibiotic biosynthesis monooxygenase family protein OSEnterococcus durans OX53345 GNycnE PE4 SV1	0.28988	0.24302	0.28859
trA0A248V8B3A0A248V8B3_9ENTE Peptide deformylase OSEnterococcus durans OX53345 GNdef PE3 SV1	0.28909	0.2296	0.27696
trA0A377KGD2A0A377KGD2_9ENTE Glutamine synthetase OSEnterococcus durans OX53345 GNglnA PE3 SV1	0.28832	0.81845	0.73975



trA0A2A7SPB4A0A2A7SPB4_9ENTE GntR family transcriptional regulator OSEnterococcus durans OX53345 GNyvoA_2 PE4 SV1	0.28781	0.32058	0.30366
trA0A377KIC0A0A377KIC0_9ENTE Signal recognition particle receptor FtsY OSEnterococcus durans OX53345 GNftsY PE3 SV1	0.2864	0.4154	0.37091
trA0A377KNL8A0A377KNL8_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNNCTC8129_03060 PE4 SV1	0.2862	0.70273	0.6365
trA0A2A7SS73A0A2A7SS73_9ENTE ABC transporter permease OSEnterococcus durans OX53345 GNgsiC PE3 SV1	0.28453	0.55056	0.59734
trA0A377KLN4A0A377KLN4_9ENTE Branched-chain-amino-acid aminotransferase OSEnterococcus durans OX53345 GNilvE PE3 SV1	0.28343	0.23689	0.37745
trA0A377KN89A0A377KN89_9ENTE Pseudouridine synthase OSEnterococcus durans OX53345 GNrluB_2 PE3 SV1	0.28282	0.29679	0.26844
trA0A2A7SPB9A0A2A7SPB9_9ENTE ATP synthase subunit c OSEnterococcus durans OX53345 GNatpE PE3 SV1	0.2809	1.52	15.872
trA0A377KKS3A0A377KKS3_9ENTE Maltose O-acetyltransferase OSEnterococcus durans OX53345 GNmaa PE4 SV1	0.28051	0.23725	0.35477
trA0A2A7SR58A0A2A7SR58_9ENTE 30S ribosomal protein S19 OSEnterococcus durans OX53345 GNrpsS PE3 SV1	0.27697	0.22015	0.20627
trA0A377KKG7A0A377KKG7_9ENTE Acetate kinase OSEnterococcus durans OX53345 GNackA PE3 SV1	0.27565	0.68741	0.63584
trA0A377KGA7A0A377KGA7_9ENTE CTP synthase OSEnterococcus durans OX53345 GNpyrG PE3 SV1	0.27551	0.49347	0.4749
trA0A367CF36A0A367CF36_9ENTE Protein of uncharacterized function DUF3042 OSEnterococcus durans OX53345 GNEA71_01726 PE4 SV1	0.27525	0.43057	0.39863
trA0A377KMU3A0A377KMU3_9ENTE Glycosyl transferase OSEnterococcus durans OX53345 GNNCTC8129_01892 PE4 SV1	0.27416	0.52674	0.47248
trA0A377KR41A0A377KR41_9ENTE KH domain-containing protein OSEnterococcus durans OX53345 GNybeZ PE4 SV1	0.27197	0.35091	0.31345
trA0A377KMB1A0A377KMB1_9ENTE HAD superfamily hydrolase OSEnterococcus durans OX53345 GNyhaX_3 PE4 SV1	0.2705	0.43421	0.61643
trA0A377KLQ9A0A377KLQ9_9ENTE Penicillin-binding protein 1A OSEnterococcus durans OX53345 GNponA_3 PE4 SV1	0.27032	0.25855	0.41178
trA0A377KGZ1A0A377KGZ1_9ENTE Pfpl family intracellular protease OSEnterococcus durans OX53345 GNyfkM PE4 SV1	0.26942	0.427	0.38122
trA0A2A7SKR3A0A2A7SKR3_9ENTE Universal stress protein OSEnterococcus durans OX53345 GNuspA4 PE3 SV1	0.26675	0.49057	0.53621
trA0A2A7SRZ0A0A2A7SRZ0_9ENTE Organic hydroperoxide resistance protein OSEnterococcus durans OX53345 GNohrB_1 PE4 SV1	0.26663	0.37737	0.46774
trA0A2A7SQD4A0A2A7SQD4_9ENTE L-serine dehydratase iron-sulfur-dependent subunit beta OSEnterococcus durans OX53345 GNsdhB PE4 SV1	0.26604	0.21699	0.61181
trA0A2A7SM55A0A2A7SM55_9ENTE RNA polymerase sigma factor SigA OSEnterococcus durans OX53345 GNrpoD PE3 SV1	0.26543	0.22943	0.20683
trA0A377KML8A0A377KML8_9ENTE UDP-N-acetylmuramoylalanine--D-glutamate ligase OSEnterococcus durans OX53345 GNmurD PE3 SV1	0.26284	0.64769	0.75809
trA0A367CGQ7A0A367CGQ7_9ENTE Ribosome-binding ATPase YchF OSEnterococcus durans OX53345 GNengD PE3 SV1	0.26279	0.2154	0.20522
trA0A2A7SSF6A0A2A7SSF6_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNEA71_00207 PE3 SV1	0.26237	0.25236	0.40179
trA0A377KLA7A0A377KLA7_9ENTE Phosphoribosylformylglycinamide synthase subunit PurL OSEnterococcus durans OX53345 GNpurL PE3 SV1	0.25927	12.104	11.467
trA0A377KIC9A0A377KIC9_9ENTE Glutathione reductase OSEnterococcus durans OX53345 GNgor PE3 SV1	0.25903	0.25612	0.34678
trA0A377KH97A0A377KH97_9ENTE DJ-1/Pfpl family protein OSEnterococcus durans OX53345 GNhchA_1 PE4 SV1	0.25842	0.97925	13.609
trA0A2A7SS33A0A2A7SS33_9ENTE 50S ribosomal protein L23 OSEnterococcus durans OX53345 GNrplW PE3 SV1	0.25797	0.52529	0.47716
trA0A377KCC9A0A377KCC9_9ENTE tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG OSEnterococcus durans OX53345 GNgidA_1 PE3 SV1	0.25734	0.25075	0.30294
trA0A377KLA4A0A377KLA4_9ENTE Dipeptidase OSEnterococcus durans OX53345 GNNCTC8129_02097 PE4 SV1	0.25537	0.68844	0.62886
trA0A377KNN5A0A377KNN5_9ENTE Bifunctional purine biosynthesis protein PurH OSEnterococcus durans OX53345 GNpurH PE3 SV1	0.25316	10.702	10.045
trA0A377KIR1A0A377KIR1_9ENTE UTP--glucose-1-phosphate uridylyltransferase OSEnterococcus durans OX53345 GNgtab PE3 SV1	0.25307	0.20265	0.18298
trA0A377KI00A0A377KI00_9ENTE 30S ribosomal protein S6 OSEnterococcus durans OX53345 GNrpsF PE3 SV1	0.25215	0.39292	0.35492
trA0A2A7SRM7A0A2A7SRM7_9ENTE 50S ribosomal protein L22 OSEnterococcus durans OX53345 GNrplV PE3 SV1	0.25185	0.26501	0.2399
trA0A377KIZ0A0A377KIZ0_9ENTE 50S ribosomal protein L6 OSEnterococcus durans OX53345 GNrplF PE3 SV1	0.25101	0.48443	0.43776
trA0A2A7SQJ6A0A2A7SQJ6_9ENTE Elongation factor P OSEnterococcus durans OX53345 GNepf PE3 SV1	0.25062	0.38087	0.36663

trAOA377KL04A0A377KL04_9ENTE Xanthine/uracil/vitamin C permease OSEnterococcus durans OX53345 GNpbuG_3 PE4 SV1	0.24754	0.19945	0.18305
trAOA377KHS3A0A377KHS3_9ENTE Glutathione peroxidase OSEnterococcus durans OX53345 GNbsaA PE3 SV1	0.24455	0.31813	0.48793
trAOA2A7SR57A0A2A7SR57_9ENTE Acetyltransferase OSEnterococcus durans OX53345 GNEA71_02900 PE4 SV1	0.24288	0.27345	0.35597
trAOA248VBJ4A0A248VBJ4_9ENTE Amino acid ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNartM_3 PE4 SV1	0.24206	0.33898	0.44944
trAOA377LOB2A0A377LOB2_9ENTE 2 3-bisphosphoglycerate-dependent phosphoglycerate mutase OSEnterococcus durans OX53345 GNrpmA_2 PE3 SV1	0.24199	0.42479	0.38757
trAOA377L6Z8A0A377L6Z8_9ENTE ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNyhel_7 PE4 SV1	0.24154	0.21033	0.25503
trAOA377KGQ8A0A377KGQ8_9ENTE 6-phospho-beta-galactosidase OSEnterococcus durans OX53345 GNlacG_1 PE3 SV1	0.24088	0.20581	0.68195
trAOA377KLS1A0A377KLS1_9ENTE DEAD-box ATP-dependent RNA helicase CshB OSEnterococcus durans OX53345 GNcshB PE3 SV1	0.24078	0.19728	0.23377
trAOA377KN05A0A377KN05_9ENTE 3-oxoacyl-acyl-carrier-protein synthase 3 OSEnterococcus durans OX53345 GNfabH PE3 SV1	0.24061	0.44335	0.43464
trAOA377KK05A0A377KK05_9ENTE RNA methyltransferase OSEnterococcus durans OX53345 GNNCTC8129_00860 PE3 SV1	0.23791	0.19118	0.19196
trAOA2A7SK31A0A2A7SK31_9ENTE Regulatory protein Spx OSEnterococcus durans OX53345 GNspxA PE3 SV1	0.23682	0.25111	0.33698
trAOA377KNP7A0A377KNP7_9ENTE D-alanyl-D-alanine carboxypeptidase OSEnterococcus durans OX53345 GNdacA PE3 SV1	0.23234	0.22103	0.31948
trAOA2A7SPL0A0A2A7SPL0_9ENTE Ribosome maturation factor RimM OSEnterococcus durans OX53345 GNrimM PE3 SV1	0.22994	0.22849	0.20582
trAOA2A7SQ73A0A2A7SQ73_9ENTE DNA-binding response regulator OSEnterococcus durans OX53345 GNvicR PE4 SV1	0.2295	0.33881	0.30567
trAOA377KHCSA0A377KHCS_9ENTE DNA topoisomerase III OSEnterococcus durans OX53345 GNtopB_2 PE4 SV1	0.22766	0.33016	0.29539
trAOA377KJL4A0A377KJL4_9ENTE FeS assembly protein SufD OSEnterococcus durans OX53345 GNsufD PE4 SV1	0.22717	0.36765	0.73411
trAOA2A7SQN9A0A2A7SQN9_9ENTE Methylglyoxal synthase OSEnterococcus durans OX53345 GNmgsA PE3 SV1	0.22223	0.29823	0.3205
trAOA2A7SR59A0A2A7SR59_9ENTE DNA-directed RNA polymerase subunit beta OSEnterococcus durans OX53345 GNrpoC PE3 SV1	0.22173	0.26233	0.241
trAOA2A7SP81A0A2A7SP81_9ENTE Protein translocase subunit SecA OSEnterococcus durans OX53345 GNsecA PE3 SV1	0.22015	0.18335	0.22147
trAOA377KLM0A0A377KLM0_9ENTE Transketolase OSEnterococcus durans OX53345 GNTkt_1 PE3 SV1	0.21904	0.17318	0.20817
trAOA2A7SLJ6A0A2A7SLJ6_9ENTE TPR domain-containing protein OSEnterococcus durans OX53345 GNEA71_01038 PE4 SV1	0.21761	0.23127	0.25181
trAOA377KLQ5A0A377KLQ5_9ENTE Carbamoyl-phosphate synthase large chain OSEnterococcus durans OX53345 GNcarB PE3 SV1	0.21584	14.519	12.966
trAOA2A7SQE5A0A2A7SQE5_9ENTE Transcription termination/antitermination protein NusG OSEnterococcus durans OX53345 GNnusG PE3 SV1	0.21147	0.55897	0.50725
trAOA377KNU7A0A377KNU7_9ENTE Bifunctional phosphoglucomutase/phosphomannomutase OSEnterococcus durans OX53345 GNpgm2 PE3 SV1	0.21115	0.22466	0.25309
trAOA367CD71A0A367CD71_9ENTE Phosphoribosylformylglycinamide synthase subunit PurS OSEnterococcus durans OX53345 GNyexA PE3 SV1	0.21014	13.052	11.961
trAOA2A7SRT0A0A2A7SRT0_9ENTE Ribonuclease 3 OSEnterococcus durans OX53345 GNrc PE3 SV1	0.20922	0.16593	0.15652
trAOA377KGT7A0A377KGT7_9ENTE Class II aminotransferase OSEnterococcus durans OX53345 GNpatB PE4 SV1	0.20918	0.31764	0.28965
trAOA377KJW7A0A377KJW7_9ENTE Adenine deaminase OSEnterococcus durans OX53345 GNadeC PE3 SV1	0.20916	0.17054	0.42781
trAOA377KLE2A0A377KLE2_9ENTE Elongation factor EF1A OSEnterococcus durans OX53345 GNtufA3 PE4 SV1	0.20904	0.72981	0.65155
trAOA2S7ME31A0A2S7ME31_9ENTE Ribosome biogenesis GTPase A OSEnterococcus durans OX53345 GNylqF PE3 SV1	0.20616	0.25047	0.2398
trAOA377KKC1A0A377KKC1_9ENTE Hemolysin A OSEnterococcus durans OX53345 GNtlyA PE4 SV1	0.20572	0.16813	0.15316
trAOA2A7SQB6A0A2A7SQB6_9ENTE 50S ribosomal protein L11 OSEnterococcus durans OX53345 GNrpK PE3 SV1	0.20509	0.30986	0.306
trAOA248V7L9A0A248V7L9_9ENTE 60 kDa chaperonin OSEnterococcus durans OX53345 GNgroL PE3 SV1	0.20445	0.28529	0.27006
trAOA2A7SPU0A0A2A7SPU0_9ENTE 30S ribosomal protein S21 OSEnterococcus durans OX53345 GNrpsU PE3 SV1	0.20386	0.26891	0.33188
trAOA2A7SQ54A0A2A7SQ54_9ENTE 10 kDa chaperonin OSEnterococcus durans OX53345 GNgroS PE3 SV1	0.19991	0.15804	0.14522
trAOA377KK91A0A377KK91_9ENTE Transcription antitermination protein NusB OSEnterococcus durans OX53345 GNnusB PE3 SV1	0.19604	0.16591	0.46761

trAOA377KN75A0A377KN75_9ENTE Calcium-translocating P-type ATPase OSEnterococcus durans OX53345 GNNCTC8129_02044 PE4 SV1	0.19585	0.43606	0.82273
trAOA2A7SKA5A0A2A7SKA5_9ENTE Phosphate acetyltransferase OSEnterococcus durans OX53345 GNpta PE4 SV1	0.19325	1.51	14.193
trAOA2A7SR87A0A2A7SR87_9ENTE RNA-binding protein OSEnterococcus durans OX53345 GNNCTC8129_01009 PE4 SV1	0.19288	0.22896	0.20441
trAOA2A7SPX2A0A2A7SPX2_9ENTE ABC transporter ATP-binding protein/permease OSEnterococcus durans OX53345 GNNCTC8129_00365 PE4 SV1	0.19158	0.33506	0.30532
trAOA377CLK2A0A377CLK2_9ENTE Tryptophan--tRNA ligase OSEnterococcus durans OX53345 GNtrpS PE3 SV1	0.19	0.28282	0.25269
trAOA2A7SJH7A0A2A7SJH7_9ENTE UPF0342 protein EA71_00425 OSEnterococcus durans OX53345 GNEA71_00425 PE3 SV1	0.18977	0.15405	0.71878
trAOA2A7SL31A0A2A7SL31_9ENTE 50S ribosomal protein L27 OSEnterococcus durans OX53345 GNrpmA PE3 SV1	0.18795	0.21551	0.20957
trAOA377KI05A0A377KI05_9ENTE U32 family peptidase OSEnterococcus durans OX53345 GNYhbU_2 PE4 SV1	0.18771	0.16754	0.16932
trAOA2A7SSG7A0A2A7SSG7_9ENTE DEAD-box ATP-dependent RNA helicase CshA OSEnterococcus durans OX53345 GNcshA_1 PE3 SV1	0.18752	0.75075	0.67204
trAOA2A7SQK5A0A2A7SQK5_9ENTE 50S ribosomal protein L10 OSEnterococcus durans OX53345 GNrpIJ PE3 SV1	0.18512	0.28872	0.30436
trAOA377KI83A0A377KI83_9ENTE Peptide chain release factor 2 OSEnterococcus durans OX53345 GNprfB PE3 SV1	0.18475	0.16599	0.2707
trAOA377KHM2A0A377KHM2_9ENTE Methionyl-tRNA formyltransferase OSEnterococcus durans OX53345 GNfmt PE3 SV1	0.18367	0.1548	0.13877
trAOA2A7SL20A0A2A7SL20_9ENTE DNA-binding protein HU OSEnterococcus durans OX53345 GNhupA PE3 SV1	0.18076	0.50054	0.44735
trAOA377KHM8A0A377KHM8_9ENTE Gamma-glutamyl phosphate reductase OSEnterococcus durans OX53345 GNproA PE3 SV1	0.18058	0.22164	0.22561
trAOA377KLS8A0A377KLS8_9ENTE Alanine--tRNA ligase OSEnterococcus durans OX53345 GNalaS PE3 SV1	0.18042	0.19982	0.22663
trAOA248V8X1A0A248V8X1_9ENTE 50S ribosomal protein L24 OSEnterococcus durans OX53345 GNrpIX PE3 SV1	0.1801	0.32099	0.2873
trAOA2A7SP49A0A2A7SP49_9ENTE Thymidylate kinase OSEnterococcus durans OX53345 GNtmk PE3 SV1	0.17935	0.46294	0.42849
trAOA377L630A0A377L630_9ENTE tRNA-specific 2-thiouridylase MnmA OSEnterococcus durans OX53345 GNmnmA PE3 SV1	0.17742	0.33061	0.51374
trAOA377KIV6A0A377KIV6_9ENTE Phenylalanine--tRNA ligase beta subunit OSEnterococcus durans OX53345 GNpht_1 PE3 SV1	0.17152	0.30174	0.33535
trAOA2A7SRN1A0A2A7SRN1_9ENTE 50S ribosomal protein L28 OSEnterococcus durans OX53345 GNrpmB PE3 SV1	0.17061	0.31707	0.2877
trAOA377KMD2A0A377KMD2_9ENTE GTP cyclohydrolase 1 type 2 homolog OSEnterococcus durans OX53345 GNNCTC8129_02618 PE3 SV1	0.1704	0.15247	0.4947
trAOA377KN74A0A377KN74_9ENTE Carbamate kinase OSEnterococcus durans OX53345 GNarcC1 PE3 SV1	0.17005	15.698	14.286
trAOA377KLC0A0A377KLC0_9ENTE Thymidylate synthase OSEnterococcus durans OX53345 GNthyB PE3 SV1	0.16854	0.2048	0.40013
trAOA2A7SRH1A0A2A7SRH1_9ENTE 30S ribosomal protein S12 OSEnterococcus durans OX53345 GNrpsL PE3 SV1	0.16782	0.15484	0.15332
trAOA377KI20A0A377KI20_9ENTE Chromosomal replication initiator protein DnaA OSEnterococcus durans OX53345 GNdnaA PE3 SV1	0.16761	0.20847	0.25079
trAOA2A7SS52A0A2A7SS52_9ENTE Translation initiation factor IF-1 OSEnterococcus durans OX53345 GNinfA PE3 SV1	0.16675	0.468	0.42354
trAOA2A7SQ59A0A2A7SQ59_9ENTE Folate family ECF transporter S component OSEnterococcus durans OX53345 GNfoIT PE4 SV1	0.16606	0.22967	0.33765
trAOA377KI96A0A377KI96_9ENTE Oligopeptide/dipeptide ABC transporter ATP-binding protein domain protein OSEnterococcus durans OX53345 GNgsiA_3 PE3 SV1	0.16547	0.17404	0.43396
trAOA377KIA1A0A377KIA1_9ENTE ATP synthase subunit beta OSEnterococcus durans OX53345 GNatpD PE3 SV1	0.16451	0.31388	0.46092
trAOA377KIT2A0A377KIT2_9ENTE UPF0145 protein NCTC8129_01267 OSEnterococcus durans OX53345 GNYbjQ PE3 SV1	0.16444	0.38075	0.41481
trAOA377KM37A0A377KM37_9ENTE Fibronectin/fibrinogen-binding protein OSEnterococcus durans OX53345 GNNCTC8129_02406 PE4 SV1	0.16335	0.14384	0.13673
trAOA2A7LS4A0A2A7LS4_9ENTE MazG nucleotide pyrophosphohydrolase OSEnterococcus durans OX53345 GNypjD PE4 SV1	0.16259	0.1985	0.50203
trAOA2A7SRN6A0A2A7SRN6_9ENTE 50S ribosomal protein L35 OSEnterococcus durans OX53345 GNrpmI PE3 SV1	0.16222	0.49823	0.44893
trAOA2A7SRK1A0A2A7SRK1_9ENTE 50S ribosomal protein L2 OSEnterococcus durans OX53345 GNrpIB PE3 SV1	0.16165	0.34087	0.34188
trAOA377KKP8A0A377KKP8_9ENTE ATPase OSEnterococcus durans OX53345 GNydiB PE4 SV1	0.16132	0.22063	0.19704
trAOA2A7SKJ3A0A2A7SKJ3_9ENTE Peptide chain release factor 1 OSEnterococcus durans OX53345 GNprfA_1 PE3 SV1	0.15936	0.52589	0.47118

trA0A367CBQ0A0A367CBQ0_9ENTE S4 domain-containing protein OSEnterococcus durans OX53345 GNEA71_00807 PE4 SV1	0.15935	0.12797	0.35028
trA0A377KKL9A0A377KKL9_9ENTE 2-dehydropantoate 2-reductase OSEnterococcus durans OX53345 GNpanE_3 PE3 SV1	0.15913	0.1961	0.25692
trA0A2A7SRL1A0A2A7SRL1_9ENTE 30S ribosomal protein S14 type Z OSEnterococcus durans OX53345 GNrpsZ PE3 SV1	0.15905	0.78629	0.70225
trA0A377KHB7A0A377KHB7_9ENTE S-Hydroxymethylglutathione dehydrogenase OSEnterococcus durans OX53345 GNfdh PE3 SV1	0.15804	0.49852	0.66529
trA0A2A7SS43A0A2A7SS43_9ENTE 50S ribosomal protein L5 OSEnterococcus durans OX53345 GNrplE PE3 SV1	0.15189	0.4836	0.49728
trA0A248V8V4A0A248V8V4_9ENTE 50S ribosomal protein L4 OSEnterococcus durans OX53345 GNrplD PE3 SV1	0.15072	0.31461	0.28129
trA0A377KI79A0A377KI79_9ENTE 50S ribosomal protein L17 OSEnterococcus durans OX53345 GNrplQ PE3 SV1	0.15002	0.31484	0.32239
trA0A2A7SLE7A0A2A7SLE7_9ENTE Peptide chain release factor 3 OSEnterococcus durans OX53345 GNprfC PE3 SV1	0.14842	0.60311	0.61015
trA0A2A7SKH5A0A2A7SKH5_9ENTE Arginine repressor OSEnterococcus durans OX53345 GNargR PE3 SV1	0.14613	0.12513	0.11467
trA0A377KNY0A0A377KNY0_9ENTE Multifunctional fusion protein OSEnterococcus durans OX53345 GNrph PE3 SV1	0.14588	0.12507	0.17664
trA0A377KKF3A0A377KKF3_9ENTE dTDP-glucose 4 6-dehydratase OSEnterococcus durans OX53345 GNrmlB PE3 SV1	0.14563	0.68306	0.6173
trA0A2A7SR41A0A2A7SR41_9ENTE Adenylate kinase OSEnterococcus durans OX53345 GNadk PE3 SV1	0.14488	0.63806	0.59085
trA0A2A7SRJ3A0A2A7SRJ3_9ENTE 50S ribosomal protein L29 OSEnterococcus durans OX53345 GNrpmC PE3 SV1	0.14396	0.63187	0.56579
trA0A2A7SLL6A0A2A7SLL6_9ENTE 30S ribosomal protein S20 OSEnterococcus durans OX53345 GNrpsT PE3 SV1	0.1421	0.24708	0.25128
trA0A377KIR9A0A377KIR9_9ENTE Acetyltransferase OSEnterococcus durans OX53345 GNydaF PE4 SV1	0.14175	0.39963	0.49485
trA0A377KNP6A0A377KNP6_9ENTE 50S ribosomal protein L19 OSEnterococcus durans OX53345 GNrplS PE3 SV1	0.14158	0.59164	0.54247
trA0A2A7SRK7A0A2A7SRK7_9ENTE 50S ribosomal protein L30 OSEnterococcus durans OX53345 GNrpmD PE3 SV1	0.13987	0.52904	0.52637
trA0A2A7SQV3A0A2A7SQV3_9ENTE Transcriptional regulator CtsR OSEnterococcus durans OX53345 GNctsR PE3 SV1	0.13964	0.24456	0.23427
trA0A367CAP5A0A367CAP5_9ENTE GNAT family acetyltransferase OSEnterococcus durans OX53345 GNEA71_02865 PE4 SV1	0.13857	0.28977	0.30932
trA0A367CAH7A0A367CAH7_9ENTE 33 kDa chaperonin OSEnterococcus durans OX53345 GNhsI O PE3 SV1	0.13799	0.13864	0.20715
trA0A2A7SQA1A0A2A7SQA1_9ENTE UPF0297 protein CUM72_01225 OSEnterococcus durans OX53345 GNCUM72_01225 PE3 SV1	0.13498	0.10906	0.20128
trA0A377KLQ2A0A377KLQ2_9ENTE UPF0291 protein NCTC8129_02336 OSEnterococcus durans OX53345 GNNCTC8129_02336 PE3 SV1	0.13454	0.38879	0.34712
trA0A377KND3A0A377KND3_9ENTE DNA topoisomerase 1 OSEnterococcus durans OX53345 GNtopA PE3 SV1	0.13391	0.52756	0.51283
trA0A377KJ1A0A377KJ1_9ENTE FMN-dependent NADH-azoreductase OSEnterococcus durans OX53345 GNacpH PE3 SV1	0.1339	0.35679	0.43981
trA0A377KJC1A0A377KJC1_9ENTE Prephenate dehydrogenase OSEnterococcus durans OX53345 GNtyrC PE4 SV1	0.13212	0.51029	0.48225
trA0A2A7SQY2A0A2A7SQY2_9ENTE Replicative DNA helicase OSEnterococcus durans OX53345 GNdnaC PE3 SV1	0.12968	0.17818	0.37445
trA0A377L0C5A0A377L0C5_9ENTE Galactose-6-phosphate isomerase subunit LacA OSEnterococcus durans OX53345 GNlacA PE3 SV1	0.12932	13.218	1.471
trA0A377KKQ4A0A377KKQ4_9ENTE Cadmium-translocating P-type ATPase OSEnterococcus durans OX53345 GNzosA_1 PE3 SV1	0.12927	0.63289	1.107
trA0A377KMW8A0A377KMW8_9ENTE Hemolysin OSEnterococcus durans OX53345 GNyfdJ PE4 SV1	0.12892	0.4997	0.45537
trA0A377KLZ7A0A377KLZ7_9ENTE Penicillin-binding protein 2B OSEnterococcus durans OX53345 GNpenA PE4 SV1	0.12862	0.14406	0.1658
trA0A2A7SS10A0A2A7SS10_9ENTE Translation initiation factor IF-3 OSEnterococcus durans OX53345 GNinfC PE3 SV1	0.12661	0.31797	0.35806
trA0A2A7SRJ8A0A2A7SRJ8_9ENTE Uridine kinase OSEnterococcus durans OX53345 GNudk PE3 SV1	0.12611	0.66811	0.59648
trA0A2A7SP54A0A2A7SP54_9ENTE 50S ribosomal protein L33 OSEnterococcus durans OX53345 GNrpmG PE3 SV1	0.12503	0.85459	0.80905
trA0A2A7SPW1A0A2A7SPW1_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNCUM72_06245 PE4 SV1	0.12453	0.10247	0.21768
trA0A2A7SR9A0A2A7SR9_9ENTE 30S ribosomal protein S3 OSEnterococcus durans OX53345 GNrpsC PE3 SV1	0.12403	0.34586	0.34216
trA0A377L1G8A0A377L1G8_9ENTE Cytidine deaminase OSEnterococcus durans OX53345 GNcdd PE3 SV1	0.12402	0.50719	0.45639
trA0A377KGM0A0A377KGM0_9ENTE Isoaspartyl dipeptidase OSEnterococcus durans OX53345 GNiadA PE3 SV1	0.12227	0.4091	0.36774

trA0A377KMJ1A0A377KMJ1_9ENTE Phosphoribosylformylglycinamide cyclo-ligase OSEnterococcus durans OX53345 GNpurM PE3 SV1	0.12183	11.463	11.312
trA0A377L2W0A0A377L2W0_9ENTE Small secreted protein OSEnterococcus durans OX53345 GNNCTC8129_01366 PE4 SV1	0.12012	0.21525	0.19416
trA0A2A7SPH3A0A2A7SPH3_9ENTE Cell division ATP-binding protein FtsE OSEnterococcus durans OX53345 GNftsE PE4 SV1	0.11812	0.23499	0.26727
trA0A2A7SPC4A0A2A7SPC4_9ENTE ATP synthase epsilon chain OSEnterococcus durans OX53345 GNatpC PE3 SV1	0.11747	0.23175	0.27473
trA0A2A7SRH7A0A2A7SRH7_9ENTE 30S ribosomal protein S10 OSEnterococcus durans OX53345 GNrpsJ PE3 SV1	0.11654	0.17669	0.15896
trA0A377KKN6A0A377KKN6_9ENTE GNAT family acetyltransferase OSEnterococcus durans OX53345 GNNCTC8129_01938 PE4 SV1	0.11503	0.15799	0.18566
trA0A2A7SS14A0A2A7SS14_9ENTE 30S ribosomal protein S15 OSEnterococcus durans OX53345 GNrpsO PE3 SV1	0.11438	0.13446	0.12139
trA0A377KNH7A0A377KNH7_9ENTE MarR family transcriptional regulator OSEnterococcus durans OX53345 GNyodC PE4 SV1	0.11313	0.11724	0.1145
trA0A377KQ94A0A377KQ94_9ENTE Uridylate kinase OSEnterococcus durans OX53345 GNpyrH PE3 SV1	0.1118	0.62715	0.57172
trQ6KJ7Q6KJ7_9ENTE Protein RecA OSEnterococcus durans OX53345 GNrecA PE3 SV1	0.10961	0.089333	0.092053
trA0A377KGL0A0A377KGL0_9ENTE ATP synthase subunit alpha OSEnterococcus durans OX53345 GNatpA PE3 SV1	0.10731	0.21528	0.34154
trA0A377KJT3A0A377KJT3_9ENTE ErfK/YbiS/Ycfs/YnhG family protein OSEnterococcus durans OX53345 GNNCTC8129_00812 PE4 SV1	0.1065	0.11793	0.17849
trA0A377KJZ8A0A377KJZ8_9ENTE Putative competence-damage inducible protein OSEnterococcus durans OX53345 GNYgaD PE3 SV1	0.10646	0.20126	0.4436
trA0A367CID2A0A367CID2_9ENTE YtxH-like protein OSEnterococcus durans OX53345 GNEA71_00453 PE4 SV1	0.10606	0.084682	0.25894
trA0A2A7SMB6A0A2A7SMB6_9ENTE ATP-dependent protease subunit HslV OSEnterococcus durans OX53345 GNhslV PE3 SV1	0.10595	0.348	0.32284
trA0A377KJF5A0A377KJF5_9ENTE Cystathionine gamma-synthase OSEnterococcus durans OX53345 GNmetB PE3 SV1	0.10471	0.73475	0.81116
trA0A377KNY5A0A377KNY5_9ENTE Acylphosphatase OSEnterococcus durans OX53345 GNacpY PE3 SV1	0.10446	0.087794	0.093207
trA0A377KHL3A0A377KHL3_9ENTE Peptidase M16 inactive domain protein OSEnterococcus durans OX53345 GNEA71_02863 PE4 SV1	0.10416	0.082319	0.088107
trA0A377KGB9A0A377KGB9_9ENTE ABC transporter ATP-binding protein/permease OSEnterococcus durans OX53345 GNNCTC8129_00364 PE4 SV1	0.10185	0.19269	0.18911
trA0A2A7SIN8A0A2A7SIN8_9ENTE 1-acyl-sn-glycerol-3-phosphate acyltransferase OSEnterococcus durans OX53345 GNplsC PE4 SV1	0.099349	0.10545	0.19048
trA0A367CFW2A0A367CFW2_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNEA71_02270 PE4 SV1	0.098371	0.13979	0.35688
trA0A377KID3A0A377KID3_9ENTE DNA mismatch repair protein MutS OSEnterococcus durans OX53345 GNmutS_2 PE3 SV1	0.097463	0.090743	0.35846
trA0A2A7SRI9A0A2A7SRI9_9ENTE Pur operon repressor OSEnterococcus durans OX53345 GNpurR_1 PE4 SV1	0.096919	0.42273	0.38463
trA0A2A7SLH2A0A2A7SLH2_9ENTE S1 domain RNA-binding protein OSEnterococcus durans OX53345 GNyitL PE4 SV1	0.096859	0.55095	0.51234
trA0A377KP57A0A377KP57_9ENTE Elongation factor Ts OSEnterococcus durans OX53345 GNtsf PE3 SV1	0.09661	0.52846	0.47421
trA0A248V8A4A0A248V8A4_9ENTE Transcription repressor NadR OSEnterococcus durans OX53345 GNnadR PE4 SV1	0.093418	0.10187	0.091313
trA0A377KH24A0A377KH24_9ENTE Glutamine amidotransferase OSEnterococcus durans OX53345 GNpuuD PE4 SV1	0.091476	0.46524	0.81635
trA0A377KI32A0A377KI32_9ENTE Mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase OSEnterococcus durans OX53345 GNNCTC8129_00876 PE4 SV1	0.089821	0.3513	0.64301
trA0A2A7SR40A0A2A7SR40_9ENTE 50S ribosomal protein L1 OSEnterococcus durans OX53345 GNrplA PE3 SV1	0.088141	0.44739	0.44317
trA0A377KJK4A0A377KJK4_9ENTE Foldase protein PrsA OSEnterococcus durans OX53345 GNprsA PE3 SV1	0.086873	0.56239	0.70322
trA0A377KMW1A0A377KMW1_9ENTE Methylase OSEnterococcus durans OX53345 GNrlmL PE3 SV1	0.084878	0.096787	0.13938
trA0A377KNU5A0A377KNU5_9ENTE Amino acid/peptide transporter OSEnterococcus durans OX53345 GNdtpT PE3 SV1	0.084689	0.26116	0.54283
trA0A377KK13A0A377KK13_9ENTE Putative flavoprotein NrdI OSEnterococcus durans OX53345 GNnrdI PE3 SV1	0.084555	0.089639	0.731
trA0A367CAE9A0A367CAE9_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNEA71_03070 PE4 SV1	0.084121	0.15052	0.21285
trA0A2A7SKI4A0A2A7SKI4_9ENTE Nucleoside diphosphate kinase OSEnterococcus durans OX53345 GNndk PE3 SV1	0.08322	0.33143	0.2959
trA0A377KMQ9A0A377KMQ9_9ENTE ATP-dependent helicase/deoxyribonuclease subunit B OSEnterococcus durans OX53345 GNaddB PE3 SV1	0.081185	0.098157	0.26399

trAOA377KQ25A0A377KQ25_9ENTE Trigger factor OSEnterococcus durans OX53345 GNtig PE3 SV1	0.080696	0.24307	0.21701
trAOA377KFT6A0A377KFT6_9ENTE Glycosyl transferase OSEnterococcus durans OX53345 GNponA_1 PE4 SV1	0.080253	0.072105	0.3452
trAOA377KNW2A0A377KNW2_9ENTE Peptidyl-prolyl cis-trans isomerase OSEnterococcus durans OX53345 GNNCTC8129_02318 PE3 SV1	0.07815	0.067163	0.50239
trAOA377KHG8A0A377KHG8_9ENTE HAD superfamily hydrolase OSEnterococcus durans OX53345 GNyutF PE4 SV1	0.077836	0.44852	0.40095
trAOA367CGE8A0A367CGE8_9ENTE Nicotinamidase/pyrazinamidase OSEnterococcus durans OX53345 GNycaC PE4 SV1	0.077674	0.3399	0.42198
trAOA377KJV5A0A377KJV5_9ENTE Transcription-repair-coupling factor OSEnterococcus durans OX53345 Gnmfd PE3 SV1	0.076214	0.06859	0.43003
trAOA367CDI5A0A367CDI5_9ENTE Cell cycle protein GpsB OSEnterococcus durans OX53345 GNdivIVA2 PE3 SV1	0.074857	0.13862	0.12508
trAOA377KPH3A0A377KPH3_9ENTE Tagatose 1 6-diphosphate aldolase OSEnterococcus durans OX53345 GnlacD PE3 SV1	0.074685	32.509	29.095
trAOA2A7SP21A0A2A7SP21_9ENTE 30S ribosomal protein S2 OSEnterococcus durans OX53345 GNrpsB PE3 SV1	0.073292	0.29576	0.29228
trAOA2A7SL12A0A2A7SL12_9ENTE Methionine import ATP-binding protein MetN OSEnterococcus durans OX53345 GNmetN2_1 PE3 SV1	0.072371	0.089722	0.52635
trAOA377KK48A0A377KK48_9ENTE D-alanine--D-alanyl carrier protein ligase OSEnterococcus durans OX53345 GndIta PE3 SV1	0.072199	0.47266	0.42225
trAOA377KJ33A0A377KJ33_9ENTE 2 5-diketo-D-gluconate reductase OSEnterococcus durans OX53345 GndkgB_1 PE4 SV1	0.072159	0.21309	0.27803
trAOA377KI73A0A377KI73_9ENTE Elongation factor G OSEnterococcus durans OX53345 GNfusA PE3 SV1	0.072031	0.27596	0.29903
trAOA377KI07A0A377KI07_9ENTE Pyruvate oxidase OSEnterococcus durans OX53345 GNpox5 PE3 SV1	0.070522	0.55821	0.69006
trAOA2A7SMP3A0A2A7SMP3_9ENTE Protein GrpE OSEnterococcus durans OX53345 GNgrpE PE3 SV1	0.067738	0.15669	0.13998
trAOA2A7SKP5A0A2A7SKP5_9ENTE Phosphoenolpyruvate-protein phosphotransferase OSEnterococcus durans OX53345 GNptsl PE3 SV1	0.063416	0.060763	0.20319
trAOA2A7SLR8A0A2A7SLR8_9ENTE GTPase Der OSEnterococcus durans OX53345 GNder PE3 SV1	0.061671	0.40713	0.40722
trAOA377KGF3A0A377KGF3_9ENTE Probable tRNA sulfurtransferase OSEnterococcus durans OX53345 GNthil PE3 SV1	0.061141	0.55615	0.93658
trAOA2A7SSB5A0A2A7SSB5_9ENTE 30S ribosomal protein S5 OSEnterococcus durans OX53345 GNrpsE PE3 SV1	0.060381	0.34466	0.31025
trAOA377KM21A0A377KM21_9ENTE Dihydroorotate dehydrogenase B NAD electron transfer subunit OSEnterococcus durans OX53345 GNpyrK PE3 SV1	0.059584	1.352	12.192
trAOA2A7SRJ0A0A2A7SRJ0_9ENTE 30S ribosomal protein S18 OSEnterococcus durans OX53345 GNrpsR PE3 SV1	0.058423	0.31996	0.41465
trAOA2A7SR72A0A2A7SR72_9ENTE Chorismate synthase OSEnterococcus durans OX53345 GNaroC PE3 SV1	0.05783	0.71988	0.70303
trAOA2A7SRR3A0A2A7SRR3_9ENTE 50S ribosomal protein L14 OSEnterococcus durans OX53345 GNrplN PE3 SV1	0.057485	0.084782	0.11054
trAOA2A7SP37A0A2A7SP37_9ENTE 30S ribosomal protein S16 OSEnterococcus durans OX53345 GNrpsP PE3 SV1	0.055983	0.14889	0.26239
trAOA377KG50A0A377KG50_9ENTE HAD superfamily hydrolase OSEnterococcus durans OX53345 GNyidA_1 PE4 SV1	0.055783	0.06426	0.32205
trAOA2A7SMT9A0A2A7SMT9_9ENTE Ornithine carbamoyltransferase OSEnterococcus durans OX53345 GNarcB PE3 SV1	0.053207	14.772	13.218
trAOA377KLJ4A0A377KLJ4_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNNCTC8129_02293 PE4 SV1	0.052774	0.13463	0.15587
trAOA377KKP4A0A377KKP4_9ENTE GTPase YqeH OSEnterococcus durans OX53345 GNyqeH PE4 SV1	0.049861	0.26922	0.64547
trAOA377KIA6A0A377KIA6_9ENTE HAD superfamily hydrolase OSEnterococcus durans OX53345 GNyidA_2 PE4 SV1	0.049488	0.36883	0.33861
trAOA377KGX3A0A377KGX3_9ENTE Glucosamine-6-phosphate deaminase OSEnterococcus durans OX53345 GNnagB PE3 SV1	0.049123	0.29991	0.34882
trAOA377KMI5A0A377KMI5_9ENTE DAACS family dicarboxylate/amino acidsodium Na symporter OSEnterococcus durans OX53345 GNtcyP_1 PE3 SV1	0.048015	0.27255	0.43481
trAOA2A7SKF5A0A2A7SKF5_9ENTE 50S ribosomal protein L21 OSEnterococcus durans OX53345 GNrplU PE3 SV1	0.046485	0.27722	0.2536
trAOA377KKP2A0A377KKP2_9ENTE 3-hydroxyacyl-acyl-carrier-protein dehydratase FabZ OSEnterococcus durans OX53345 GNfabZ2 PE3 SV1	0.046164	0.25516	0.25509
trAOA377KMP6A0A377KMP6_9ENTE Cell division protein FtsA OSEnterococcus durans OX53345 GNftsA PE3 SV1	0.045275	0.063052	0.19531
trAOA2A7SLF2A0A2A7SLF2_9ENTE Phosphocarrier protein HPr OSEnterococcus durans OX53345 GNptsH PE4 SV1	0.044545	0.20324	0.19925
trAOA2A7SPR8A0A2A7SPR8_9ENTE Protein from nitrogen regulatory protein P-II GLNB family OSEnterococcus durans OX53345 GNEA71_02224 PE4 SV1	0.044309	0.21268	0.29771
trAOA377KL38A0A377KL38_9ENTE Aldose 1-epimerase OSEnterococcus durans OX53345 GNgalM4 PE4 SV1	0.044271	0.41754	0.39503

trA0A377KKM3A0A377KKM3_9ENTE ATP-dependent Clp protease ATP-binding protein ClpE OSEnterococcus durans OX53345 GNclpE PE3 SV1	0.036704	0.15118	0.14786
trA0A377L7J2A0A377L7J2_9ENTE tRNA-dihydrouridine synthase OSEnterococcus durans OX53345 GNdus_2 PE3 SV1	0.033134	0.030859	0.33042
trA0A2A7SRM1A0A2A7SRM1_9ENTE 50S ribosomal protein L36 OSEnterococcus durans OX53345 GNrpmJ PE3 SV1	0.031548	0.44111	0.3952
trA0A367CEM5A0A367CEM5_9ENTE Chaperone protein DnaK OSEnterococcus durans OX53345 GNdnaK PE2 SV1	0.031514	0.084209	0.098665
trA0A2A7SRP0A0A2A7SRP0_9ENTE 30S ribosomal protein S9 OSEnterococcus durans OX53345 GNrpsI PE3 SV1	0.02904	0.30799	0.29
trA0A367CB54A0A367CB54_9ENTE 50S ribosomal protein L13 OSEnterococcus durans OX53345 GNrplM PE3 SV1	0.028657	0.35584	0.31846
trA0A377L6H0A0A377L6H0_9ENTE DUF2188 family protein OSEnterococcus durans OX53345 GNNCTC8129_02826 PE4 SV1	0.028626	0.40496	0.37913
trA0A377KJU1A0A377KJU1_9ENTE Ribulose-phosphate 3-epimerase OSEnterococcus durans OX53345 GNrpe PE3 SV1	0.028436	0.51241	0.60816
trA0A377KJ48A0A377KJ48_9ENTE 2-dehydropantoate 2-reductase OSEnterococcus durans OX53345 GNpanE_2 PE3 SV1	0.027755	0.38954	0.34927
trA0A2A7SQF4A0A2A7SQF4_9ENTE GTP diphosphokinase OSEnterococcus durans OX53345 GNrelA_3 PE4 SV1	0.026531	0.028739	0.049662
trA0A2A7SRP9A0A2A7SRP9_9ENTE 30S ribosomal protein S11 OSEnterococcus durans OX53345 GNrpsK PE3 SV1	0.026276	0.31245	0.28358
trA0A2A7SS17A0A2A7SS17_9ENTE Elongation factor Tu OSEnterococcus durans OX53345 GntufA PE3 SV1	0.025859	0.094945	0.11385
trA0A377KLJ6A0A377KLJ6_9ENTE Adenine-specific methyltransferase OSEnterococcus durans OX53345 GNNCTC8129_01465 PE4 SV1	0.025498	0.98598	0.94525
trA0A367CH01A0A367CH01_9ENTE ABC transporter ATP-binding protein OSEnterococcus durans OX53345 GNykpA PE4 SV1	0.024927	0.3633	0.35258
trA0A377KG27A0A377KG27_9ENTE Endonuclease Muts2 OSEnterococcus durans OX53345 GNmutS2_1 PE3 SV1	0.024413	0.21795	0.23496
trA0A377KI92A0A377KI92_9ENTE Phosphate acyltransferase OSEnterococcus durans OX53345 GNplsX PE3 SV1	0.022697	0.4856	0.43352
trA0A2A7SPI5A0A2A7SPI5_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNEA71_02098 PE4 SV1	0.022468	0.22449	0.27911
trA0A377KKS6A0A377KKS6_9ENTE Tautomerase OSEnterococcus durans OX53345 GNNCTC8129_01978 PE3 SV1	0.02076	0.087099	0.29915
trA0A2A7SPA6A0A2A7SPA6_9ENTE GTPase Era OSEnterococcus durans OX53345 GNera PE3 SV1	0.020678	0.4923	0.50394
trA0A377KMT8A0A377KMT8_9ENTE Chaperone protein DnaJ OSEnterococcus durans OX53345 GNdnaJ PE3 SV1	0.020005	0.080059	0.071557
trA0A377KLD6A0A377KLD6_9ENTE Anaerobic ribonucleoside triphosphate reductase OSEnterococcus durans OX53345 GNnrdD PE4 SV1	0.019887	20.559	18.477
trA0A2A7SNE2A0A2A7SNE2_9ENTE Ribosome-recycling factor OSEnterococcus durans OX53345 GNfr PE3 SV1	0.018096	0.2756	0.24644
trA0A367CD28A0A367CD28_9ENTE Response regulator receiver domain-containing protein OSEnterococcus durans OX53345 GNagrA_2 PE4 SV1	0.017988	0.45911	0.4263
trA0A2A7SRI4A0A2A7SRI4_9ENTE 30S ribosomal protein S17 OSEnterococcus durans OX53345 GNrpsQ PE3 SV1	0.017475	0.29068	0.25999
trA0A377KLN1A0A377KLN1_9ENTE 30S ribosomal protein S1 OSEnterococcus durans OX53345 GNrpsA PE4 SV1	0.017022	0.37862	0.36554
trA0A377KPY6A0A377KPY6_9ENTE Aminotransferase OSEnterococcus durans OX53345 GNNCTC8129_03056 PE3 SV1	0.016925	0.44627	0.43135
trA0A377KM64A0A377KM64_9ENTE ATP-dependent helicase/nuclease subunit A OSEnterococcus durans OX53345 GNaddA PE3 SV1	0.016311	0.093567	0.087697
trA0A2A7SRK6A0A2A7SRK6_9ENTE DegV family protein OSEnterococcus durans OX53345 GNNCTC8129_01026 PE4 SV1	0.015805	0.41649	0.38433
trA0A377KLJ8A0A377KLJ8_9ENTE 3-hydroxyacyl-acyl-carrier-protein dehydratase FabZ OSEnterococcus durans OX53345 GNfabZ1 PE3 SV1	0.015788	0.77628	0.69578
trA0A377KMU6A0A377KMU6_9ENTE Translation initiation factor IF-2 OSEnterococcus durans OX53345 GNinfB PE3 SV1	0.015672	0.11053	0.24994
trA0A2A7SPM5A0A2A7SPM5_9ENTE Virion core protein OSEnterococcus durans OX53345 GNEA71_02229 PE4 SV1	0.015062	0.086425	0.10451
trA0A377KM24A0A377KM24_9ENTE Phosphomethylpyrimidine kinase OSEnterococcus durans OX53345 GNthiD PE4 SV1	0.014367	0.036991	0.67394
trA0A377KGU5A0A377KGU5_9ENTE General stress protein OSEnterococcus durans OX53345 GNNCTC8129_00164 PE4 SV1	0.013552	0.36377	0.52189
trA0A377L7S0A0A377L7S0_9ENTE tRNA modification GTPase MnmE OSEnterococcus durans OX53345 GNtrmE PE3 SV1	0.01181	0.09341	0.5609
trA0A377KFU5A0A377KFU5_9ENTE Glycerol-3-phosphate dehydrogenase NADP OSEnterococcus durans OX53345 GNgpsA PE3 SV1	0.010335	0.37028	0.35585
trA0A377KNE2A0A377KNE2_9ENTE DNA topoisomerase 4 subunit A OSEnterococcus durans OX53345 GNparC PE3 SV1	0.0080364	0.41575	0.52811

trA0A2A7SM65A0A2A7SM65_9ENTE Uncharacterized protein conserved in bacteria with the myosin-like domain OSEnterococcus durans OX53345 GNEA71_01397 PE4 SV1	0.0076743	0.18121	0.24168
trA0A377KG23A0A377KG23_9ENTE Ribonuclease R OSEnterococcus durans OX53345 GNrnR PE3 SV1	0.006855	0.17933	0.33265
trA0A377KMN7A0A377KMN7_9ENTE Methionine synthase II Cobalamin-independent OSEnterococcus durans OX53345 GNmetE_2 PE3 SV1	0.0052527	0.21676	0.42295
trA0A2A7SRQ5A0A2A7SRQ5_9ENTE 50S ribosomal protein L3 OSEnterococcus durans OX53345 GNrplC PE3 SV1	0.0043077	0.098134	0.15776
trA0A367CEQ4A0A367CEQ4_9ENTE Cell division protein FtsZ OSEnterococcus durans OX53345 GNftsZ PE3 SV1	0.0020847	0.1076	0.10226
trA0A2A7SRJ7A0A2A7SRJ7_9ENTE 50S ribosomal protein L15 OSEnterococcus durans OX53345 GNrpLO PE3 SV1	0.0018124	0.047259	0.084496
trA0A377KCC8A0A377KCC8_9ENTE Gfo/Idh/MocA family oxidoreductase OSEnterococcus durans OX53345 GNycjS PE4 SV1	0.0013203	0.022657	0.16193
trA0A2A7SRQ0A0A2A7SRQ0_9ENTE 30S ribosomal protein S4 OSEnterococcus durans OX53345 GNrpsD PE3 SV1	0.0003664		
	8	0.074343	0.088715



**Table S2.** PLS-DA values used to identify differentially expressed proteins related to the use of different carbon sources (FOS, GOS and GLU) and the presence or absence of oxygen in *E. durans* supernatant with the VIP threshold > 1.0 in the first component of PLS-DA.

Protein Group	Comp. 1	Comp. 2	Comp. 3
trA0A248V5I4A0A248V5I4_9ENTE GatB/YqeY domain-containing protein OSEnterococcus durans OX53345 GNYqeY PE4 SV1	40.767	0.52577	0.52064
trA0A248V5S1A0A248V5S1_9ENTE Triosephosphate isomerase OSEnterococcus durans OX53345 GNtpiA PE3 SV1	40.311	0.50019	0.49415
trA0A248V6U6A0A248V6U6_9ENTE 50S ribosomal protein L7/L12 OSEnterococcus durans OX53345 GNrpIL PE3 SV1	36.591	0.62244	0.62107
trA0A248V7D7A0A248V7D7_9ENTE Lysine--tRNA ligase OSEnterococcus durans OX53345 GNlysS PE3 SV1	34.962	0.72418	0.71732
trA0A248V7L9A0A248V7L9_9ENTE 60 kDa chaperonin OSEnterococcus durans OX53345 GNgroL PE3 SV1	34.917	0.17837	0.20495
trA0A248V826A0A248V826_9ENTE Tyrosine--tRNA ligase OSEnterococcus durans OX53345 GNTyrS1 PE3 SV1	29.588	0.69603	0.69835
trA0A248V8V4A0A248V8V4_9ENTE 50S ribosomal protein L4 OSEnterococcus durans OX53345 GNrpID PE3 SV1	29.273	0.24293	0.25791
trA0A248V8X1A0A248V8X1_9ENTE 50S ribosomal protein L24 OSEnterococcus durans OX53345 GNrpIX PE3 SV1	29.255	0.39249	0.40124
trA0A248VAA1A0A248VAA1_9ENTE Dihydrolipoyl dehydrogenase OSEnterococcus durans OX53345 GNpdhD_2 PE3 SV1	26.873	11.617	11.498
trA0A248VC59A0A248VC59_9ENTE Glyceraldehyde-3-phosphate dehydrogenase OSEnterococcus durans OX53345 GNgapA_1 PE3 SV1	24.751	0.88902	0.88279
trA0A2A7SJH7A0A2A7SJH7_9ENTE UPF0342 protein EA71_00425 OSEnterococcus durans OX53345 GNEA71_00425 PE3 SV1	23.126	0.70901	0.70589
trA0A2A7SJX2A0A2A7SJX2_9ENTE Uracil phosphoribosyltransferase OSEnterococcus durans OX53345 GNupp PE3 SV1	21.858	0.71357	0.70539
trA0A2A7SK26A0A2A7SK26_9ENTE Aggregation promoting factor OSEnterococcus durans OX53345 GNNCTC8129_01509 PE4 SV1	21.421	24.155	23.863
trA0A2A7SKA5A0A2A7SKA5_9ENTE Phosphate acetyltransferase OSEnterococcus durans OX53345 GNpta PE4 SV1	21.237	0.92378	0.91529
trA0A2A7SKC4A0A2A7SKC4_9ENTE PTS system mannose/fructose/sorbose-specific IIAB component OSEnterococcus durans OX53345 GNmanX_2 PE4 SV1	20.752	10.522	10.961
trA0A2A7SKF5A0A2A7SKF5_9ENTE 50S ribosomal protein L21 OSEnterococcus durans OX53345 GNrpIU PE3 SV1	19.628	0.69431	0.69103
trA0A2A7SKI4A0A2A7SKI4_9ENTE Nucleoside diphosphate kinase OSEnterococcus durans OX53345 GNndk PE3 SV1	19.266	0.41436	0.40993
trA0A2A7SKN6A0A2A7SKN6_9ENTE Pyrrolidone-carboxylate peptidase OSEnterococcus durans OX53345 GNpcp PE3 SV1	19.241	0.52558	0.52694
trA0A2A7SKP5A0A2A7SKP5_9ENTE Phosphoenolpyruvate-protein phosphotransferase OSEnterococcus durans OX53345 GNptsI PE3 SV1	18.104	0.62016	0.61714
trA0A2A7SKR3A0A2A7SKR3_9ENTE Universal stress protein OSEnterococcus durans OX53345 GNuspA4 PE3 SV1	17.519	0.80481	0.79546
trA0A2A7SL20A0A2A7SL20_9ENTE DNA-binding protein HU OSEnterococcus durans OX53345 GNhupA PE3 SV1	16.372	0.55886	0.55677
trA0A2A7SL31A0A2A7SL31_9ENTE 50S ribosomal protein L27 OSEnterococcus durans OX53345 GNrpmA PE3 SV1	16.113	0.25373	0.30881
trA0A2A7SL88A0A2A7SL88_9ENTE Acyl carrier protein OSEnterococcus durans OX53345 GNacpA PE3 SV1	15.548	0.56407	0.57386
trA0A2A7SL95A0A2A7SL95_9ENTE DUF1797 domain-containing protein OSEnterococcus durans OX53345 GNYkuJ PE4 SV1	15.188	0.19077	0.19514
trA0A2A7SLE1A0A2A7SLE1_9ENTE Elongation factor Tu OSEnterococcus durans OX53345 GNtuf PE3 SV1	14.974	0.24574	0.28973
trA0A2A7SLF2A0A2A7SLF2_9ENTE Phosphocarrier protein HPr OSEnterococcus durans OX53345 GNptsH PE4 SV1	14.789	12.925	12.996
trA0A2A7SLH9A0A2A7SLH9_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNCUM72_12610 PE4 SV1	13.457	0.63115	0.62553
trA0A2A7SLJ7A0A2A7SLJ7_9ENTE ATP-dependent 6-phosphofructokinase OSEnterococcus durans OX53345 GNpfkA PE3 SV1	13.418	0.41262	0.42122
trA0A2A7SLK1A0A2A7SLK1_9ENTE 50S ribosomal protein L32 OSEnterococcus durans OX53345 GNrpmF PE3 SV1	13.281	0.65749	0.64996
trA0A2A7SL6A0A2A7SL6_9ENTE 30S ribosomal protein S20 OSEnterococcus durans OX53345 GNrpsT PE3 SV1	13.222	0.59159	0.58599
trA0A2A7SLR8A0A2A7SLR8_9ENTE GTPase Der OSEnterococcus durans OX53345 GNder PE3 SV1	12.786	0.2784	0.28399
trA0A2A7SLW7A0A2A7SLW7_9ENTE Bifunctional protein PyrR OSEnterococcus durans OX53345 GNpyrR PE3 SV1	12.473	0.17369	0.17709

trA0A2A7SLZ7A0A2A7SLZ7_9ENTE DNA-binding response regulator OSEnterococcus durans OX53345 GNarIR PE4 SV1	12.253	0.37324	0.36952
trA0A2A7SMP3A0A2A7SMP3_9ENTE Protein GrpE OSEnterococcus durans OX53345 GNgrpE PE3 SV1	10.604	0.37934	0.39606
trA0A2A7SMT9A0A2A7SMT9_9ENTE Ornithine carbamoyltransferase OSEnterococcus durans OX53345 GNarcB PE3 SV1	4.046	15.626	15.447
trA0A2A7SMW4A0A2A7SMW4_9ENTE Asp23/Gls24 family envelope stress response protein OSEnterococcus durans OX53345 GNCUM72_00245 PE4 SV1	3.572	0.61557	0.60984
trA0A2A7SNE2A0A2A7SNE2_9ENTE Ribosome-recycling factor OSEnterococcus durans OX53345 GNfrf PE3 SV1	2.502	0.48968	0.48916
trA0A2A7SNZ0A0A2A7SNZ0_9ENTE Lactoylglutathione lyase OSEnterococcus durans OX53345 GNgloA_2 PE4 SV1	1.596	0.28664	0.28632
trA0A2A7SP06A0A2A7SP06_9ENTE Glyceraldehyde-3-phosphate dehydrogenase OSEnterococcus durans OX53345 GNgap PE3 SV1	1.554	0.51402	0.52421
trA0A2A7SP18A0A2A7SP18_9ENTE Arginine deiminase OSEnterococcus durans OX53345 GNarcA PE3 SV1	1.221	16.798	16.617
trA0A2A7SP21A0A2A7SP21_9ENTE 30S ribosomal protein S2 OSEnterococcus durans OX53345 GNrpsB PE3 SV1	1.167	0.59088	0.58376
trA0A2A7SP37A0A2A7SP37_9ENTE 30S ribosomal protein S16 OSEnterococcus durans OX53345 GNrpsP PE3 SV1	0.9869	0.40909	0.4436
trA0A2A7SP54A0A2A7SP54_9ENTE 50S ribosomal protein L33 OSEnterococcus durans OX53345 GNrpmG PE3 SV1	0.97689	0.35139	0.4355
trA0A2A7SP81A0A2A7SP81_9ENTE Protein translocase subunit SecA OSEnterococcus durans OX53345 GNsecA PE3 SV1	0.97185	0.54975	0.55202
trA0A2A7SP93A0A2A7SP93_9ENTE UPF0356 protein CUM72_10905 OSEnterococcus durans OX53345 GNCUM72_10905 PE3 SV1	0.96474	0.17584	0.17371
trA0A2A7SPA1A0A2A7SPA1_9ENTE Ribosome hibernation promoting factor OSEnterococcus durans OX53345 GNhpf PE3 SV1	0.958	0.51955	0.52303
trA0A2A7SPD0A0A2A7SPD0_9ENTE GNAT family acetyltransferase OSEnterococcus durans OX53345 GNEA71_02120 PE4 SV1	0.94533	0.53906	0.53622
trA0A2A7SPE3A0A2A7SPE3_9ENTE Phosphoglycerate kinase OSEnterococcus durans OX53345 GNpgk PE3 SV1	0.92815	0.17045	0.17933
trA0A2A7SPM8A0A2A7SPM8_9ENTE Nucleoid-associated protein EA71_02227 OSEnterococcus durans OX53345 GNEA71_02227 PE3 SV1	0.92748	0.32681	0.32362
trA0A2A7SPP7A0A2A7SPP7_9ENTE Fructose-1 6-bisphosphate aldolase	0.92195	0.5887	0.6317
trA0A2A7SPR8A0A2A7SPR8_9ENTE Protein from nitrogen regulatory protein P-II GLNB family OSEnterococcus durans OX53345 GNEA71_02224 PE4 SV1	0.90528	0.34202	0.39381
trA0A2A7SPU0A0A2A7SPU0_9ENTE 30S ribosomal protein S21 OSEnterococcus durans OX53345 GNrpsU PE3 SV1	0.8842	0.70944	0.70748
trA0A2A7SPW4A0A2A7SPW4_9ENTE CsbD-like protein OSEnterococcus durans OX53345 GNEA71_02356 PE3 SV1	0.88016	0.66752	0.65991
trA0A2A7SPW8A0A2A7SPW8_9ENTE 50S ribosomal protein L31 type B OSEnterococcus durans OX53345 GNrpmE2 PE3 SV1	0.86978	0.19288	0.19163
trA0A2A7SQ04A0A2A7SQ04_9ENTE Peptidyl-prolyl cis-trans isomerase OSEnterococcus durans OX53345 GNppi PE3 SV1	0.86124	0.45979	0.47072
trA0A2A7SQ54A0A2A7SQ54_9ENTE 10 kDa chaperonin OSEnterococcus durans OX53345 GNgroS PE3 SV1	0.856	0.19787	0.20388
trA0A2A7SQA6A0A2A7SQA6_9ENTE Ribonucleoside-diphosphate reductase subunit beta OSEnterococcus durans OX53345 GNrdF_1 PE3 SV1	0.85475	0.74203	0.7411
trA0A2A7SQB6A0A2A7SQB6_9ENTE 50S ribosomal protein L11 OSEnterococcus durans OX53345 GNrplK PE3 SV1	0.84898	0.56526	0.57684
trA0A2A7SQE5A0A2A7SQE5_9ENTE Transcription termination/antitermination protein NusG OSEnterococcus durans OX53345 GNnusG PE3 SV1	0.84781	0.33685	0.38043
trA0A2A7SQG0A0A2A7SQG0_9ENTE ATP-dependent Clp protease proteolytic subunit OSEnterococcus durans OX53345 GNclpP PE3 SV1	0.84584	0.21248	0.21134
trA0A2A7SQG5A0A2A7SQG5_9ENTE 3-deoxy-7-phosphoheptulonate synthase OSEnterococcus durans OX53345 GNaroF_2 PE4 SV1	0.81713	23.447	2.33
trA0A2A7SQJ6A0A2A7SQJ6_9ENTE Elongation factor P OSEnterococcus durans OX53345 GNefp PE3 SV1	0.81687	0.47457	0.46913
trA0A2A7SQK5A0A2A7SQK5_9ENTE 50S ribosomal protein L10 OSEnterococcus durans OX53345 GNrplJ PE3 SV1	0.81067	0.5632	0.5569
trA0A2A7SQR5A0A2A7SQR5_9ENTE S-ribosylhomocysteine lyase OSEnterococcus durans OX53345 GNluxS PE3 SV1	0.79983	0.38051	0.376
trA0A2A7SR40A0A2A7SR40_9ENTE 50S ribosomal protein L1 OSEnterococcus durans OX53345 GNrplA PE3 SV1	0.79919	0.45717	0.48375
trA0A2A7SR41A0A2A7SR41_9ENTE Adenylate kinase OSEnterococcus durans OX53345 GNadk PE3 SV1	0.79803	0.84371	0.8489
trA0A2A7SR58A0A2A7SR58_9ENTE 30S ribosomal protein S19 OSEnterococcus durans OX53345 GNrpsS PE3 SV1	0.79097	10.166	10.303

trA0A2A7SR59A0A2A7SR59_9ENTE DNA-directed RNA polymerase subunit beta OSEnterococcus durans OX53345 GNrpoC PE3 SV1	0.78495	0.60423	0.66337
trA0A2A7SR68A0A2A7SR68_9ENTE Ribose-phosphate pyrophosphokinase OSEnterococcus durans OX53345 GNprsA4 PE3 SV1	0.76664	0.72412	0.71802
trA0A2A7SR71A0A2A7SR71_9ENTE DNA protection during starvation protein 1 OSEnterococcus durans OX53345 GNdps PE3 SV1	0.76209	0.98861	0.97883
trA0A2A7SR75A0A2A7SR75_9ENTE 30S ribosomal protein S8 OSEnterococcus durans OX53345 GNrpsH PE3 SV1	0.75773	0.67173	0.66643
trA0A2A7SR85A0A2A7SR85_9ENTE 30S ribosomal protein S13 OSEnterococcus durans OX53345 GNrpsM PE3 SV1	0.7558	0.53648	0.53037
trA0A2A7SR88A0A2A7SR88_9ENTE Single-stranded DNA-binding protein OSEnterococcus durans OX53345 GNssb PE3 SV1	0.75552	0.56005	0.56806
trA0A2A7SRF1A0A2A7SRF1_9ENTE L-lactate dehydrogenase OSEnterococcus durans OX53345 GNldh PE3 SV1	0.7431	0.4363	0.46195
trA0A2A7SRG7A0A2A7SRG7_9ENTE ABC superfamily ATP binding cassette transporter binding protein OSEnterococcus durans OX53345 GNtmpC_1 PE4 SV1	0.74209	1.924	19.116
trA0A2A7SRH1A0A2A7SRH1_9ENTE 30S ribosomal protein S12 OSEnterococcus durans OX53345 GNrpsL PE3 SV1	0.73883	0.94415	0.9366
trA0A2A7SRH7A0A2A7SRH7_9ENTE 30S ribosomal protein S10 OSEnterococcus durans OX53345 GNrpsJ PE3 SV1	0.73814	0.73254	0.72534
trA0A2A7SRI4A0A2A7SRI4_9ENTE 30S ribosomal protein S17 OSEnterococcus durans OX53345 GNrpsQ PE3 SV1	0.73629	0.62817	0.62616
trA0A2A7SRJ0A0A2A7SRJ0_9ENTE 30S ribosomal protein S18 OSEnterococcus durans OX53345 GNrpsR PE3 SV1	0.73349	0.81975	0.81034
trA0A2A7SRJ2A0A2A7SRJ2_9ENTE DNA gyrase subunit B OSEnterococcus durans OX53345 GNgyrB PE3 SV1	0.72363	0.27633	0.28944
trA0A2A7SRJ3A0A2A7SRJ3_9ENTE 50S ribosomal protein L29 OSEnterococcus durans OX53345 GNrpmC PE3 SV1	0.72064	0.75007	0.74742
trA0A2A7SRJ7A0A2A7SRJ7_9ENTE 50S ribosomal protein L15 OSEnterococcus durans OX53345 GNrpLO PE3 SV1	0.7179	0.87714	0.87101
trA0A2A7SRK1A0A2A7SRK1_9ENTE 50S ribosomal protein L2 OSEnterococcus durans OX53345 GNrpLB PE3 SV1	0.70578	0.39442	0.41105
trA0A2A7SRK7A0A2A7SRK7_9ENTE 50S ribosomal protein L30 OSEnterococcus durans OX53345 GNrpmD PE3 SV1	0.70499	0.57716	0.57466
trA0A2A7SRL1A0A2A7SRL1_9ENTE 30S ribosomal protein S14 type Z OSEnterococcus durans OX53345 GNrpsZ PE3 SV1	0.70192	0.73083	0.72255
trA0A2A7SRM1A0A2A7SRM1_9ENTE 50S ribosomal protein L36 OSEnterococcus durans OX53345 GNrpmJ PE3 SV1	0.70027	0.58964	0.58458
trA0A2A7SRM7A0A2A7SRM7_9ENTE 50S ribosomal protein L22 OSEnterococcus durans OX53345 GNrpIV PE3 SV1	0.69144	0.46188	0.46595
trA0A2A7SRN1A0A2A7SRN1_9ENTE 50S ribosomal protein L28 OSEnterococcus durans OX53345 GNrpmB PE3 SV1	0.68922	0.48211	0.48246
trA0A2A7SRN2A0A2A7SRN2_9ENTE Septum formation initiator OSEnterococcus durans OX53345 GNEA71_02816 PE4 SV1	0.68636	16.934	17.287
trA0A2A7SRN6A0A2A7SRN6_9ENTE 50S ribosomal protein L35 OSEnterococcus durans OX53345 GNrpmI PE3 SV1	0.68616	0.57411	0.57734
trA0A2A7SRP0A0A2A7SRP0_9ENTE 30S ribosomal protein S9 OSEnterococcus durans OX53345 GNrpsI PE3 SV1	0.68435	0.50752	0.50895
trA0A2A7SRP7A0A2A7SRP7_9ENTE 30S ribosomal protein S7 OSEnterococcus durans OX53345 GNrpsG PE3 SV1	0.67958	0.62996	0.62397
trA0A2A7SRP9A0A2A7SRP9_9ENTE 30S ribosomal protein S11 OSEnterococcus durans OX53345 GNrpsK PE3 SV1	0.67871	0.75982	0.80585
trA0A2A7SRQ0A0A2A7SRQ0_9ENTE 30S ribosomal protein S4 OSEnterococcus durans OX53345 GNrpsD PE3 SV1	0.66947	0.41703	0.41529
trA0A2A7SRQ5A0A2A7SRQ5_9ENTE 50S ribosomal protein L3 OSEnterococcus durans OX53345 GNrpIC PE3 SV1	0.66482	0.40543	0.40293
trA0A2A7SRR3A0A2A7SRR3_9ENTE 50S ribosomal protein L14 OSEnterococcus durans OX53345 GNrpIN PE3 SV1	0.66298	0.44913	0.44831
trA0A2A7SRS9A0A2A7SRS9_9ENTE 30S ribosomal protein S3 OSEnterococcus durans OX53345 GNrpsC PE3 SV1	0.65999	0.60429	0.59996
trA0A2A7SRU2A0A2A7SRU2_9ENTE 50S ribosomal protein L18 OSEnterococcus durans OX53345 GNrpLR PE3 SV1	0.64311	0.72175	0.71415
trA0A2A7SRV0A0A2A7SRV0_9ENTE DNA-directed RNA polymerase subunit alpha OSEnterococcus durans OX53345 GNrpoA PE3 SV1	0.64171	0.3534	0.35015
trA0A2A7SRW7A0A2A7SRW7_9ENTE 50S ribosomal protein L9 OSEnterococcus durans OX53345 GNrpII PE3 SV1	0.63927	0.45233	0.45115
trA0A2A7SRZ0A0A2A7SRZ0_9ENTE Organic hydroperoxide resistance protein OSEnterococcus durans OX53345 GNohrB_1 PE4 SV1	0.63514	0.38743	0.38387

trA0A2A7SS10A0A2A7SS10_9ENTE Translation initiation factor IF-3 OSEnterococcus durans OX53345 GNinfC PE3 SV1	0.63016	15.758	15.609
trA0A2A7SS14A0A2A7SS14_9ENTE 30S ribosomal protein S15 OSEnterococcus durans OX53345 GNrpsO PE3 SV1	0.62161	0.74635	0.7383
trA0A2A7SS17A0A2A7SS17_9ENTE Elongation factor Tu OSEnterococcus durans OX53345 GNtufA PE3 SV1	0.62	0.56762	0.62345
trA0A2A7SS33A0A2A7SS33_9ENTE 50S ribosomal protein L23 OSEnterococcus durans OX53345 GNrplW PE3 SV1	0.60127	0.36689	0.38456
trA0A2A7SS43A0A2A7SS43_9ENTE 50S ribosomal protein L5 OSEnterococcus durans OX53345 GNrplE PE3 SV1	0.59756	0.5865	0.59587
trA0A2A7SSA5A0A2A7SSA5_9ENTE 50S ribosomal protein L16 OSEnterococcus durans OX53345 GNrplP PE3 SV1	0.59639	0.49401	0.48854
trA0A2A7SSB5A0A2A7SSB5_9ENTE 30S ribosomal protein S5 OSEnterococcus durans OX53345 GNrpsE PE3 SV1	0.59181	0.45868	0.46664
trA0A2A7SSF6A0A2A7SSF6_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNEA71_00207 PE3 SV1	0.58769	0.82802	0.81875
trA0A2A7SSG7A0A2A7SSG7_9ENTE DEAD-box ATP-dependent RNA helicase CshA OSEnterococcus durans OX53345 GNcshA_1 PE3 SV1	0.58656	0.60508	0.59833
trA0A2S7MFM9A0A2S7MFM9_9ENTE Pyruvate kinase OSEnterococcus durans OX53345 GNpyk PE3 SV1	0.58015	0.63301	0.63774
trA0A2S7MJP1A0A2S7MJP1_9ENTE Alpha-ketoacid dehydrogenase subunit beta OSEnterococcus durans OX53345 GNpdhB PE4 SV1	0.57923	2.183	21.879
trA0A367CB54A0A367CB54_9ENTE 50S ribosomal protein L13 OSEnterococcus durans OX53345 GNrplM PE3 SV1	0.5677	0.7001	0.698
trA0A367CDI5A0A367CDI5_9ENTE Cell cycle protein GpsB OSEnterococcus durans OX53345 GNdivIVA2 PE3 SV1	0.55825	0.22811	0.24661
trA0A367CEM5A0A367CEM5_9ENTE Chaperone protein DnaK OSEnterococcus durans OX53345 GNdnaK PE2 SV1	0.55327	0.21692	0.24312
trA0A367CEQ4A0A367CEQ4_9ENTE Cell division protein FtsZ OSEnterococcus durans OX53345 GNftsZ PE3 SV1	0.55048	0.21624	0.25876
trA0A367CF36A0A367CF36_9ENTE Protein of uncharacterized function DUF3042 OSEnterococcus durans OX53345 GNEA71_01726 PE4 SV1	0.54413	0.29794	0.30434
trA0A367CFB0A0A367CFB0_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNEA71_02076 PE4 SV1	0.54179	0.74377	0.73676
trA0A367CGQ7A0A367CGQ7_9ENTE Ribosome-binding ATPase YchF OSEnterococcus durans OX53345 GNengD PE3 SV1	0.54039	0.37108	0.37103
trA0A377KFS1A0A377KFS1_9ENTE ATPases with chaperone activity ATP-binding subunit OSEnterococcus durans OX53345 GNclpC_1 PE4 SV1	0.53586	0.73736	0.73301
trA0A377KFT2A0A377KFT2_9ENTE Catabolite control protein A OSEnterococcus durans OX53345 GNccpA_1 PE4 SV1	0.52873	0.26367	0.28022
trA0A377KFT6A0A377KFT6_9ENTE Glycosyl transferase OSEnterococcus durans OX53345 GNponA_1 PE4 SV1	0.52507	10.253	1.051
trA0A377KG32A0A377KG32_9ENTE Enolase OSEnterococcus durans OX53345 GNeno PE3 SV1	0.52457	0.4971	0.51891
trA0A377KG89A0A377KG89_9ENTE Phosphate ABC transporter substrate-binding protein OSEnterococcus durans OX53345 GNpst_1 PE4 SV1	0.52222	0.96694	0.96509
trA0A377KGA7A0A377KGA7_9ENTE CTP synthase OSEnterococcus durans OX53345 GNpyrG PE3 SV1	0.5207	0.85206	0.84714
trA0A377KGD2A0A377KGD2_9ENTE Glutamine synthetase OSEnterococcus durans OX53345 GNglnA PE3 SV1	0.51775	10.314	10.205
trA0A377KGD6A0A377KGD6_9ENTE Aminopeptidase C OSEnterococcus durans OX53345 GNpepC PE4 SV1	0.51468	0.61311	0.60675
trA0A377KGE4A0A377KGE4_9ENTE Valine--tRNA ligase OSEnterococcus durans OX53345 GNvalS PE3 SV1	0.51383	0.45071	0.51065
trA0A377KGQ8A0A377KGQ8_9ENTE 6-phospho-beta-galactosidase OSEnterococcus durans OX53345 GNlacG_1 PE3 SV1	0.51263	0.60061	0.5934
trA0A377KGT6A0A377KGT6_9ENTE NH3-dependent NAD synthetase OSEnterococcus durans OX53345 GNnadE PE3 SV1	0.51169	0.49461	0.49785
trA0A377KGU4A0A377KGU4_9ENTE DNA-entry nuclease OSEnterococcus durans OX53345 GNendA_1 PE4 SV1	0.50967	0.51457	0.68176
trA0A377KGU5A0A377KGU5_9ENTE General stress protein OSEnterococcus durans OX53345 GNNCTC8129_00164 PE4 SV1	0.50951	0.30338	0.40212
trA0A377KGX3A0A377KGX3_9ENTE Glucosamine-6-phosphate deaminase OSEnterococcus durans OX53345 GNnagB PE3 SV1	0.5054	0.66353	0.65949
trA0A377KGZ1A0A377KGZ1_9ENTE Pfpl family intracellular protease OSEnterococcus durans OX53345 GNyfkM PE4 SV1	0.49915	0.08741	0.13007
trA0A377KH06A0A377KH06_9ENTE Primosomal protein N OSEnterococcus durans OX53345 GNNCTC8129_00238 PE4 SV1	0.49733	8	0.36784
trA0A377KH40A0A377KH40_9ENTE NADH peroxidase OSEnterococcus durans OX53345 GNnpr_2 PE4 SV1	0.49136	0.35506	23.937
trA0A377KH51A0A377KH51_9ENTE Uncharacterized protein conserved in bacteria OSEnterococcus durans OX53345 GNyxeA_1 PE4 SV1	0.49075	24.196	0.93713

trA0A377KHG5A0A377KHG5_9ENTE S1 RNA binding domain-containing protein OSEnterococcus durans OX53345 GNyugl_2 PE4 SV1	0.4844	0.5533	0.55324
trA0A377KHG6A0A377KHG6_9ENTE Hypoxanthine phosphoribosyltransferase OSEnterococcus durans OX53345 GNhpt PE3 SV1	0.47916	0.28433	0.33871
trA0A377KHH3A0A377KHH3_9ENTE NlpC/P60 family lipoprotein OSEnterococcus durans OX53345 GNNCTC8129_00818 PE4 SV1	0.47598	2.8	2.769
trA0A377KHH9A0A377KHH9_9ENTE Autolysin OSEnterococcus durans OX53345 GNNCTC8129_00758 PE4 SV1	0.47091	31.387	3.102
trA0A377KH8A0A377KH8_9ENTE Ribonucleoside-diphosphate reductase OSEnterococcus durans OX53345 GNrdE2 PE3 SV1	0.46863	31.486	31.448
trA0A377KHM4A0A377KHM4_9ENTE Methionine--tRNA ligase OSEnterococcus durans OX53345 GNmetG_1 PE3 SV1	0.46617	0.44947	0.50652
trA0A377KHV3A0A377KHV3_9ENTE DNA-directed RNA polymerase subunit beta OSEnterococcus durans OX53345 GNrpoB PE3 SV1	0.46416	0.3729	0.37823
trA0A377KHX2A0A377KHX2_9ENTE FMN-binding domain-containing protein OSEnterococcus durans OX53345 GNNCTC8129_00960 PE4 SV1	0.45451	18.286	1.807
trA0A377KHZ0A0A377KHZ0_9ENTE Pheromone cAD1 lipoprotein OSEnterococcus durans OX53345 GNNCTC8129_00959 PE4 SV1	0.4515	15.881	15.695
trA0A377KI00A0A377KI00_9ENTE 30S ribosomal protein S6 OSEnterococcus durans OX53345 GNrpsF PE3 SV1	0.44669	0.79949	0.78995
trA0A377KI07A0A377KI07_9ENTE Pyruvate oxidase OSEnterococcus durans OX53345 GNpox5 PE3 SV1	0.44102	0.40946	0.40461
trA0A377KI13A0A377KI13_9ENTE Beta sliding clamp OSEnterococcus durans OX53345 GNdnaB_1 PE3 SV1	0.44074	0.2966	0.29312
trA0A377KI32A0A377KI32_9ENTE Mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase OSEnterococcus durans OX53345 GNNCTC8129_00876 PE4 SV1	0.43448	16.378	16.184
trA0A377KI73A0A377KI73_9ENTE Elongation factor G OSEnterococcus durans OX53345 GNfusA PE3 SV1	0.41197	0.39848	0.41197
trA0A377KI79A0A377KI79_9ENTE 50S ribosomal protein L17 OSEnterococcus durans OX53345 GNrplQ PE3 SV1	0.40759	0.67911	0.6787
trA0A377KIA1A0A377KIA1_9ENTE ATP synthase subunit beta OSEnterococcus durans OX53345 GNatpD PE3 SV1	0.406	0.30295	0.32704
trA0A377KIJ5A0A377KIJ5_9ENTE D-alanine--D-alanine ligase OSEnterococcus durans OX53345 GNddl PE3 SV1	0.40545	0.08540	0.17048
trA0A377KIP0A0A377KIP0_9ENTE Tyrosine decarboxylase OSEnterococcus durans OX53345 GNddc_2 PE4 SV1	0.40508	8	0.17048
trA0A377KIP6A0A377KIP6_9ENTE LPXTG family cell surface protein Fms3 OSEnterococcus durans OX53345 GNfms3 PE4 SV1	0.40351	14.734	14.612
trA0A377KIS7A0A377KIS7_9ENTE Glutamyl aminopeptidase OSEnterococcus durans OX53345 GNpepA_1 PE3 SV1	0.40311	31.304	31.081
trA0A377KIW4A0A377KIW4_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNNCTC8129_01354 PE4 SV1	0.40311	0.56021	0.56492
trA0A377KIX2A0A377KIX2_9ENTE 50S ribosomal protein L25 OSEnterococcus durans OX53345 GNrply PE3 SV1	0.4029	0.82335	0.81382
trA0A377KIZ0A0A377KIZ0_9ENTE 50S ribosomal protein L6 OSEnterococcus durans OX53345 GNrplF PE3 SV1	0.38894	0.20822	0.34987
trA0A377KJ12A0A377KJ12_9ENTE DAK2 domain fusion protein YloV OSEnterococcus durans OX53345 GNyloV PE4 SV1	0.38337	0.388	0.43254
trA0A377KJ39A0A377KJ39_9ENTE Dipeptidase PepV OSEnterococcus durans OX53345 GNpepV_2 PE4 SV1	0.382	0.36448	0.36334
trA0A377KJ82A0A377KJ82_9ENTE Aspartate--tRNA ligase OSEnterococcus durans OX53345 GNaspS PE3 SV1	0.37569	0.53845	0.56735
trA0A377KJB0A0A377KJB0_9ENTE Oligoendopeptidase F plasmid OSEnterococcus durans OX53345 GNpepF1_2 PE3 SV1	0.37455	0.36255	0.36318
trA0A377KJB4A0A377KJB4_9ENTE Pheromone binding domain protein OSEnterococcus durans OX53345 GNoppA_2 PE4 SV1	0.37383	0.3281	0.32423
trA0A377KJE7A0A377KJE7_9ENTE Polysaccharide deacetylase OSEnterococcus durans OX53345 GNNCTC8129_01271 PE4 SV1	0.36634	0.67287	0.66518
trA0A377KJF6A0A377KJF6_9ENTE N-acetylmuramoyl-L-alanine amidase OSEnterococcus durans OX53345 GNNCTC8129_00658 PE4 SV1	0.36511	0.66591	0.67596
trA0A377KJG7A0A377KJG7_9ENTE 3-carboxymuconate cyclase OSEnterococcus durans OX53345 GNNCTC8129_01543 PE4 SV1	0.36246	30.881	30.517
trA0A377KJI1A0A377KJI1_9ENTE FMN-dependent NADH-azoreductase OSEnterococcus durans OX53345 GNacpH PE3 SV1	0.35563	19.784	19.573
trA0A377KJI8A0A377KJI8_9ENTE 5-methylthioadenosine/S-adenosylhomocysteine nucleosidase OSEnterococcus durans OX53345 GNmntN PE3 SV1	0.35291	0.87526	0.87881
trA0A377KJJ5A0A377KJJ5_9ENTE Serine hydroxymethyltransferase OSEnterococcus durans OX53345 GNglyA PE3 SV1	0.34897	0.13192	0.13855
trA0A377KJK4A0A377KJK4_9ENTE Foldase protein PrsA OSEnterococcus durans OX53345 GNprsA PE3 SV1	0.34846	11.544	11.606
	0.34451	13.498	13.418

trA0A377KJS9A0A377KJS9_9ENTE Basic membrane family protein OSEnterococcus durans OX53345 GNtmpC_2 PE4 SV1	0.34451	17.964	18.109
trA0A377KJT3A0A377KJT3_9ENTE ErfK/YbiS/Ycf5/YnhG family protein OSEnterococcus durans OX53345 GNNCTC8129_00812 PE4 SV1	0.33913	21.748	21.486
trA0A377KJX1A0A377KJX1_9ENTE Sulfatase OSEnterococcus durans OX53345 GNltaS1_1 PE4 SV1	0.33486	15.317	15.637
trA0A377KJY5A0A377KJY5_9ENTE Bifunctional protein GlmU OSEnterococcus durans OX53345 GNglmU PE3 SV1	0.32692	0.22208	0.24909
trA0A377KK17A0A377KK17_9ENTE Succinate-semialdehyde dehydrogenase OSEnterococcus durans OX53345 GNgabD PE4 SV1	0.32629	0.31197	0.34619
trA0A377KK67A0A377KK67_9ENTE Protein DltD OSEnterococcus durans OX53345 GNdltD PE3 SV1	0.3239	0.5894	0.59711
trA0A377KK85A0A377KK85_9ENTE M42 glutamyl aminopeptidase OSEnterococcus durans OX53345 GNysdC PE3 SV1	0.3221	0.12872	0.19273
trA0A377KKA3A0A377KKA3_9ENTE Inosine-5-monophosphate dehydrogenase OSEnterococcus durans OX53345 GNguaB_1 PE3 SV1	0.31966	0.73046	0.74286
trA0A377KKC2A0A377KKC2_9ENTE DNA gyrase subunit A OSEnterococcus durans OX53345 GNgyrA PE3 SV1	0.31647	0.34168	0.34383
trA0A377KKD3A0A377KKD3_9ENTE Aminopeptidase OSEnterococcus durans OX53345 GNpepS PE4 SV1	0.31477	0.10061	0.15643
trA0A377KKE1A0A377KKE1_9ENTE Peptidoglycan glycosyltransferase OSEnterococcus durans OX53345 GNpbpX PE4 SV1	0.31171	0.95253	0.96589
trA0A377KKE3A0A377KKE3_9ENTE Isoleucine--tRNA ligase OSEnterococcus durans OX53345 GNileS PE3 SV1	0.31111	0.21868	0.2344
trA0A377KKFOA0A377KKFO_9ENTE Adenylosuccinate synthetase OSEnterococcus durans OX53345 GNpurA PE3 SV1	0.3082	20.494	20.274
trA0A377KKF3A0A377KKF3_9ENTE dTDP-glucose 4 6-dehydratase OSEnterococcus durans OX53345 GNrmlB PE3 SV1	0.3074	0.43016	0.48944
trA0A377KKF9A0A377KKF9_9ENTE 2 3-bisphosphoglycerate-dependent phosphoglycerate mutase OSEnterococcus durans OX53345 GNgpmA_1 PE3 SV1	0.3029	0.7484	0.75366
trA0A377KKG7A0A377KKG7_9ENTE Acetate kinase OSEnterococcus durans OX53345 GNackA PE3 SV1	0.2985	0.99428	0.98234
trA0A377KKH9A0A377KKH9_9ENTE Phosphopentomutase OSEnterococcus durans OX53345 GNdeoB PE3 SV1	0.29768	0.20215	0.21567
trA0A377KKI2A0A377KKI2_9ENTE dTDP-4-dehydrorhamnose reductase OSEnterococcus durans OX53345 GNrmlD PE3 SV1	0.29658	0.55457	0.59311
trA0A377KKM1A0A377KKM1_9ENTE OsmC/Ohr family protein OSEnterococcus durans OX53345 GNohrB_2 PE4 SV1	0.29318	0.41504	0.41105
trA0A377KKM3A0A377KKM3_9ENTE ATP-dependent Clp protease ATP-binding protein ClpE OSEnterococcus durans OX53345 GNclpE PE3 SV1	0.28602	0.34438	0.38299
trA0A377KKM5A0A377KKM5_9ENTE Family 5 extracellular solute-binding protein OSEnterococcus durans OX53345 GNappA PE4 SV1	0.28572	22.758	22.484
trA0A377KKM9A0A377KKM9_9ENTE 3-oxoacyl-acyl-carrier-protein synthase 2 OSEnterococcus durans OX53345 GNfabF PE3 SV1	0.28352	0.12268	0.17611
trA0A377KKP3A0A377KKP3_9ENTE Endopeptidase PepO OSEnterococcus durans OX53345 GNpepO PE4 SV1	0.28334	0.12379	0.12233
trA0A377KKQ7A0A377KKQ7_9ENTE Biotin carboxyl carrier protein of acetyl-CoA carboxylase OSEnterococcus durans OX53345 GNaccB PE4 SV1	0.27889	0.23199	0.23538
trA0A377KKR7A0A377KKR7_9ENTE 6-phosphogluconate dehydrogenase decarboxylating OSEnterococcus durans OX53345 GNnd PE3 SV1	0.27877	0.20544	0.23825
trA0A377KKS6A0A377KKS6_9ENTE Tautomerase OSEnterococcus durans OX53345 GNNCTC8129_01978 PE3 SV1	0.27853	0.19102	0.19274
trA0A377KKX2A0A377KKX2_9ENTE Malonyl CoA-acyl carrier protein transacylase OSEnterococcus durans OX53345 GNfabD_2 PE3 SV1	0.27211	0.72381	0.8187
trA0A377KL37A0A377KL37_9ENTE ATP-dependent protease ATPase subunit HslU OSEnterococcus durans OX53345 GNhslU PE3 SV1	0.27156	0.42997	0.42765
trA0A377KL65A0A377KL65_9ENTE Transcription elongation factor GreA OSEnterococcus durans OX53345 GNgreA PE3 SV1	0.26866	0.11815	0.20802
trA0A377KL69A0A377KL69_9ENTE Oxidoreductase aldo/keto reductase OSEnterococcus durans OX53345 GNyvgN PE4 SV1	0.26599	0.54814	0.54221
trA0A377KL87A0A377KL87_9ENTE Chitin binding protein OSEnterococcus durans OX53345 GNgbpA_3 PE4 SV1	0.25764	32.118	32.154
trA0A377KL98A0A377KL98_9ENTE Adenylosuccinate lyase OSEnterococcus durans OX53345 GNpurB PE3 SV1	0.25677	0.76065	0.7607
trA0A377KLA4A0A377KLA4_9ENTE Dipeptidase OSEnterococcus durans OX53345 GNNCTC8129_02097 PE4 SV1	0.25334	0.70492	0.70644
trA0A377KLB2A0A377KLB2_9ENTE Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OSEnterococcus durans OX53345 GNaccA PE3 SV1	0.25324	0.04852	0.05373
trA0A377KLB8A0A377KLB8_9ENTE D-alanyl-D-alanine carboxypeptidase OSEnterococcus durans OX53345 GNNCTC8129_02202 PE4 SV1	6	1	1
trA0A377KLD6A0A377KLD6_9ENTE Anaerobic ribonucleoside triphosphate reductase OSEnterococcus durans OX53345 GNrdD PE4 SV1	0.25003	2.705	27.143
	0.24314	22.128	21.922

trA0A377KLE2A0A377KLE2_9ENTE Elongation factor EF1A OSEnterococcus durans OX53345 GNtufA3 PE4 SV1	0.22033	0.08170 4	0.13194
trA0A377KLE7A0A377KLE7_9ENTE Lipoate--protein ligase OSEnterococcus durans OX53345 GNlplJ_1 PE4 SV1	0.22018	0.29549	0.29883
trA0A377KLE8A0A377KLE8_9ENTE Predicted outer membrane protein OSEnterococcus durans OX53345 GNNCTC8129_02242 PE4 SV1	0.21898	28.014	27.702
trA0A377KLJ3A0A377KLJ3_9ENTE Nitroreductase family protein OSEnterococcus durans OX53345 GNNCTC8129_02296 PE4 SV1	0.21223	0.85062	0.85476
trA0A377KLJ8A0A377KLJ8_9ENTE 3-hydroxyacyl-acyl-carrier-protein dehydratase FabZ OSEnterococcus durans OX53345 GNfabZ1 PE3 SV1	0.2092	0.50513	0.5192
trA0A377KLL5A0A377KLL5_9ENTE Histidine--tRNA ligase OSEnterococcus durans OX53345 GNhisS PE3 SV1	0.2078	0.29741	0.30978
trA0A377KLM0A0A377KLM0_9ENTE Transketolase OSEnterococcus durans OX53345 GNtkt_1 PE3 SV1	0.20464	0.23458	0.25469
trA0A377KLM6A0A377KLM6_9ENTE Enoyl-acyl-carrier-protein reductase NADH OSEnterococcus durans OX53345 GNfabI PE3 SV1	0.20004	0.36141	0.3629
trA0A377KLN1A0A377KLN1_9ENTE 30S ribosomal protein S1 OSEnterococcus durans OX53345 GNrpsA PE4 SV1	0.19436	0.6471	0.65264
trA0A377KLQ2A0A377KLQ2_9ENTE UPF0291 protein NCTC8129_02336 OSEnterococcus durans OX53345 GNNCTC8129_02336 PE3 SV1	0.19417	0.10922	0.10933
trA0A377KLQ5A0A377KLQ5_9ENTE Carbamoyl-phosphate synthase large chain OSEnterococcus durans OX53345 GNcarB PE3 SV1	0.19268	0.74987	0.74159
trA0A377KLS8A0A377KLS8_9ENTE Alanine--tRNA ligase OSEnterococcus durans OX53345 GNalaS PE3 SV1	0.1885	0.45086	0.50756
trA0A377KLT2A0A377KLT2_9ENTE Superoxide dismutase OSEnterococcus durans OX53345 GNsodA PE3 SV1	0.18554	0.34715	0.51895
trA0A377KLU7A0A377KLU7_9ENTE Orotate phosphoribosyltransferase OSEnterococcus durans OX53345 GNpyrE PE3 SV1	0.18496	0.52936	0.52846
trA0A377KLV0A0A377KLV0_9ENTE Orotidine 5-phosphate decarboxylase OSEnterococcus durans OX53345 GNpyrF PE3 SV1	0.18315	0.46225	0.46251
trA0A377KLV6A0A377KLV6_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNNCTC8129_02407 PE4 SV1	0.18295	0.98681	0.98528
trA0A377KLY8A0A377KLY8_9ENTE Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex OSEnterococcus durans OX53345 GNpdhC PE3 SV1	0.17611	13.905	13.846
trA0A377KLZ7A0A377KLZ7_9ENTE Penicillin-binding protein 2B OSEnterococcus durans OX53345 GNpenA PE4 SV1	0.17609	0.97801	1
trA0A377KLZ9A0A377KLZ9_9ENTE Pyruvate carboxylase OSEnterococcus durans OX53345 GNcfiB PE4 SV1	0.17466	0.27124	0.34326
trA0A377KM63A0A377KM63_9ENTE Cyclopropane-fatty-acyl-phospholipid synthase OSEnterococcus durans OX53345 GNcfa PE4 SV1	0.16964	0.17859	0.22888
trA0A377KMB2A0A377KMB2_9ENTE General stress protein Gls33 OSEnterococcus durans OX53345 GNglS33 PE4 SV1	0.16766	12.909	12.753
trA0A377KMC7A0A377KMC7_9ENTE Fumarate reductase flavoprotein subunit OSEnterococcus durans OX53345 GNfccA PE4 SV1	0.16685	10.224	10.103
trA0A377KMD0A0A377KMD0_9ENTE Formate acetyltransferase OSEnterococcus durans OX53345 GNpfiB PE4 SV1	0.16352	33.849	33.474
trA0A377KME6A0A377KME6_9ENTE Dihydroorotase OSEnterococcus durans OX53345 GNpyrC PE3 SV1	0.16325	0.67436	0.66976
trA0A377KMN9A0A377KMN9_9ENTE Aspartyl/glutamyl-tRNAAsn/Gln amidotransferase subunit B OSEnterococcus durans OX53345 GNgatB_3 PE3 SV1	0.15372	0.24978	0.32144
trA0A377KMP6A0A377KMP6_9ENTE Cell division protein FtsA OSEnterococcus durans OX53345 GNftsA PE3 SV1	0.15093	0.12942	0.18108
trA0A377KMP9A0A377KMP9_9ENTE Tagatose-6-phosphate kinase OSEnterococcus durans OX53345 GNlacC_1 PE3 SV1	0.14971	13.151	13.029
trA0A377KMS1A0A377KMS1_9ENTE Glutamyl-tRNAIn amidotransferase subunit A OSEnterococcus durans OX53345 GNgatA PE3 SV1	0.14922	0.34664	0.36296
trA0A377KMU2A0A377KMU2_9ENTE Beta-lactamase OSEnterococcus durans OX53345 GNfmtA PE4 SV1	0.14548	0.68339	0.7187
trA0A377KMU6A0A377KMU6_9ENTE Translation initiation factor IF-2 OSEnterococcus durans OX53345 GNinfB PE3 SV1	0.14295	0.4599	0.47344
trA0A377KMV3A0A377KMV3_9ENTE Arginine--tRNA ligase OSEnterococcus durans OX53345 GNargS PE3 SV1	0.13108	0.59773	0.64631
trA0A377KN05A0A377KN05_9ENTE 3-oxoacyl-acyl-carrier-protein synthase 3 OSEnterococcus durans OX53345 GNfabH PE3 SV1	0.13001	0.05384 4	0.08454 9
trA0A377KN07A0A377KN07_9ENTE Amino acid ABC transporter amino acid-binding/permease OSEnterococcus durans OX53345 GNartQ_2 PE3 SV1	0.12746	0.91956 0.08108	0.93011 0.08777
trA0A377KN56A0A377KN56_9ENTE Proline--tRNA ligase OSEnterococcus durans OX53345 GNproS PE3 SV1	0.12499	3	6
trA0A377KN69A0A377KN69_9ENTE Sulfatase OSEnterococcus durans OX53345 GNltaS1_2 PE4 SV1	0.12057	22.804	22.535
trA0A377KN74A0A377KN74_9ENTE Carbamate kinase OSEnterococcus durans OX53345 GNarcC1 PE3 SV1	0.11299	20.535	20.415

trA0A377KN82A0A377KN82_9ENTE ATP-dependent Clp protease ATP-binding subunit ClpX OSEnterococcus durans OX53345 GNclpX_2 PE3 SV1	0.11138	0.39675	0.39207
trA0A377KN94A0A377KN94_9ENTE Glucokinase OSEnterococcus durans OX53345 GNglcK PE4 SV1	0.11061	0.37265	0.38102
trA0A377KNB1A0A377KNB1_9ENTE Uncharacterized conserved protein OSEnterococcus durans OX53345 GNNCTC8129_02084 PE4 SV1	0.11003	23.946	23.675
trA0A377KND0A0A377KND0_9ENTE Glucose-6-phosphate isomerase OSEnterococcus durans OX53345 GNpgi PE3 SV1	0.10163	0.36917	0.40444
trA0A377KNE8A0A377KNE8_9ENTE Transcription termination/antitermination protein NusA OSEnterococcus durans OX53345 GNnusA PE3 SV1	0.09872	7	0.17824
trA0A377KNF0A0A377KNF0_9ENTE Putative phage-encoded protein-like protein OSEnterococcus durans OX53345 GNNCTC8129_02877 PE4 SV1	0.09693	4	0.71539
trA0A377KNF1A0A377KNF1_9ENTE Short chain dehydrogenase/reductase family oxidoreductase OSEnterococcus durans OX53345 GNfad PE4 SV1	0.09572	5	1.587
trA0A377KNG7A0A377KNG7_9ENTE Probable manganese-dependent inorganic pyrophosphatase OSEnterococcus durans OX53345 GNppaC PE3 SV1	0.09087	4	0.28105
trA0A377KNJ3A0A377KNJ3_9ENTE DNA-entry nuclease OSEnterococcus durans OX53345 GNendA_2 PE4 SV1	0.08964	10.062	1.094
trA0A377KNJ5A0A377KNJ5_9ENTE Flavin reductase OSEnterococcus durans OX53345 GNazr PE4 SV1	0.08922	5	0.73624
trA0A377KNK5A0A377KNK5_9ENTE Xanthine phosphoribosyltransferase OSEnterococcus durans OX53345 GNxpt PE3 SV1	0.08844	9	0.50518
trA0A377KNK6A0A377KNK6_9ENTE N-acetylglucosamine-6-phosphate deacetylase OSEnterococcus durans OX53345 GNnagA PE3 SV1	0.08763	5	0.17258
trA0A377KNL8A0A377KNL8_9ENTE Uncharacterized protein OSEnterococcus durans OX53345 GNNCTC8129_03060 PE4 SV1	0.08621	0.75901	0.75545
trA0A377KNM9A0A377KNM9_9ENTE Pyruvate dehydrogenase E1 component subunit alpha OSEnterococcus durans OX53345 GNpdhA PE4 SV1	0.08230	1	12.221
trA0A377KNN5A0A377KNN5_9ENTE Bifunctional purine biosynthesis protein PurH OSEnterococcus durans OX53345 GNpurH PE3 SV1	0.08072	3	0.74145
trA0A377KNP6A0A377KNP6_9ENTE 50S ribosomal protein L19 OSEnterococcus durans OX53345 GNrplS PE3 SV1	0.07568	1	0.51449
trA0A377KNP7A0A377KNP7_9ENTE D-alanyl-D-alanine carboxypeptidase OSEnterococcus durans OX53345 GNdacA PE3 SV1	0.07514	5	10.983
trA0A377KNT7A0A377KNT7_9ENTE Oxidoreductase zinc-binding protein OSEnterococcus durans OX53345 GNNCTC8129_03104 PE4 SV1	0.07047	5	0.16785
trA0A377KNU7A0A377KNU7_9ENTE Bifunctional phosphoglucomutase/phosphomannomutase OSEnterococcus durans OX53345 GNpgm2 PE3 SV1	0.06621	2	0.62548
trA0A377KNW2A0A377KNW2_9ENTE Peptidyl-prolyl cis-trans isomerase OSEnterococcus durans OX53345 GNNCTC8129_02318 PE3 SV1	0.06401	3	19.433
trA0A377KNX9A0A377KNX9_9ENTE N-acetylmuramoyl-L-alanine amidase OSEnterococcus durans OX53345 GNEA71_01260 PE4 SV1	0.05417	2.729	27.004
trA0A377KP08A0A377KP08_9ENTE NADH oxidase OSEnterococcus durans OX53345 GNnox_3 PE4 SV1	0.05375	9	18.612
trA0A377KP54A0A377KP54_9ENTE Carbamoyl-phosphate synthase small chain OSEnterococcus durans OX53345 GNcarA PE3 SV1	0.04854	3	0.36912
trA0A377KP57A0A377KP57_9ENTE Elongation factor Ts OSEnterococcus durans OX53345 GNtsf PE3 SV1	0.04587	0.50651	0.50807
trA0A377KQ13A0A377KQ13_9ENTE Phosphoglucoamine mutase OSEnterococcus durans OX53345 GNglmM PE3 SV1	0.04151	6	0.24085
trA0A377KQ25A0A377KQ25_9ENTE Trigger factor OSEnterococcus durans OX53345 GNtig PE3 SV1	0.03802	8	0.2368
trA0A377KQ46A0A377KQ46_9ENTE UvrABC system protein A OSEnterococcus durans OX53345 GNuvrA PE3 SV1	0.03198	4	0.23276
trA0A377KR02A0A377KR02_9ENTE Asparagine--tRNA ligase OSEnterococcus durans OX53345 GNasnS PE3 SV1	0.03162	6	0.37543
trA0A377KR58A0A377KR58_9ENTE Glycine--tRNA ligase beta subunit OSEnterococcus durans OX53345 GNglyS PE3 SV1	0.02837	5	0.26613
trA0A377L0K4A0A377L0K4_9ENTE Mannose-6-phosphate isomerase OSEnterococcus durans OX53345 GNNCTC8129_00093 PE4 SV1	0.02809	7	15.528
trA0A377L328A0A377L328_9ENTE Aspartate carbamoyltransferase OSEnterococcus durans OX53345 GNpyrB PE3 SV1	0.02299	4	0.5939
trA0A377L368A0A377L368_9ENTE GMP reductase OSEnterococcus durans OX53345 GNguaC PE3 SV1	0.02184	7	0.26966
trA0A377L56A0A377L56_9ENTE UTP--glucose-1-phosphate uridylyltransferase OSEnterococcus durans OX53345 GNgalU PE3 SV1	0.01501	7	0.43186
trA0A377MS03A0A377MS03_9ENTE Pyridine nucleotide-disulfide oxidoreductase OSEnterococcus durans OX53345 GNpdh_3 PE3 SV1	0.01157	7	0.5782



trA0A377MS14A0A377MS14_9ENTE N-acetylmuramoyl-L-alanine amidase OSEnterococcus durans OX53345 GNlytA_3 PE4 SV1	0.01082		
	1	0.38086	0.37
	0.00764		
trQ6KCJ7Q6KCJ7_9ENTE Protein RecA OSEnterococcus durans OX53345 GNrecA PE3 SV1	2	0.79366	0.79337

## ARTIGO CIENTÍFICO 4

---

**Metaproteomics reveals the action of proteins secreted by probiotic *E. durans* LAB18S in an *ex vivo* culture of the human gut microbiome.**

A ser submetido.

**Metaproteomics reveals the action of proteins secreted by probiotic *E. durans* LAB18S in an *ex vivo* culture of the human gut microbiome.**

Carolina Baldisserotto Comerlato<sup>1</sup>

Xu Zhang<sup>2</sup>

Krystal Walker<sup>2</sup>

Adriano Brandelli<sup>1</sup>

Daniel Figeys<sup>2\*</sup>

<sup>1</sup> Laboratório de Bioquímica e Microbiologia Aplicada, Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, 91510-970 Porto Alegre, Brazil

<sup>2</sup> Ottawa Institute of Systems Biology and Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada

## Abstract

The microbiome is important to all animals and humans, playing a critical role in health. *Ex vivo* culture based approaches are time and cost-effective solutions for rapidly evaluating the effects of probiotics against microbiomes. In this study, proteins secreted by probiotic *E. durans* LAB18S in symbiosis with fructooligosaccharide (FOS) and galactooligosaccharide (GOS) were subjected to an *ex vivo* culture of the intestinal microbiome of a healthy individual. Metaproteomics was used to evaluate changes in microbial communities of the human intestinal microbiome. The results revealed that microbial growth and protein expression varied on the presence of different *E. durans* secretome concentrations. These findings suggest that proteins secreted by the probiotic in symbiosis with FOS and GOS have different effects on the modulation of microbiota functional activities during cultivation. Hierarchical clustering analysis showed 654 differential proteins from the metaproteome samples. The higher concentration of probiotics secretome used considerably modifies the protein expression of the intestinal microbiome. The treatment of *E. durans* secretomes showed an increase of microorganisms in Firmicutes and Bacteroidetes phyla. The study results were suggestive of the favorable effects of the secretome in improving the intestinal microbial composition, stimulating bacterial growth and different protein expression.

**Keywords:** *Enterococcus durans*; intestinal human microbiome, FOS, GOS, probiotics, *ex vivo* culture.

## INTRODUCTION

The human gut harbors thousands of microbial species and plays important roles in host health. Much evidence has been associated with changes in microbiota composition and diseases such as inflammatory bowel disease (IBD), obesity, diabetes and cancer. In addition, modulations of the intestinal microbiota using probiotics / prebiotics were also reported to improve host metabolic phenotypes (VELASQUEZ-MANOFF, 2015).

The term "probiotics" first appeared in 1974 and its definition refers to living microorganisms that confer health benefit when consumed in adequate quantities (HILL *et al.*, 2014). Probiotics are currently one of the most popular dietary supplements consumed worldwide (CLARKE *et al.*, 2015). Consumption of these supplements is widely supported by doctors, specifically gastroenterologists (DRAPER *et al.*, 2017). The ability of probiotics to promote health benefits have been fueled a growing scientific interest for several decades. The use of these microorganisms is often associated with beneficial microbiota modulation and normalization of an intestinal dysbiosis, obtaining favorable results alone or as a mechanism by which probiotics protect the host against disease (IRWIN *et al.*, 2017).

New generation sequencing (NGS), such as metagenomics and metatranscriptomics, are used to examine microbiota composition and predict potential functions. However, it does not provide direct evidence as to whether genes are translated into proteins or not (JUSTE *et al.*, 2014). Metaproteomics can provide valuable information about the microbiome functional activities through direct protein profile expression levels. In contrast to metagenomics, the metaproteomic approach is less applied in studies of the intestinal microbiota (MAYNE *et al.*, 2016).

Although research has shown positive health effects on probiotic use, most of the published literature is conducted in populations with pathologies. Evidence supporting the effects of probiotics in healthy adults is limited and less consistent (KRISTENSEN *et al.*, 2016). Despite this, probiotic manufacturers promote the wider use of your product in the consumer market than those with specific health conditions. Therefore, this study aimed to evaluate the action of the *Enterococcus durans* 18S probiotic secretome (proteins secreted in the extracellular environment) in symbiosis with two widely used prebiotics (FOS and GOS) and their effect on the human intestinal microbiota of a healthy individual.

## **MATERIAL AND METHODS**

### **Culture of *Enterococcus durans* LAB 18S and secretome extraction**

*E. durans* LAB18S, which has probiotic properties (PIENIZ *et al.*, 2014), was grown in a synthetic culture medium (SM) described by ROSSI *et al.*, 2005 with FOS, GOS and Glucose (as a carbon source control). A concentration of 1 % of the carbon sources in 30 mL of SM was prepared in triplicate. *E. durans* cultured in 24 hour Luria-Bertani (LB) were inoculated (2%, v / v) with an initial OD at 600 nm between 0.8 and 0.9. Incubation was performed at 37 ° C for 8 h (mid-log phase) under anaerobic conditions (5% H<sub>2</sub>, 5% CO<sub>2</sub> and 90% N<sub>2</sub> at 37 ° C) until protein extraction was performed. In addition to these treatments, the isolate was cultured in the SM medium with the carbon sources containing 200 µg of bovine serum albumin (BSA) as protein control.

Extraction of proteins secreted by *E. durans* after incubation was performed as described by DEEKE *et al.* (2018). Proteins were extracted from culture medium supernatants after centrifugation at 14,000 g for 20 min at 4 ° C. Supernatants were carefully collected and filtered through 0.22 µm membranes into new tubes. Precipitation was performed using 20% (w / v) trichloroacetic acid (TCA) and centrifuged again at 16,000 g and 4 ° C for 20 min. Then the supernatants were discarded and the pellets were washed twice with acetone. The extracted proteins were kept at -20 ° C until further use.

### ***Ex vivo* gut microbiome culturing**

The Research Ethics Board protocol for stool sample collection was approved by the Ottawa Health Science Network Research Ethics Board at the Ottawa Hospital. In order to prepare the culture of the human intestinal microbiome *in vitro*, fecal sample was obtained from a healthy volunteer. Stool samples (~ 3 g) were mixed in pre-reduced PBS with 0.1% (w / v) L-cysteine hydrochloride, weighted in an anaerobic workstation where the tube was uncapped to remove O<sub>2</sub>, filtered with sterile gauze to remove large particles and obtain the microbiome inoculum. The microbiome inoculum was immediately inoculated at a concentration of 10 % (w / v) into a 96 well deep well plate containing 1 ml culture medium and the proteins secreted by *E. durans* in two different concentrations: 10 µL (low concentration) and 50 µL (high concentration). The culture media contained 2.0 g L<sup>-1</sup> peptone water, 2.0 g L<sup>-1</sup> yeast extract, 0.5 g L<sup>-1</sup> L-cysteine hydrochloride, 2 mL L<sup>-1</sup> Tween 40, 5 mg L<sup>-1</sup> hemin and 10 µL L<sup>-1</sup> vitamin K1. This medium was sterile and had been pre-reduced overnight in an anaerobic workstation. In place of proteins secreted by *E. durans*,

PBS was used as control for culture medium. After inoculation, the 96-well deep plate was covered with a silicone cap with a vent hole for each well made by a sterile syringe needle. The plate was incubated in anaerobic chamber at 37 ° C and shaken with a digital shaker (MS3, IKA, Germany) at 500 rpm.

### **Metaproteomic sample processing and LC-MS/MS analysis**

Briefly, the culture samples were centrifuged at 300g and 4 ° C for 5 min to remove debris. Supernatants were collected and subjected to two more centrifugations. The supernatants were transferred to 2 mL tubes and centrifuged at 14000g and 4 ° C for 20 min. Next, the lysis buffer was freshly prepared, containing 8 M urea in 100 mM Tris-HCl buffer (pH = 8.0), plus Roche cOmplete™ Mini tablets. Microbial cell pellets were then re-suspended in 150 µl lysis buffer and lysed on ice using a sonicator (Q125 Qsonica, USA). The protein lysates were precipitated with acetic acid, acetone and ethanol buffer at -20 ° C overnight. The pellets were washed three times with ice-cold acetone and centrifuged at 16,000 g for 25 min. Protein concentrations of the samples were measured in triplicate using a detergent compatible (DC) assay (Bio-Rad, USA). Trypsin digestion and desalting was performed following the procedures described by ZHANG et al. (2016). The samples were reduced and alkylated with 10 mM dithiothreitol (DTT) and 20 mM iodoacetamide (IAA), followed by a 10× dilution using 100 mM Tris-HCl (pH = 8.0) and tryptic digestion under 37°C for 18 hours using 1 µg of trypsin per sample (Worthington Biochemical Corp., Lakewood, NJ). Tryptic peptides were dissolved in 0.1% formic acid, and 4 µg of protein was loaded for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis



with an Agilent 1100 Capillary LC system (Agilent Technologies, San Jose, CA) and an LTQ-Orbitrap XL mass spectrometer (Thermo Electron, Waltham, MA).

### **Metaproteomic data processing**

Protein/peptide identification and quantification, taxonomic assignment and functional annotations were done using the MetaLab software (version 1.1.0) (CHENG *et al.*, 2017). MetaLab is a software that automates an iterative database search strategy, i.e. MetaPro-IQ (ZHANG *et al.*, 2016). The search was based on a human gut microbial gene catalog containing 9,878,647 sequences from <http://meta.genomics.cn/>. A spectral clustering strategy were used for database construction from all raw files, then the peptide and protein lists were generated by applying strict filtering based on a FDR of 0.01, and quantitative information of proteins were obtained with the maxLFQ algorithm on MaxQuant (version 1.5.3.30). Carbamidomethyl (C) was set as a fixed modification and oxidation (M) and N-terminal acetylation (Protein N-term) were set as variable modifications. Instrument resolution was set as “High-High”.

The quantified protein groups were first filtered according to the criteria that the protein should be identified by  $\geq 1$  unique peptides in  $\geq 50\%$  of the samples (Q50). LFQ protein group intensities of the filtered file was  $\log_{10}$  transformed. Functional annotations of protein groups, including COG and KEGG information, were obtained in the MetaLab software.

## Statistical analysis

All missing values of the  $\log_{10}$  transformed and Q50-filtered protein group abundance data were imputed using the KNN algorithm, and then partial least-squares discriminant analyses (PLS-DA) was performed in MetaboAnalyst (<http://www.metaboanalyst.ca/>) (CHONG *et al.*, 2018) for discriminating proteins differentially abundant in response to secreted proteins by *E. durans*. Cross-validation with  $R^2$  and  $Q^2$  were used to evaluate the performance of the PLS-DA models. Identification of the differential proteins in response to *E. durans* secretomes were achieved using the variable importance in projection (VIP); a protein with a VIP score higher than one was considered as an important feature for group discrimination in the model. Hierarchical clustering of samples was performed with Pearson's correlation of the normalized data. Each cluster represents a group of proteins with a similar expression pattern in response to *E. durans* excreted proteins grown on different substrates.

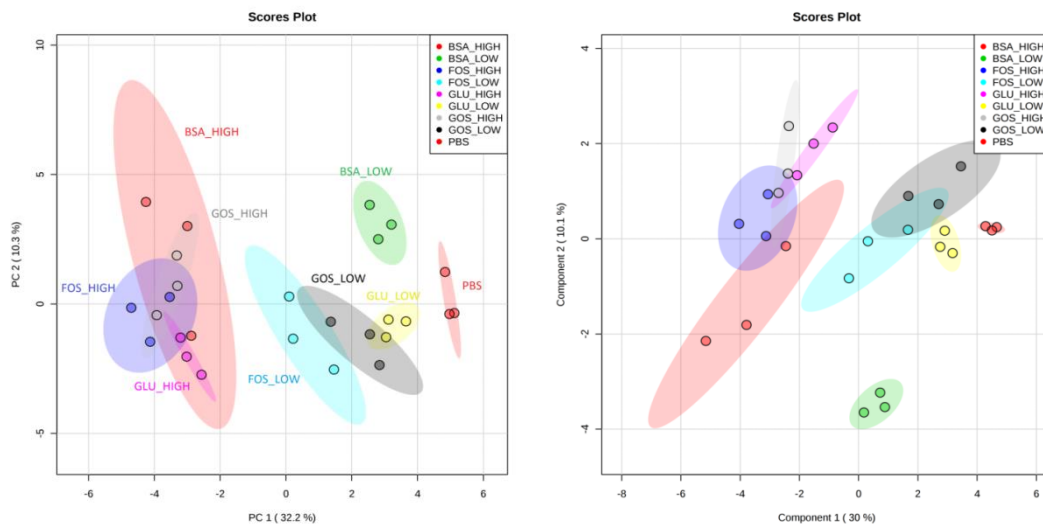
## RESULTS

*E. durans* secretome was treated with FOS, GOS and glucose and inoculated in human intestinal microbiome. Metaproteomic results showed a total of 21,519 peptides corresponding to 6,470 protein groups were identified across all samples with a false discovery rate (FDR) threshold of 1%. To obtain an accurate assessment of the effects of *E.*

*durans* secretomes on the human microbiome, data filtering criteria were used to identify 6.470 protein groups present in more than 50% of the samples.

Principal component analysis (PCA) using the log-transformed LFQ intensity of protein groups showed a secretome concentration-dependent effect on human microbiome culture (Figure 1A). This revealed that the microbial communities clearly separate on the presence of different secretome concentrations. These findings suggest that proteins secreted by the probiotic in symbiosis with the prebiotics have different effects on the modulation of microbiota functional activities during *in vitro* cultivation.

In addition, we can observe that the culture medium with PBS is isolated in Figure 1, showing that the culture used has no influence on the response of the *Enterococcus* secretome on the human microbiome. The use of BSA showed that the response of the human microbiome was different when using the secretome of the probiotic isolate.

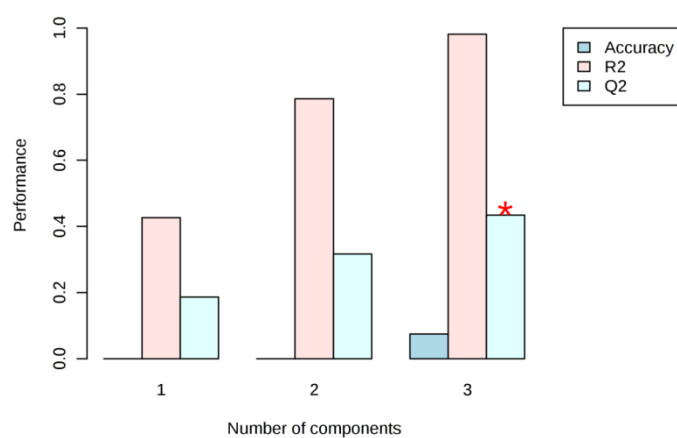


**Figure 1. (A)** PCA with all samples based on protein groups' LFQ intensities showed trends of protein changes over high and low concentrations of *E. durans* secretomes treated in different carbon sources. **(B)** PLS-DA

A PLS-DA approach was employed to identify the differentially expressed proteins related to the presence of different *E. durans* secretomes (Figure 1B) which identified 654 differential proteins with the threshold of VIP > 1.0 in the first component of the PLS-DA (Table S1). Cross validation showed acceptable performance for secretome PLS-DA model ( $R^2 > 0.98$  and  $Q^2 > 0.43$ ; Figures S1).

**PLS-DA cross validation details:**

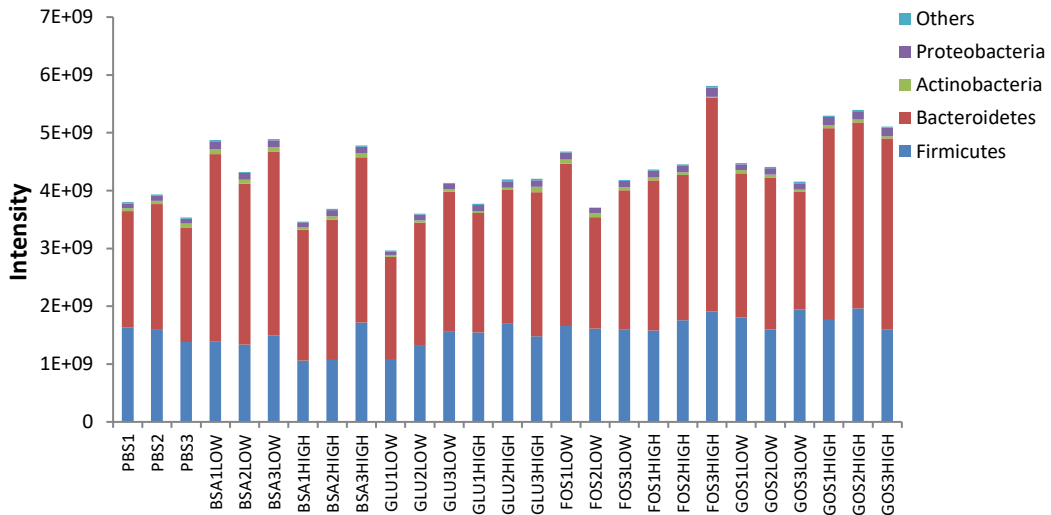
Measure	1 comps	2 comps	3 comps
Accuracy	0.0	0.0	0.074074
R2	0.42621	0.78578	0.98156
Q2	0.18583	0.31623	0.43408



**Figure S1.** Cross validation with  $R^2$  and  $Q^2$  were used to evaluate the performance of the PLS-DA model.

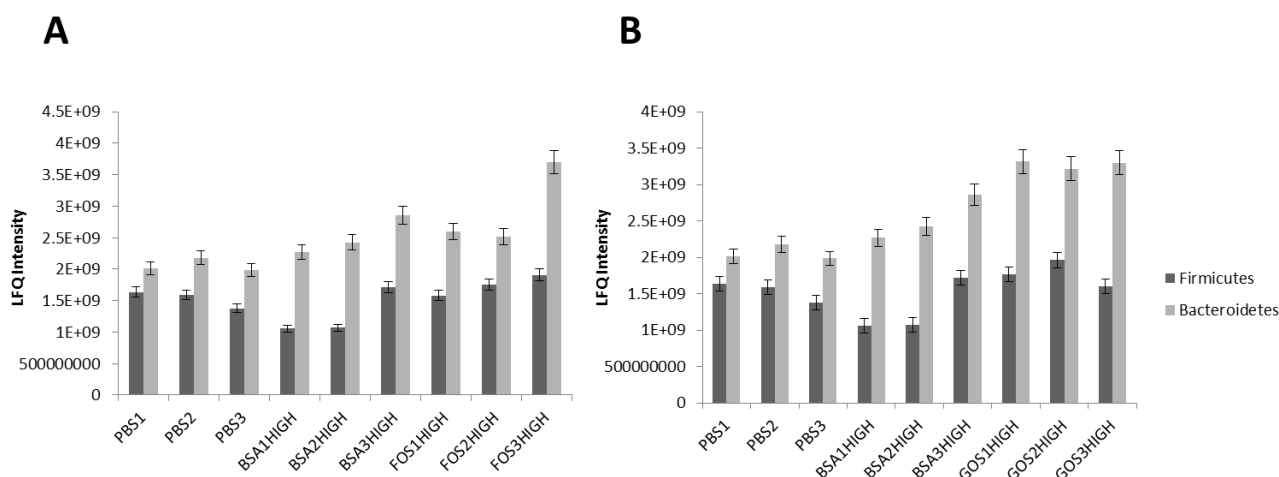


is used. Through this analysis, we can conclude that a higher concentration of probiotics considerably modifies the protein expression of the intestinal microbiome.



**Figure 3.** (A) PCA with all samples based on protein groups' LFQ intensities showed trends of protein changes over high and low concentrations of *E. durans* secretomes treated in different carbon sources. (B) PLS-DA with all samples based on protein groups' LFQ intensities.

The treatment of *E. durans* secretomes with glucose, FOS and GOS showed an increase in the amount of microorganisms especially in Bacteroidetes phyla compared with PBS treatment (Figure 3). In general, *E. durans* secretome treated with FOS and GOS in high concentrations increased the amount of Firmicutes and Bacteroidetes phyla (Figure 4).



**Figure 4.** Histogram showing the intensity of bacterial phyla Firmicutes and Bacteroidetes from human microbiome (A) using the secretome in high concentration treated with FOS and (B) the secretome in high concentration treated with GOS in symbiosis with *E. durans* secretome.

## DISCUSSION

The species found in the human intestinal microbiome mainly include four phyla: Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria. The change in the composition of the intestinal microbiota can lead to various diseases in humans and animals (LYE et al., 2017; NAKAMOTO et al., 2017; YU et al., 2017). In this study, the taxonomic profiles of fecal microbiota outlined remarkable changes in the gut bacterial composition and the rate of Firmicutes and Bacteroidetes was higher when *E. durans* was treated with FOS and GOS. Species from the two major bacterial phyla, Bacteroidetes and Firmicutes, have been identified that break down polysaccharides and complex oligosaccharides (SALAYERS et al., 1977). Mechanisms besides carbohydrate utilization, like cross-feeding, allow homogeneous bacterial populations to give rise to more diverse populations where metabolites from one strain provide a niche for the other (TURNBAUGH et al., 2008). MORSHEDI (2020) evaluated the human microbiome in three different groups: the D-Syn group treated with the inulin symbiosis and the probiotic *Lactobacillus plantarum*, the D-

Pro group only treated with the probiotic, and the D-Pre group treated only with inulin. The results showed a significant increase in the proportion of Firmicutes in D-Syn and D-Pro groups.

Many studies show the ability of probiotic species to change and control the population of microorganisms in the intestinal microbiota (KITAZAWA, 2015; DEEKE et al., 2018; MCNULTY et al., 2013). In this study we were able to observe an increase in the microbial population when the culture medium, in which the probiotic *Enterococcus durans* grew, was supplemented with FOS and GOS, especially in the phylum Bacteroidetes. Recently, a symbiotic mixture of FOS, GOS and probiotics promoted the diversity of the microbiota in babies who had been compromised since birth (CHUA et al., 2017). KOSUWON (2018) observed a bacterial increase in the groups belonging to *Lactobacillus* and *Enterococcus* in children supplemented with a mixture of FOS and GOS. The high abundance of the phylum Firmicutes and the decrease in the amount of Bacteroidetes are associated with the unbalanced composition of microbiomes (intestinal dysbiosis) and several diseases are associated with dysbiosis, such as diabetes, obesity, cancer and irritable bowel diseases (SCHMIDT et al., 2018).

In general, the results of the present study were suggestive of the favorable effects of the secretome in improving the intestinal microbial composition, stimulating bacterial growth. The administration of the secretome with the carbon sources had an result in relation to the controls with PBS and BSA. In addition, the different concentrations of the secretome used showed important changes in the expression of proteins. As a final point, the manipulation of the intestinal microbiota can be considered a therapeutic target for the prevention and treatment of metabolic disorders. Due to the highly limited research in this field, further studies are needed.



## REFERENCES

- CHENG, K.; NING, Z.; ZHANG, X.; LI, L.; LIAO, B.; MAYNE, J.; STINTZI, A. & FIGEYS, D. MetaLab: an automated pipeline for metaproteomic data analysis. *Microbiome*, 5:157, 2017.
- CHONG, J.; SOUFAN, O.; LI, C.; CARAUS, I.; LI, S.; BOURQUE, G.; WISHART, D. S. & XIA, J. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Research*, 46:486-94, 2018.
- CHUA, M. C.; BEN-AMOR, K.; LAY, C. Effect of Synbiotic on the Gut Microbiota of Cesarean Delivered Infants: A Randomized, Double-blind, Multicenter Study. *J Pediatr Gastroenterol Nutr*. 65(1):102–106, 2017.
- CLARKE, T. C.; BLACK, L. I.; STUSSMAN, B. J.; BARNES, P. M. & NAHIN, R. L. Trends in the use of complementary health approaches among adults: United States, 2002–2012. *National Health State Report*, 79: 1–16, 2015.
- DEEKE, S. A.; STARR, A. E.; NING, Z.; AHMADI, S.; ZHANG, X.; MAYNE, J.; CHIANG, C. K.; SINGLETON, R.; BENCHIMOL, E. I.; MACK, D. R.; STINTZI, A. & FIGEYS, D. Mucosal-luminal interface proteomics reveals biomarkers of pediatric inflammatory bowel disease-associated colitis. *The American Journal of Gastroenterology*, 113:713–724, 2018.
- DRAPER, K.; LEY, C. & PARSONNET, J. Probiotic guidelines and physician practice: a cross-sectional survey and overview of the literature. *Beneficial Microbes*, 8: 507–519, 2017.
- EL KAOUTARI, A.; ARMOUGOM, F.; GORDON, J. I.; RAOULT, D.; HENRISSAT, B. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nat Rev Microbiol*, 11:497–504, 2013.
- HILL, C.; GUARNER, F.; REID, G.; GIBSON, G. R.; MERENSTEIN, D. J.; POT, B.; MORELLI, L.; CANANI, R. B.; FLINT, H. J.; SALMINEN, S.; CALDER, P. C.; SANDERS, M. E. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Review Gastroenterology and Hepatology*, 11: 506–514, 2014.
- IRWIN, C.; KHALESII, S.; COX, A. J.; GRANT, G.; DAVEY, A. K.; BULMER, A. C. & DESBROW, B. Effect of 8-weeks prebiotics/probiotics supplementation on alcohol metabolism and blood biomarkers of healthy adults: a pilot study. *Europe Journal of Nutrition*, 57(4):1523-1534, 2017.
- JUSTE, C.; KREIL, D. P.; BEAUVALLET, C.; GUILLOT, A.; VACA, S.; CARAPITO, C.; MONDOT, S.; SYKACEK, P.; SOKOL, H.; BLON, F.; LEPERCQ, P.; LEVENEZ, F.; VALOT, B.; CARRE, W.; LOUX, V.; PONS, N.; DAVID, O.; SCHAEFFER, B.; LEPAGE, P.; MARTIN, P.; MONNET, V.; SEKSIK, P.; BEAUGERIE, L.; EHRLICH, S. D.; GIBRAT, J. F.; VAN DORSSELAER, A. & DORE, J. Bacterial protein signals are associated with Crohn's disease. *Gut*, 63: 1566-1577, 2014.
- KITAZAWA, H.; ALVAREZ, S.; SUVOROV, A.; MELNIKOV, V.; VILLENA, J.; S'ANCHEZ, B. Recent advances and future perspective in microbiota and probiotics. *BioMed Research International*, 2015, Article ID 275631, 2015.

- KOSUWON, P.; LAO-ARAYA, M.; UTHAISANGSOOK, S. A synbiotic mixture of scGOS/lcFOS and *Bifidobacterium breve* M-16V increases faecal *Bifidobacterium* in healthy young children. *Benef Microbes*. 9(4):541–552, 2018.
- KRISTENSEN, N. B.; BRYRUP, T.; ALLIN, K. H.; NIELSEN, T.; HANSEN, T. H. & PEDERSEN, O. Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. *Genome Medicine*, 8:52, 2016.
- LYE, H. S.; KATO, T.; LOW W. Y. *Lactobacillus fermentum* FTDC 8312 combats hypercholesterolemia via alteration of gut microbiota. *Journal of Biotechnology*, 262:75–83, 2017.
- MAYNE, J.; NING, Z.; ZHANG, X.; STARR, A. E.; CHEN, R.; DEEKE, S.; CHIANG, C. K.; XU, B.; WEN, M.; CHENG, K.; SEEBUN, D.; STAR, A.; MOORE, J. I. & FIGEYS, D. Bottom-Up Proteomics (2013-2015): Keeping up in the Era of Systems Biology. *Analytical Chemistry*, 88-121, 2016.
- MCNULTY, N. P.; WU, M.; ERICKSON, A. R.; PAN, C.; ERICKSON, B. K.; MARTENS, E. C.; PUDLO, N. A.; MUEGGE, B. D.; HENRISSAT, B.; HETTICH, R. L.; GORDON, J. I. Effects of diet on resource utilization by a model human gut microbiota containing *Bacteroides cellulosilyticus* WH2, a symbiont with an extensive glycobiome. *PLoS Biol*, 11:e1001637, 2013.
- MORSHEDI, M.; SAGHAFI-ASL, M.; HOSSEINIFARD, E. S. The potential therapeutic effects of the gut microbiome manipulation by synbiotic containing-*Lactobacillus plantarum* on neuropsychological performance of diabetic rats. *J Transl Med.*, 18(1):18, 2020.
- NAKAMOTO, N.; AMIYA, T.; AOKI, R. Commensal *Lactobacillus* Controls Immune Tolerance during Acute Liver Injury in Mice. *Cell Reports*, 21(5):1215–1226, 2017.
- PIENIZ, S.; ANDREAZZA, R.; ANGHINONI, T.; CAMARGO, F. & BRANDELLI, A. Probiotic potential, antimicrobial and antioxidant activities of *Enterococcus durans* strain LAB18S. *Food Control*, 37: 251-256, 2014.
- SALYERS, A. A.; VERCELLOTTI, J. R.; WEST, S. E.; WILKINS, T. D. Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon. *Appl Environ Microbiol*, 33:319–322, 1977.
- SCHMIDT, T. S. B.; RAES, J. & BORK, P. The Human Gut Microbiome: From Association to Modulation. *Cell*, 172: 1198–1215, 2018.
- TURNBAUGH, P. J.; BÄCKHED, F.; FULTON, L.; GORDON, J. I. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe*, 3:213–223, 2008.
- UNDERWOOD, M. A.; GAERLAN, S.; DE LEOZ, M. L. A.; DIMAPASOC, L.; KALANETRA, K. M.; LEMAY, D. G.; GERMAN, J. B.; MILLS, D. A.; LEBRILLA, C. B. Human milk oligosaccharides in premature infants: absorption, excretion, and influence on the intestinal microbiota. *Pediatr Res.*, 78:670–677, 2015.

YU, L.; ZHAO, X. K.; CHENG, M. L. *Saccharomyces boulardii* Administration Changes Gut Microbiota and Attenuates DGalactosamine-Induced Liver Injury. *Scientific Reports*, 7(1):1359, 2017.

ZHANG, X.; NING, Z.; MAYNE, J.; MOORE, J. I.; LI, J.; BUTCHER, J.; DEEKE, S. A.; CHEN, R.; CHIANG, C. K.; WEN, M.; MACK, D.; STINTZI, A. & FIGEYS, D. MetaPro-IQ: a universal metaproteomic approach to studying human and mouse gut microbiota. *Microbiome*, 4:31, 2016.

## 6. DISCUSSÃO

A presente tese engloba a avaliação do probiótico *Enterococcus durans* LAB18S através de um estudo genômico e proteômico a fim de estudar a ação simbiótica com os prebióticos FOS e GOS e sua interação com o microbioma humano. Através dos resultados obtidos é possível fazer algumas inferências de modo a unificar os desfechos observados e sua repercussão no microbioma intestinal humano.

De um modo geral, a maioria dos microrganismos exibem diferentes níveis de resistência contra um ou mais agentes antimicrobianos. Algumas das resistências foram definidas como intrínsecas, quando desenvolvidas ao longo da especiação do microrganismo; ou definidas como adquiridas, se obtidas pela transferência horizontal de genes de outros microrganismos (HOLZAPFEL *et al.*, 2018). O potencial patogênico de isolados clínicos de enterococos tem sido principalmente correlacionado à produção de substâncias de agregação e outros componentes favoráveis à propagação da bactéria, como hemolisina, gelatinase, hialuronidase, antígeno EfaAfs / EfaAfm, proteína de superfície Espfm e proteína de adesão Ace / Acm-adesina (LIASKOV'S'KYĚ *et al.*, 2008; LI *et al.*, 2015). A análise do genoma do probiótico LAB18S contribuiu para o conhecimento dos genes associados à resistência e à virulência, sendo que os principais genes de resistência, virulência e plasmídeos associados a esse gênero estavam ausentes no isolado LAB18S.

Muitos estudos estão associando o gênero *Enterococcus* ao grupo dos probióticos (BONACINA *et al.*, 2017; HOLZAPFEL *et al.*, 2018; YERLIKAYA & AKBULUT, 2019). Algumas características probióticas promissoras foram encontradas no genoma do isolado de estudo. A sobrevivência dos probióticos no trato gastrointestinal (TGI) é um fator importante e está associada com genes que conferem resistência ao meio ácido

(GUO *et al.*, 2015). O LAB18S possui um gene que contribui para a regulação do pH intracelular e demonstrou alta capacidade de sobreviver na presença de suco gástrico simulado (PIENIZ *et al.*, 2014). Além da habilidade de se manter em meio ácido, esse isolado apresentou um gene que pode estar associado à tolerância ao sal biliar. Essa tolerância também confere sobrevivência do isolado no TGI e tem sido recomendada para probióticos (LAVERMICOCCA *et al.*, 2008, HAMON *et al.*, 2011).

Embora a capacidade de adesão da bactéria probiótica no intestino do hospedeiro não garanta necessariamente um benefício à saúde, essa aderência pode ter um papel protetor contra enteropatógenos através da competição por sítios de ligação a células hospedeiras. Além disso, a capacidade de adesão das bactérias probióticas poderia aumentar a oportunidade de interagir com o hospedeiro, resultando em uma colonização temporária e aumentando seu tempo de trânsito no intestino para exercer seus efeitos benéficos (CELEBIOGLU *et al.*, 2017). Além disso, os oligossacarídeos que agem como prebióticos podem melhorar a capacidade de inibir a adesão de alguns agentes patogênicos no intestino, conforme revisado por HICKEY (2012). O genoma do LAB18S não apresentou genes associados à ligação ao muco, e / ou genes de adesão. No entanto, apresenta duas proteínas que podem contribuir para a adesão e um fator promotor de agregação, sugerindo que esse isolado pode se ligar a receptores no ambiente intestinal (SENAN *et al.*, 2015).

Outros benefícios probióticos foram encontrados como a produção de bacteriocinas que possuem um importante papel contra bactérias patogênicas no TGI (GILLOR *et al.*, 2008). Dentre as bacteriocinas encontradas estão a colicina V, produzida por muitas bactérias Gram-negativas (HÅVARSTEIN *et al.*, 1994) mostrando que pode ter ocorrido a transferência de genes de bactérias Gram-negativas para o LAB18S (LANGA *et al.*,

2017). Além de peptídeos com atividade antimicrobiana, no genoma do LAB18S foram encontrados genes do sistema toxina-antitoxina, associados à sobrevivência sob condições de estresse (FERNÁNDEZ-GRACÍA *et al.*, 2016); genes relacionados ao metabolismo de moléculas prebióticas, como o FOS; o gene BGL que codifica uma enzima com atividades de hidrolase e transglicosilase e é utilizada em aplicações como culturas probióticas na indústria de laticínios ou para síntese de GOS (MEIRA *et al.*, 2012).

Visto que em um estudo anterior, observou-se que o LAB18S possui capacidade de acumulação do selênio (PIENIZ *et al.*, 2017), houve o interesse pela busca de genes relacionados a essa característica. O selênio é essencial para a expressão de selenoproteínas que possuem propriedades antioxidantes (LIN *et al.*, 2015) e o LAB18S apresentou alguns genes relacionados. A partir da premissa que os prebióticos estimulam o crescimento e / ou a produção de diferentes substâncias oriundas das bactérias probióticas, foi demonstrado, nesse estudo, que FOS e GOS além de serem metabolizados pela LAB18S estimularam esse isolado a produzir proteínas de interesse industrial. Esse interesse industrial está vinculado a enzima  $\beta$ -galactosidase que é produzida pelo LAB18S e através da hidrólise reversa de lactose produz GOS (PARK & OH, 2010). Assim, a análise do genoma do probiótico LAB18S foi de extrema importância para guiar outras avaliações desse estudo.

A capacidade do probiótico em aderir às células epiteliais intestinais foi, agora, avaliada estimulando o islado com os prebióticos FOS e GOS. Através da análise do proteoma do LAB18S foram observadas duas proteínas associadas à adesão que estavam superexpressas em cultivo com GOS. Uma delas foi a EF-Tu que funciona como uma GTPase que garante precisão de tradução (SPRINZL, 1994) e mostrou adesão à mucina

e às células epiteliais humanas em outros estudos (BERGONZELLI *et al.*, 2006; GILAD *et al.*, 2011). A outra proteína, associada à adesão, é a GAPDH (gliceraldeído 3-fosfato desidrogenase), uma importante enzima glicolítica que pode ser secretada fora da parede celular (KINOSHITA *et al.*, 2012). Podemos observar que além de GOS ser capaz de superexpressar proteínas relacionadas à adesão no epitélio intestinal, esse prebiótico estimulou a produção de proteínas diferentes àquelas descritas no estudo do genoma do LAB18S.

As proteases do tipo Clp estão envolvidas no sistema de degradação de proteínas relacionadas ao estresse e mostraram uma expressão aumentada nos cultivos com FOS. NEHER *et al.* (2006) demonstraram que um isolado de *E. coli* apresentou a produção de ClpX, que controlou os níveis de muitas proteínas de resposta ao estresse, contribuindo para a sua sobrevivência. FOS também estimulou uma proteína relacionada à divisão celular que é multifuncional em *Enterococcus* spp. Essa está relacionada à viabilidade do isolado, à biossíntese do peptidoglicano, fechamento completo do septo de divisão, morfogênese e segregação cromossômica (BOHLE *et al.*, 2010; OLIVIA *et al.*, 2010).

Os prebióticos FOS e GOS são, atualmente, os mais estudados e os mais amplamente utilizados pela indústria. Quando falamos em microbioma e sua interação com os prebióticos, podemos notar que esses compostos parecem promover um aumento no crescimento de bactérias benéficas (SIMPSON & CAMPBELL, 2015). O prebiótico apenas é metabolizado por bactérias aptas a essa metabolização e através disso podem desencadear efeitos que promovem a saúde. Em outras palavras, a mudança da fonte de carbono promove nos microrganismos probióticos uma mudança nas vias metabólicas (SKALKAM *et al.*, 2016). Portanto, a abordagem proteômica desse estudo avaliando a

simbiose com prebióticos pode esclarecer que o sucesso de um isolado probiótico irá depender dos substratos disponíveis que ele encontrará no lúmen intestinal.

Após investigar o proteoma do LAB18S e sua interação simbiótica com FOS e GOS, as proteínas secretadas no meio extracelular por esse microrganismo foram objeto de estudo, assim como a influência do oxigênio na expressão de proteínas do isolado. O termo secretoma foi cunhado por TJALSMA *et al.*, (2000) e definido como o coletivo para todas as proteínas secretadas e maquinaria secretora das bactérias. O secretoma possui proteínas que podem estar envolvidas em diversos processos biológicos vitais, incluindo adesão celular, migração celular, comunicação célula-célula, diferenciação, proliferação, morfogênese, sobrevivência e defesa, fatores de virulência em bactérias e respostas imunes (KOPPENOL-RAAB *et al.*, 2017). As expressões de genes específicos na cepa ou comunidade microbiana durante a utilização de diferentes fontes de carbono envolvem uma complexa rede genética e diferem com o tipo e a complexidade das fontes de carbono. As bactérias devem conter transportadores e enzimas específicas que permitam o metabolismo de carboidratos diferentes como fonte de energia (LEBLANC *et al.*, 2017).

Este estudo mostra que o FOS foi eficaz na estimulação da capacidade fermentativa do LAB18S em condições anaeróbicas. Muitas proteínas foram expressas diferencialmente na ausência de oxigênio que participam da divisão celular e da desintoxicação de H<sub>2</sub>O<sub>2</sub>. Assim, FOS parece possuir um efeito protetor, visto que em um estudo de MURTINI *et al.* (2016), o efeito de FOS no perfil proteico de *Lactobacillus rhamnosus* mostrou que o dano celular causado pelo procedimento de extração foi evitado em mais de 80%. Além do efeito de desintoxicação estimulado por FOS na ausência de oxigênio, os microrganismos probióticos possuem o poder de neutralização



de espécies reativas de oxigênio e a repressão do estresse oxidativo no hospedeiro (MARTARELLI *et al.*, 2011; EJTAHED *et al.*, 2012). O gênero *Enterococcus* spp. produz diferentes proteínas de estresse que são induzidas em resposta ao oxigênio, tais como a NADH peroxidase e a NADH oxidase (PORTELA *et al.*, 2014). Nesse estudo, a NADH peroxidase foi mais diferencialmente expressa em amostras de *pellets* com FOS na presença e na ausência de oxigênio e a expressão da NADH oxidase só foi estimulada na presença de oxigênio. A oxidação de NADH a NAD + via NADH oxidase produz H<sub>2</sub>O<sub>2</sub>, que é reduzido à água pela NADH peroxidase. LU *et al.* (2018) investigaram cepas de *Lactobacillus* em relação à sua capacidade antioxidante com suplementação de diferentes prebióticos e encontraram níveis mais altos de atividade antioxidante na amostra suplementada com FOS.

A proteína mais abundante identificada no secretoma do *E. durans* foi uma peptidase da família NlpC / P60. Essa peptidase está relacionada a mecanismos de proteção contra patógenos entéricos (RANGAN *et al.*, 2016). O mecanismo de ação consiste na atividade de hidrolase que forma fragmentos de peptidoglicanos que ativam o sistema imunológico do hospedeiro, aumentando a integridade da barreira epitelial e aprisionando os patógenos no lúmen intestinal, promovendo tolerância à infecção. Neste estudo, a proteína NlpC / P60 está presente em abundância no secretoma, sendo o GOS e a glicose estimuladores da sua produção.

Um dos efeitos de maior impacto na saúde e que mais vem chamando a atenção para o uso de probióticos é sua ação anticâncer. Já foi relatado que o uso de FOS pode apresentar um efeito quimiopreventivo. Em um estudo, a redução da proliferação celular e o número de lesões pré-neoplásicas em câncer de cólon de ratos machos que receberam dieta com raízes yacon foi observada (DE MOURA *et al.*, 2012). Não só os prebióticos

podem possuir ação anticâncer como também as próprias bactérias probióticas podem produzir proteínas com importância biológica para o tratamento do câncer. Nesse estudo, podemos encontrar duas proteínas relacionadas a essa característica: a arginina deiminase (gene *arcA*) e a L-asparaginase (gene *ansA*). As duas possuem função regulatória dos processos celulares limitando o crescimento de células tumorais (LU *et al.*, 2006; WARANGKAR & KHOBRADE, 2009; JONES *et al.*, 2009). Nossos resultados mostraram que estas enzimas foram expressas diferencialmente apenas no secretoma do LAB18S cultivado em FOS, independentemente da presença ou ausência de oxigênio.

O microbioma intestinal humano abrange em torno de  $10^{14}$  microrganismos, incluindo bactérias, vírus, fungos e protozoários, vivendo de maneira simbiótica (GILL *et al.*, 2006). Os microrganismos intestinais facilitam a absorção e o metabolismo de nutrientes complexos por meio de seu arsenal enzimático e sua capacidade biossintética (HUMAN MICROBIOME PROJECT, 2012; VELASQUEZ-MANOFF, 2015). Estudos recentes demonstraram várias consequências adversas do microbioma intestinal anormal ou alterado (disbiose intestinal), incluindo doenças gastrointestinais crônicas (HOLLISTER *et al.*, 2014), neurológicas (TREMLET *et al.*, 2017) e metabólicas (MAZIDI *et al.*, 2016).

As bactérias são o grupo mais estudado e foi o foco principal desse estudo. O secretoma do LAB18S foi tratado com FOS e GOS e inoculado no microbioma intestinal humano *in vitro*. Através dos resultados de metaproteômica podemos identificar que os prebióticos e a concentração do secretoma utilizados no ensaio alteraram as comunidades microbianas presentes no microbioma intestinal. Muitos estudos sobre a utilização de prebióticos por microrganismos intestinais foram realizados utilizando isolados únicos

ou amostras fecais (MOENS *et al.*, 2016; DOSTAL *et al.*, 2015; TAKAGI *et al.*, 2015). No entanto, a microbiota intestinal programa mecanismos complexos para atingir substratos alimentares, o que pode incluir competição ou cooperação. Portanto, a utilização microbiana de prebióticos provavelmente depende de interações microbianas (MEDINA *et al.*, 2017).

No geral, os grupos bacterianos predominantes no microbioma são Firmicutes e Bacteroidetes (WALKER *et al.*, 2011). No presente estudo, o tratamento com os secretomas do LAB18S em simbiose com FOS e GOS mostrou um aumento na quantidade de microrganismos, especialmente do filo Bacteroidetes. A alta abundância do filo Firmicutes e a diminuição da quantidade de Bacteroidetes estão associados à composição desequilibrada de microbiomas (disbiose intestinal) e várias doenças associadas à disbiose, incluindo diabetes, obesidade, câncer e doenças do intestino irritável (SCHMIDT *et al.*, 2018). Segundo NAGPAL *et al.* (2018), lactobacilos e enterococos, pertencentes ao filo Firmicutes, foram inoculados na microbiota intestinal de camundongos e no microbioma fecal humano mostrando uma diminuição no número de microrganismos do filo Firmicutes e aumento do filo Bacteroidetes. Esses achados sugerem que o tratamento com esses probióticos de origem humana pode ajudar a melhorar a disbiose do microbioma intestinal, melhorando a diversidade microbiana, a abundância de Bacteroidetes (NAGPAL *et al.* 2018).

## 7. CONCLUSÕES

Portanto, tendo em vista todos os resultados desse trabalho através da análise do genoma, do proteoma e das interações do LAB18S com os prebióticos e com o microbioma intestinal humano pode-se inferir que esse isolado possui um potencial probiótico para aplicação à saúde.

O genoma do LAB18S apresenta diferentes genes associados a propriedades probióticas, como adesão, viabilidade em baixo pH, tolerância ao sal biliar, produção de bacteriocinas e metabolização de moléculas prebióticas. Além disso, esse isolado apresenta genes que codificam selenoproteínas conhecidas, auxiliando na ação antioxidante. Esse genoma, em comparação com outros da mesma espécie, não apresentou genes de virulência, resistência antimicrobiana e plasmídeos, além de ser o único genoma de origem alimentar com essas características encontrado no banco de dados do NCBI.

Os resultados obtidos com o uso dos prebióticos FOS e GOS sugerem que o sucesso dos mesmos depende de diferenças nas preferências do substrato pelo isolado probiótico. Esses oligossacarídeos, usados como fonte de carbono pelo LAB18S, estimularam o microrganismo a produzir diferentes proteínas e / ou diferentes níveis de expressão proteica, como a produção de proteínas de adesão da mucosa intestinal, degradação do biofilme, controle dos níveis de resposta ao estresse, entre outros. Esse estudo contribuiu para o entendimento de bactérias probióticas à nível de metabolismo.

FOS, de um modo geral, se mostrou mais eficiente na modulação do proteoma e do secretoma do isolado LAB18S comparado com GOS. Além disso, o estudo que avalia a ação do oxigênio na expressão de proteínas do isolado probiótico mostrou que o cultivo na ausência

de oxigênio produziu proteínas relacionadas à multiplicação celular, integridade e resistência da parede celular e desintoxicação. Além disso, a expressão de duas enzimas de importância clínica para o tratamento do câncer, L-asparaginase e arginina deiminase, foram superexpressas no cultivo do isolado em FOS. Assim, pode-se concluir que o LAB18S em simbiose com FOS foi estimulado a produzir biomoléculas de importância clínica, incluindo proteínas que foram investigadas como potenciais agentes antineoplásicos.

A interação das proteínas secretadas pelo LAB18S, cultivado em FOS e GOS, com o microbioma intestinal humano foi avaliado. Os resultados mostraram diferenças na expressão proteica do microbioma intestinal quando se utilizou altas e baixas concentrações do secretoma do isolado probiótico, além de diferenças quando esse foi cultivado em simbiose com FOS e GOS. Esses resultados são promissores e mostram não só a diferença na influência dos prebióticos FOS e GOS na expressão de proteínas do LAB18S, mas também diferenças na interação das proteínas secretadas por esse isolado no microbioma intestinal humano.

## 8. REFERÊNCIAS BIBLIOGRÁFICAS

ACNFP. Report on *Enterococcus faecium*, strain K77D. In: MAFF Advisory Committee on Novel Foods and Processes, Report, Ergon House c/o Nobel House, 17 Smith Square, London SW1 3JR, United Kingdom, 1996.

ALWAN, A. Global Status Report on Noncommunicable Diseases 2010. World Health Organization: Geneva, Switzerland, 2011.

AMER, M. N.; MANSOUR, N. M.; EL-DIWANY, A. I.; DAWOUD, I. E. & RASHAD, F. M. Isolation of probiotic lactobacilli strains harboring L-asparaginase and arginine deiminase genes from human infant feces for their potential application in cancer prevention. *Annals of Microbiology*, 7(1): 1-2, 2012.

ARROYO, R.; MARTÍN, V.; MALDONADO, A.; JIMENEZ, E.; FERNANDEZ, L. & RODRÍGUEZ, J. M. Treatment of infectious mastitis during lactation: antibiotics versus oral administration of lactobacilli isolated from breast milk. *Clinical Infectious Diseases*, 50(12): 1551-1558, 2010.

BALI, V.; PANESAR, P. S.; BERA, M. B. & PANESAR, R. Fructooligosaccharides: production, purification and potential applications. *Critical Reviews in Food Science and Nutrition*, 55(11): 1475–1490, 2015.

BARBONETTI, A.; VASSALLO, M. R.; CINQUE, B.; FILIPPONI, S.; MASTROMARINO, P.; CIFONE, M. G.; FRANCAVILLA, S. & FRANCAVILLA, F. Soluble products of *Escherichia coli* induce mitochondrial dysfunction-related sperm membrane lipid peroxidation which is prevented by lactobacilli. *PLoS One*, 8(12):831 - 836, 2013.

BARCELONA-ANDRES, B.; MARINA, A. & RUBIO, V. Gene structure, organization, expression and potential regulatory mechanisms of arginine catabolism in *Enterococcus faecalis*. *Journal of Bacteriology*, 184: 6289–6300, 2002.

BENCHIMOL, E.I.; FORTINSKY, K. J.; GOZDYRA, P.; VAN DEN HEUVEL, M.; VAN LIMBERGEN, J. & GRIFFITHS, A. M. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. *Inflammatory Bowel Diseases*, 17: 423–39, 2011.

BERGONZELLI, G. E.; GRANATO, D.; PRIDMORE, R. D.; MARVIN-GUY, L. F.; DONNICOLA, D. & CORTHESEY-THEULAZ, I. E. GroEl of *Lactobacillus johnsonii* La1 (NCC533) is cell surface associated: potential role in interactions with the host and the gastric pathogen *Helicobacter pylori*. *Infection and Immunity*, 74: 425-434, 2006.

BERMUDEZ-BRITO, M.; PLAZA-DÍAZ, J.; MUÑOZ-QUEZADA, S.; GÓMEZ-LLORENTE, C. & GIL, A. Probiotic Mechanisms of Action. *Annals of Nutrition and Metabolism*, 61(2): 160–174, 2012.

BHATIA, S.; PRABHU, P. N.; BENEFIEL, A. C.; MILLER, M. J.; CHOW, J.; DAVIS, S. R. & GASKINS, H. R. Galactooligosaccharides may directly enhance intestinal

barrier function through the modulation of goblet cells. *Molecular Nutrition & Food Research*, 59(3): 566–573, 2015.

BISCHOFF, S. C.; BOIRIE, Y.; CEDERHOLM, T.; CHOURDAKIS, M.; CUERDA, C.; DELZENNE, N. M.; DEUTZ, N. E.; FOUQUE, D.; GENTON, L.; GIL, C.; KOLETZKO, B.; LEON-SANZ, M.; SHAMIR, R.; SINGER, J.; SINGER, P.; STROEBELE-BENSCHOP, N.; THORELL, A.; WEIMANN, A. & BARAZZONI, R. Towards a multidisciplinary approach to understand and manage obesity and related diseases. *Clinical Nutrition*, 36: 917–938, 2017.

BOHLE, L. A.; FÆRGESTAD, E. M.; VEISETH-KENT, E.; STEINMOEN, H.; NES, I. F.; EIJSINK, V. G. & MATHIESEN, G. Identification of proteins related to the stress response in *Enterococcus faecalis* V583 caused by bovine bile. *Proteome Science*, 8: 37, 2010.

BONACINA, J.; SUAREZ, N.; HORMIGO, R.; FADDA, S.; LECHNER, M. & SAAVEDRA, L. A genomic view of food-related and probiotic *Enterococcus* strains. *DNA Research*, 24: 11–24, 2017.

BRODMANN, T.; ENDO, A.; GUEIMONDE, M.; VINDEROLA, G.; KNEIFEL, W.; DE VOS, W. M.; SALMINEN, S. & GÓMEZ-GALLEGO, C. Safety of novel microbes for human consumption: practical examples of assessment in the European Union. *Frontiers in Microbiology*, 8: 17 - 25, 2017.

CASCI, T. & RASTALL, R. A. Manufacture of prebiotic oligosaccharides. In: *Prebiotics: Development and applications*, New York, John Wiley, p. 29 – 55, 2006.

CAMPBELL, J. M.; BAUER, L. L.; FAHEY, G. C.; HOGARTH, A. J. C. L.; WOLF, B. W. & HUNTER, D. E. Selected fructooligosaccharide (1-kestose, nystose, and 1F- $\beta$ -fructofuranosyl-nystose) composition of foods and feeds. *Journal of Agricultural and Food Chemistry*, 45(8): 3076–3082, 1997.

ÇELİK, M. N. & ÜNLÜ SÖĞÜT, M. Probiotics Improve Chemerin and Metabolic Syndrome Parameters in Obese Rats. *Balkan Medical Journal*, 36(5): 270-275, 2019.

CELEBIOGLU, H. U.; OLESEN, S. V.; PREHN, K.; LAHTINEN, S. J.; BRIX, S.; ABOU HACHEM, M & SVENSSON, B. Mucin and carbohydrate-stimulated adhesion and subproteome changes of the probiotic bacterium *Lactobacillus acidophilus* NCFM. *Journal Proteome*, 163:102–110, 2017.

CELLIGOI, M. A. P. C.; DOS SANTOS, D. A.; DA SILVA, P. B. & BALDO, C. Fermented foods, part I biochemistry and biotechnology. Taylor & Francis Group: CRC Press, 2015.

CHEN, G.; LI, C. & CHEN, K. Fructooligosaccharides: A review on their mechanisms of action and effects. In: *Studies in natural products chemistry: Bioactive Natural Products*, 48: 209–229, 2016.

COXAM, V. Current data with inulin-type fructans and calcium, targeting bone health in adults. *The Journal of Nutrition*, 137: 2527–2533, 2007.

- DE ANGELIS, M.; MARIOTTI, L.; ROSSI, J.; SERVILI, M.; FOX, P.F.; ROLLÁN, G. & GOBBETTI, M. Arginine catabolism by sourdough lactic acid bacteria: purification and characterization of the arginine deiminase pathway enzymes from *Lactobacillus sanfranciscensis* CB1. *Applied and Environmental Microbiology*, 68: 6193–6201, 2002.
- DE MOURA, N. A.; CAETANO, B. F. R.; SIVIERI, K.; URBANO, L. H.; CABELLO, C.; RODRIGUES, M. A. & BARBISAN, L. F. Protective effects of yacon (*Smallanthus sonchifolius*) intake on experimental colon carcinogenesis. *Food and Chemical Toxicology*, 50: 2902–2910, 2012.
- DE PRETER, V.; HAMER, H.M.; WINDEY, K. & VERBEKE, K. The impact of pre- and/or probiotics on human colonic metabolism: Does it affect human health? *Molecular Nutrition and Food Research*, 55: 46–57, 2011.
- DOMINGUEZ, L. J.; MARTÍNEZ-GONZÁLEZ, M. A.; BASTERRA-GORTARI, F. J.; GEA, A.; BARBAGALLO, M. & BESRASTROLLO, M. Fast food consumption and gestational diabetes incidence in the SUN project. *PloS One*, 9(9): 106 - 127, 2014.
- DOSTAL, A.; LACROIX, C.; BIRCHER, L.; PHAM, V. T.; FOLLADOR, R.; ZIMMERMANN, M. B. & CHASSARD, C. Iron modulates butyrate production by a child gut microbiota in vitro. *mBio*, 6, 2015.
- EATON, T. J. & GASSON, M. J. Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. *Applied and Environmental Microbiology*, 67: 1628–1635, 2001.
- EC. Directive 2000/54/EC of the European Parliament and of the council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. Official J. Eur. Union L, 262: 21–45, 2000.
- EFSA. Guidance for assessing safety of *Enterococcus faecium* in animal feed. *EFSA Journal*, 2012a.
- EFSA. Scientific Opinion on Lactiferm R (*Enterococcus faecium*) as a feed additive for weaned piglets and calves. *EFSA Journal*, 10: 25 - 74, 2012b.
- EFSA. Scientific opinion on risk based control of biogenic amine formation in fermented foods. In: Panel on Biological Hazards. *EFSA Journal*, 9: 23 - 93, 2011.
- EFSA. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. In: RICCI, A.; ALLENDE, A.; BOLTON, D. & CHEMALY, M. Panel on Biological Hazards. *EFSA Journal*, 15: 604 - 664, 2017.
- EJTAHED, H. S.; MOHTADI-NIA, J.; HOMAYOUNI-RAD, A.; NIAFAR, M.; ASGHARI-JAFARABADI, M. & MOFID, V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition*, 28: 539-543, 2012.
- FAO (Food and Agricultural Organization) / WHO (World Health Organization). Guidelines for the Evaluation of Probiotics in Food, 2006.



FERNÁNDEZ-GARCÍA, L.; BLASCO, L.; LOPEZ, M.; BOU, G.; GARCÍA-CONTRERAS, R.; WOOD, T. & TOMAS, M. Toxin-Antitoxin systems in clinical pathogens. *Toxins*, 8: 227, 2016.

FIGUEROA-GONZALEZ, I.; CRUZ-GUERRERO, A. & QUIJANO, G. The benefits of probiotics on human health. *Journal of Microbial and Biochemical Technology*, 1: 003, 2011.

FLORES-MALTOS, D. A.; MUSSATTO, S. I.; CONTRERAS-ESQUIVEL, J. C.; RODRÍGUEZ-HERRERA, R.; TEIXEIRA, J. A. & AGUILAR, C. N. Biotechnological production and application of fructooligosaccharides. *Critical Reviews in Biotechnology*, 36(2): 259 – 267, 2016.

FORSYTHE, P.; BIENENSTOCK, J. & KUNZE, W. A. Vagal pathways for microbiome-brain-gut axis communication. *Advances in Experimental Medicine and Biology*, 817: 115- 133, 2014.

FRANZ, C. M. A. P.; HUCH, M.; ABRIOUEL, H.; HOLZAPFEL, W. & GÁLVEZ, A. Enterococci as probiotics and their implications in food safety. *International Journal of Food Microbiology*, 151: 125–140, 2011.

FUNAYAMA, R.; TANIGUCHI, H.; MIZUMA, M.; FUJISHIMA, F.; KOBAYASHI, M.; OHNUMA, S.; UNNO, M. & NAKAYAMA, K. Protein-arginine deiminase 2 suppresses proliferation of colon cancer cells through protein citrullination. *Cancer Science*, 108: 713 – 718, 2017.

GANJI-ARJENAKI, M. & RAFIEIAN-KOPAEI, M. Probiotics are a good choice in remission of inflammatory bowel diseases: A meta-analysis and systematic review. *Journal of Cellular Physiology*, 233(3): 2091–2103, 2017.

GASBARRINI, G.; BONVICINI, F. & GRAMENZI, A. Probiotics History. *Journal of Clinical Gastroenterology*, 50: 116 – 119, 2016.

GAYATHRI, D. & RASHMI, B.S. Anti-cancer properties of probiotics: a natural strategy for cancer prevention. *EC Nutrition*, 5: 1191 - 1202, 2016.

GERARD, P. Gut microbiota and obesity. *Cellular and Molecular Life Sciences*, 73: 147 – 162, 2016.

GIBSON, G.R.; HUTKINS, R.W.; SANDERS, M.E.; PRESCOTT, S.L.; REIMER, R.A.; SALMINEN, S.J.; SCOTT, K.; STANTON, C.; SWANSON, K.S. & CANI, P.D. The international scientific association for probiotics and prebiotics (isapp) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology and Hepatology*, 14(8): 491 – 502, 2017.

GIBSON, G. R. & ROBERFROID, M. B. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition*, 125: 1401 – 1412, 1995.

GILAD, O.; SVENSSON, B.; VIBORG, A. H.; STUER-LAURIDSEN, B. & JACOBSEN, S. The extracellular proteome of *Bifidobacterium animalis* subsp. lactis

BB-12 reveals proteins with putative roles in probiotic effects. *Proteomics*, 12: 2503-2514, 2011.

GILL, S. R.; POP, M.; DEBOY, R. T.; ECKBURG, P. B.; TURNBAUGH, P. J.; SAMUEL, B. S.; GORDON, J. I.; RELMAN, D. A.; FRASER-LIGGETT, C. M. & NELSON, K. E. Metagenomic Analysis of the human distal gut microbiome. *Science*, 312: 1355–1359, 2006.

GILLOR, O.; ETZION, A. & RILEY, M. A. The dual role of bacteriocins as anti- and probiotics. *Applied Microbiology and Biotechnology*, 81: 591-606, 2008.

GOLDENBERG, J.Z.; YAP, C.; LYTVYN, L.; LO, C. K.; BEARDSLEY, J.; MERTZ, D. & JOHNSTON, B. C. Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children. *Cochrane Database of Systematic Reviews*, 12: 06 – 95, 2017.

GUO, M.; CHEN, G. & CHEN, K. Fructooligosaccharides: effects, mechanisms, and applications. In: Research progress in oligosaccharins. Springer, New York, p. 51 – 63, 2016.

GUPTA, A. & TIWARI, S. K. Probiotic potential of bacteriocin-producing *Enterococcus hirae* strain LD3 isolated from dosa batter. *Annals of Microbiology*, 65: 2333 – 2342, 2015.

HABERMAN, Y.; TICKLE, T. L.; DEXHEIMER, P. J.; KIM, M. O.; TANG, D.; KARNIS, R. & DENSON, L. A. Pediatric Crohn disease patients exhibit specific ileal transcriptome and microbiome signature. *The Journal of Clinical Investigation*, 125(3): 1363, 2015.

HAMON, E.; HORVATOVICH, P.; IZQUIERDO, E.; BRINGEL, F.; MARCHIONI, E.; AOUDE-WERNER, D. & ENNAHAR, S. Comparative proteomic analysis of *Lactobacillus plantarum* for the identification of key proteins in bile tolerance. *BMC microbiology*, 11: 63, 2011.

HAVARSTEIN, L. S.; HOLO, H. & NES, I. F. The leader peptide of colicin V shares consensus sequences with leader peptides that are common among peptide bacteriocins produced by Gram positive bacteria. *Microbiology*, 140: 2383-2389, 1994.

HAVENAAR, R. & HUIS IN'T VELD, J. M. J. Lactic Acid Bacteria in Health and Disease. v. 1, p. 151–170, Elsevier Applied Science Publishers, 1992.

HENDRICKX, A. P.; VAN WAMEL, W. J.; POSTHUMA, G.; BONTEN, M. J. & WILLEMS, R. J. Five genes encoding surface-exposed LPXTG proteins are enriched in hospital-adapted *Enterococcus faecium* clonal complex 17 isolates. *Journal of Bacteriology*, 189: 8321–8332, 2007.

HICKEY, R. M. The role of oligosaccharides from human milk and other sources in prevention of pathogen adhesion. *International Dairy Journal*, 22(2): 141–146, 2012.

HILL, C.; GUARNER, F.; REID, G.; GIBSON, G. R.; MERENSTEIN, D. J.; POT, B.; MORELLI, L.; CANANI, R. B.; FLINT, H. J.; SALMINEN, S.; CALDER, P.C. &

- SANDERS, M. E. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews of Gastroenterology and Hepatology*, 11(8): 506-514, 2014.
- HOLLISTER, E. B.; GAO, C. & VERSALOVIC, J. Compositional and functional features of the gastrointestinal microbiome and their effects on human health. *Gastroenterology*, 146: 1449–1458, 2014.
- HOLZAPFEL, W.; ARINI, A.; AESCHBACHER, M.; COPPOLECCHIA, R. & POT, B. *Enterococcus faecium* SF68 as a model for efficacy and safety evaluation of pharmaceutical probiotics. *Beneficial Microbes*, 9: 375–388, 2018.
- HUMAN MICROBIOME PROJECT. Structure, function and diversity of the healthy human microbiome. *Nature*, 486: 207–214, 2012.
- HUME, M. P.; NICOLUCCI, A. C. & REIMER, R. A. Prebiotic supplementation improves appetite control in children with overweight and obesity: A randomized controlled trial. *The American Journal of Clinical Nutrition*, 105: 790–799, 2017.
- HUTKINS, R. W.; KRUMBECK, J. A.; BINDELS, L. B.; CANI, P. D.; FAHEY, G.; GOH, Y. J.; HAMAKER, B.; MARTENS, E. C.; MILLS, D. A.; RASTAL, R. A. & VAUGHAN, E. Probiotics: why definitions matter. *Current Opinion in Biotechnology*, 37: 1–7, 2016.
- HUYS, G.; BOTTELDOORN, N.; DELVIGNE, F.; DE VUYST, L.; HEYNDRICKX, M.; POT, B.; DUBOIS, J. J. & DAUBE, G. Microbial characterization of probiotics–advisory report of the working group “8651 probiotics” of the Belgian superior health council (SHC). *Molecular Nutrition and Food Research*, 57: 1479–1504, 2013.
- IQBAL, M. Z.; QADIR, M. I.; HUSSAIN, T.; JANBAZ, K. H.; KHAN, Y. H. & AHMAD, B. Probiotics and their beneficial effects against various diseases. *Pakistan Journal of Pharmaceutical Sciences*, 27: 405-415, 2014.
- ISEPPI, R.; MESSI, P.; CAMELLINI, S. & SABIA, C. Bacteriocin activity of *Lactobacillus brevis* and *Lactobacillus paracasei* ssp. *paracasei*. *Journal of Medical Microbiology*, 68(9): 1359-1366, 2019.
- ISLAM, S. U. Clinical uses of probiotics. *Medicine*, Baltimore, 95: 1-5, 2016.
- ITAYA, N. M.; ASEGA, A. F.; CARVALHO, M. A. M. & RITA DE CÁSSIA, L. Hydrolase and fructosyltransferase activities implicated in the accumulation of different chain size fructans in three Asteraceae species. *Plant Physiology and Biochemistry*, 45(9): 647–656, 2007.
- JAHAN, M.; ZHANEL, G. G.; SPARLING, R. & HOLLEY, R. A. Horizontal transfer of antibiotic resistance from *Enterococcus faecium* of fermented meat origin to clinical isolates of *E. faecium* and *Enterococcus faecalis*. *International Journal of Food Microbiology*, 199: 78–85, 2015.
- JOHANSON, J. F. & KRALSTEIN, J. Chronic constipation: a survey of the patient perspective. *Alimentary Pharmacology & Therapeutics*, 25: 599–608, 2007.

- JONES, J. E.; CAUSEY, C. P.; KNUCKLEY, B.; SLACK-NOYES, J. L. & THOMPSON, P. R. Protein arginine deiminase 4 (PAD4): Current understanding and future therapeutic potential. *Current Opinion in Drug Discovery and Development*, 12: 616-627, 2009.
- JUNG, A.; METZNER, M. & RYLL, M. Comparison of pathogenic and nonpathogenic *Enterococcus cecorum* strains from different animal species. *BMC Microbiology*, 17: 33, 2017.
- KAHOULI, I.; HANDIRI, N. R.; MALHOTRA, M.; RIAHI, A.; ALAOUJ-JAMALI, M. & PRAKASH, S. Characterization of *L. reuteri* NCIMB 701359 probiotic features for potential use as a colorectal cancer biotherapeutic by identifying fatty acid profile and anti-proliferative action against colorectal cancer cells. *Drug Designing*, 5: 1-11, 2016.
- KAMIYA, T.; WANG, L.; FORSYTHE, P.; GOETTSCHE, G.; MAO, Y.; WANG, Y.; TOUGAS, G. & BIENENSTOCK, J. Inhibitory effects of *Lactobacillus reuteri* on visceral pain induced by colorectal distension in Sprague-Dawley rats. *Gut*, 55 : 191–6, 2006.
- KAREEM, K. Y.; LING, F. H.; CHWEN, L. T.; FOONG, O. M. & ASMARA, S. A. Inhibitory activity of postbiotic produced by strains of *Lactobacillus plantarum* using reconstituted media supplemented with inulin. *Gut Pathogens*, 6: 1-7, 2014.
- KARIMI, G.; SABRAN, M. R.; JAMALUDDIN, R.; PARVANEH, K.; MOHTARRUDIN, N.; AHMAD, Z.; KHAZAAI, H. & KHODAVANDI, A. The anti-obesity effects of *Lactobacillus casei* strain Shirota versus Orlistat on high fat diet-induced obese rats. *Food & Nutrition Research*, 59: 1-8, 2015.
- KHAZAIE, K.; ZADEH, M.; KHAN, M. W.; BERE, P.; GOUNARI, F.; DENNIS, K.; BLATNER, N. R.; OWEN, J. L.; KLAENHAMMER, T. D. & MOHAMADZADEH, M. Abating colon cancer polyposis by *Lactobacillus acidophilus* deficient in lipoteichoic acid. *Proceedings of the National Academy of Sciences*, 109: 10462–10467, 2012.
- KIM, J. E.; JEONG, D. W. & LEE, H. J. Expression, purification, and characterization of arginine deiminase from *Lactococcus lactis* ssp. *lactis* ATCC 7962 in *Escherichia coli* BL21. *Protein Expression and Purification*, 53: 9–15, 2007.
- KINOSHITA, H.; IMOTO, S.; SUDA, Y.; ISHIDA, M.; WATANABE, M.; KAWAI, Y. & SAITO, T. Proposal of screening method for intestinal mucus adhesive lactobacilli using the enzymatic activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). *Animal Science Journal*, 84: 150-158, 2012.
- KOPPENOL-RAAB, M.; SJOELUND, V.; MANES, N. P.; GOTTSCHALK, R. A.; DUTTA, B.; BENET, Z. L.; FRASER, I. D. & NITA-LAZAR, A. Proteome and secretome analysis reveals differential post-transcriptional regulation of toll-like receptor responses. *Molecular & Cellular Proteomics*, 6: 172-186, 2017.
- LA FATA, G.; WEBER, P. & MOHAJERI, M. H. Probiotics and the gut immune system: indirect regulation. *Probiotics and Antimicrobial Proteins*, 10: 11–21, 2018.

- LANDETE, J. M.; DE LAS RIVAS, B.; MARCOBAL, A. & MUNOZ, R. Molecular methods for the detection of biogenic amine-producing bacteria on foods. *International Journal of Food Microbiology*, 117: 258–269, 2007.
- LANGA, S.; ARQUÉS, J.; MEDINA, M. & LANDETE, J. Coproduction of colicin V and lactic acid bacteria bacteriocins in lactococci and enterococci strains of biotechnological interest. *Journal of Applied Microbiology*, 122: 1159–1167, 2017.
- LAULUND, S.; WIND, A.; DERKX, P. & ZULIANI, V. Regulatory and safety requirements for food cultures. *Microorganisms*, 5: 28, 2017.
- LAUKOVÁ, A. Potential applications of probiotic, bacteriocin-producing enterococci and their bacteriocins. In: *Lactic Acid Bacteria Microbiological and Functional Aspects*, 4th ed., A. V. Wright, Boca Raton, FL: CRC Press, p. 39–61, 2011.
- LAVERMICOCCA, P.; VALERIO, F.; LONIGRO, S. L.; DI LEO, A. & VISCONTI, A. Antagonistic activity of potential probiotic lactobacilli against the ureolytic pathogen *Yersinia enterocolitica*. *Frontiers in Microbiology*, 56: 175–181, 2008.
- LEBLANC, J.G.; CHAIN, F.; MARTÍN, R.; BERMÚDEZ-HUMARÁN, L. G.; COURAU, S. & LANGELLA, P. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microbial Cell Factories*, 16:79, 2017.
- LIASKOVSKÝ, T. M.; PIDHORSKYĀ, V. S.; KOVALENKO, N. K.; HARMASHEVA, I. L. & MUCHNYK, F. V. Identification of probiotic lactic acid bacteria strains. *Microbiological Journal*, 70: 3–9, 2008.
- LI, J.; BUTCHER, J.; MACK, D. & STINTZI, A. Functional impacts of the intestinal microbiome in the pathogenesis of inflammatory bowel disease. *Inflammatory Bowel Diseases*, 21(1): 139–153, 2015.
- LIN, H. C.; HO, S. C.; CHEN, Y. Y.; KHOO, K. H.; HSU, P. H. & YEN, H. C. S. CRL2 aids elimination of truncated selenoproteins produced by failed UGA/Sec decoding. *Science* 349:91–95, 2015.
- LOVELL, R. M. & FORD, A. C. Global prevalence of, and risk factors for, irritable bowel syndrome: A meta-analysis. *Clinical Gastroenterology and Hepatology*, 10: 712–721, 2012.
- LU, E.; YEUNG, M. & YEUNG, C. K. Comparative analysis of lactulose and fructooligosaccharide on growth kinetics, fermentation, and antioxidant activity of common probiotics. *Food and Nutrition Sciences*, 9: 161–178, 2018.
- LU, X.; LI, L.; WU, R.; FENG, X.; LI, Z.; YANG, H.; WANG, C.; GUO, H.; GALKIN, A.; HERZBERG, O.; MARIANO, P. S.; MARTIN, B. M. & DUNAWAY-MARIANO, D. Kinetic analysis of *Pseudomonas aeruginosa* arginine deiminase mutants and alternate substrates provides insight into structural determinants of function. *Biochemistry*, 45:1162–1172, 2006.

- LYTE, M. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. *Bioessays*, 33(8): 574-581, 2011.
- MARTARELLI, D.; VERDENELLI, M. C.; SCURI, S.; COCCHIONI, M.; SILVI, S.; CECCHINI, C. & POMPEI, P. Effect of a probiotic intake on oxidant and antioxidant parameters in plasma of athletes during intense exercise training. *Current Microbiology*, 62: 1689-1696, 2011.
- MATSUMOTO, K.; KOBAYASHI, Y.; TAMURA, N.; WATANABE, T. & TATSUHIKO, K. Production of galactooligosaccharides with b-galactosidase. *Journal of the Japanese Society of Starch Science*, 36(2): 123-130, 1989.
- MAZIDI, M.; REZAIE, P.; KENGNE, A. P.; MOBARHAN, M. G. & FERNS, G. A. Gut microbiome and metabolic syndrome. *Diabetology & Metabolic Syndrome*, 10: 150-157, 2016.
- MCKENZIE, Y. A.; THOMPSON, J.; GULIA, P.; LOMER, M. C. E. British Dietetic Association systematic review of systematic reviews and evidence-based practice guidelines for the use of probiotics in the management of irritable bowel syndrome in adults. *Journal of Human Nutrition and Dietetics*, 29(5): 576-592, 2016.
- MEDINA, D.; PINTO, F.; OVALLE, ALINE.; THOMSON, P. & GARRIDO, D. Prebiotics mediate microbial interactions in a consortium of the infant gut microbiome. *International Journal of Molecular Sciences*, 18(10): 2095, 2017.
- MEDINA-VERA, I.; SANCHEZ-TAPIA, M.; NORIEGA-LOPEZ, L.; GRANADOS-PORTILLO, O.; GUEVARA-CRUZ, M.; FLORES-LOPEZ, A.; AVILA-NAVA, A.; FERNANDEZ, M.L.; TOVAR, A.R. & TORRES, N. A dietary intervention with functional foods reduces metabolic endotoxaemia and attenuates biochemical abnormalities by modifying faecal microbiota in people with type 2 diabetes. *Diabetes & Metabolism*, 45(2):122-131, 2019.
- MEIRA, S. M. M.; HELFER, V. E.; VELHO, R. V.; LOPES, F. C. & BRANDELLI, A. Probiotic potential of *Lactobacillus* spp. isolated from Brazilian regional ovine cheeses. *Journal of Dairy Research*, 79:119-127, 2012.
- MESSAOUDI, M.; VIOLLE, N.; BISSON, J. F.; DESOR, D.; JAVELOT, H. & ROUGEOT, C. Beneficial psychological effects of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in healthy human volunteers. *Gut Microbes*, 2(4): 256-261, 2011.
- MOAYYEDI, P.; FORD, A. C.; BRANDT, L. J. & QUIGLEY, E. M. The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Gut*, 59: 325-32, 2010.
- MOENS, F.; WECKX, S. & DE VUYST, L. Bifidobacterial inulin-type fructan degradation capacity determines cross-feeding interactions between bifidobacteria and *Faecalibacterium prausnitzii*. *International Journal of Food Microbiology*, 231: 76-85, 2016.

- MONTEALEGRE, M. C.; SINGH, K. V. & MURRAY, B. E. Gastrointestinal tract colonization dynamics by different *Enterococcus faecium* clades. *The Journal of Infectious Diseases*, 213: 1914–1922, 2016.
- MONTEAGUDO-MERA, A.; ARTHUR, J. C.; JOBIN, C.; KEKU, T.; BRUNO-BARCENA, J. M. & AZCARATE-PERIL, M. A. High purity galactooligosaccharides enhance specific *Bifidobacterium* species and their metabolic activity in the mouse gut microbiome. *Beneficial Microbes*, 7(2): 247–264, 2016.
- MONTET, D. & RAY, R. C. Fermented foods. Biochemistry and biotechnology. New York, NY: CRC Press, 2016.
- MUJAGIC, Z.; DE VOS, P.; BOEKSCHOTEN, M. V.; GOVERS, C.; PIETERS, H. H.; DE WIT, N. J.; BRON, P. A.; MASCLEE, A. A. & TROOST, F. J. The effects of *Lactobacillus plantarum* on small intestinal barrier function and mucosal gene transcription; a randomized double-blind placebo controlled trial. *Scientific Reports*, 7: 401-428, 2017.
- MURTINI, D.; ARYANTINI, N. P.; SUJAYA, I. N.; URASHIMA, T. & FUKUDA, K. Effects of prebiotic oligosaccharides consumption on the growth and expression profile of cell surface-associated proteins of a potential probiotic *Lactobacillus rhamnosus* FSMM15. *Bioscience Microbiota Food Health*, 35:41-49, 2016.
- NAGPAL, R.; WANG, S.; AHMADI, S.; HAYES, J.; GAGLIANO, J.; SUBASHCHANDRABOSE, S.; KITZMAN, D. W.; BECTON, T.; READ, R. & YADAV, H. Human-origin probiotic cocktail increases short-chain fatty acid production via modulation of mice and human gut microbiome. *Science Report*, 8: 126-149, 2018.
- NAKAYAMA, J.; KARIYAMA, R. & KUMON, H. Description of a 23.9- kilobase chromosomal deletion containing a region encoding *fsr* genes which mainly determines the gelatinase-negative phenotype of clinical isolates of *Enterococcus faecalis* in urine. *Applied and Environmental Microbiology*, 68: 3152–3155, 2002.
- NAMI, Y.; ABDULLAH, N.; HAGHSHENAS, B.; RADIAH, D.; ROSLI, R. & KHOSROUSHAHI, A. Y. Probiotic assessment of *Enterococcus durans* 6HL and *Lactococcus lactis* 2HL isolated from vaginal microflora. *Journal of Medical Microbiology*, 63: 1044–1051, 2014.
- NARTA, U. K.; KANWAR, S. S. & AZMI, W. Pharmacological and clinical evaluation of L-asparaginase in the treatment of leukemia. *Critical Reviews in Oncology/Hematology*, 61: 208–221, 2007.
- NEHER, S. B.; VILLÉN, J.; OAKES, E. C.; BAKALARSKI, C. E.; SAUER, R. T.; GYGI, S. P. & BAKER, T. A. Proteomic profiling of ClpXP substrates after DNA damage reveals extensive instability within SOS regulon. *Molecular Cell*, 22: 193-204, 2006.
- OELSCHLAEGER, T. A. Mechanisms of probiotic actions—A review. *International Journal of Medical Microbiology*, 300: 57–62, 2010.

- OHTSUKA, K.; OKI, S.; OZAWA, O. & UCHIDA, T. Isolation and cultural conditions of galactooligosaccharide producing yeast *Cryptococcus laurentii*. *Journal of Fermentation Technology*, 66(4): 479–480, 1988.
- OLAFSDOTTIR, L. B.; GUDJONSSON, H.; JONSDOTTIR, H. H.; JONSSON, J. S.; BJORNSSON, E. & THJODLEIFSSON, B. Irritable bowel syndrome: physicians' awareness and patients' experience. *World Journal of Gastroenterology*, 18: 3715-3720, 2012.
- OLIVA, M. A.; HALBEDEL, S.; FREUND, S. M.; DUTOW, P.; LEONARD, T. A.; VEPRINTSEV, D. B.; HAMOEN, L. W. & LÖWE, J. Features critical for membrane binding revealed by DivIVA crystal structure. *EMBO Journal*, 29: 1988-2001, 2010.
- O' MAHONY, L.; MCCARTHY, J.; KELLY, P.; HURLEY, G.; LUO, F.; CHEN, K.; O'SULLIVAN, G. C.; KIELY, B.; COLLINS, J. K.; SHANAHAN, F. & QUIGLEY, E. M. *Lactobacillus* and *Bifidobacterium* in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology*, 128: 541–551, 2005.
- OOI, L. G. & LIONG, M. T. Cholesterol-lowering effects of probiotics and prebiotics: a review of *in vivo* and *in vitro* findings. *International Journal of Molecular Sciences*, 11(6): 2499–2522, 2010.
- OTT, P. A.; HODI, F. S. & ROBERT, C. CTLA-4 and PD-1/PD-L1 blockade: new immunotherapeutic modalities with durable clinical benefit in melanoma patients. *Clinical Cancer Research*, 19: 5300–5309, 2013.
- PARK, A. R. & OH, D. K. Galactooligosaccharide production using microbial beta-galactosidase: current state and perspectives. *Applied Microbiology and Biotechnology*, 85:1279-1286, 2010.
- PARKER, R. B. Probiotics, the other half of the antibiotic story. *Animal Nutrition & Health*, 29: 4–8, 1974.
- PARKER, E.A.; ROY, T.; D'ADAMO, C. R. & WIELAND, L. S. Probiotics and gastrointestinal conditions: An overview of evidence from the Cochrane Collaboration. *Nutrition*, 45: 125–134, 2018.
- PATEL, S. & GOYAL, A. The current trends and future perspectives of prebiotics research: a review. *Biotechnology Journal*, 2(2): 115–125, 2012.
- PIENIZ, S.; ANDREAZZA, R.; ANGHINONI, T.; CAMARGO, F. & BRANDELLI, A. Probiotic potential, antimicrobial and antioxidant activities of *Enterococcus durans* strain LAB18S. *Food Control*, 37: 251–256, 2014.
- PIENIZ, S.; ANDREAZZA, R.; MANN, M. B.; CAMARGO, F. A. O. & BRANDELLI, A. Bioaccumulation and distribution of selenium in *Enterococcus durans*. *Journal of Trace Elements in Medicine and Biology*, 40:37-45, 2017.
- PORTELA, C. A.; SMART, K. F.; TUMANOV, S.; COOK, G. M. & VILLAS-BÔAS, S. G. Global metabolic response of *Enterococcus faecalis* to oxygen. *Journal of Bacteriology*, 196:2012–2022, 2014.



- QIN, X.; SINGH, K. V.; WEINSTOCK, G. M. & MURRAY, B. E. Effects of *Enterococcus faecalis* *fsr* genes on production of gelatinase and a serine protease and virulence. *Infection and Immunity*, 68: 2579–2586, 2000.
- RANGAN, K. J.; PEDICORD, V. A.; WANG, Y. C.; KIM, B.; LU, Y.; SHAHAM, S.; MUCIDA, D. & HANG, H. C. A secreted bacterial peptidoglycan hydrolase enhances tolerance to enteric pathogens. *Science*, 353:1434-1437, 2016.
- RICE, L. B.; CARIAS, L.; RUDIN, S.; VAEL, C.; GOOSSENS, H.; KONSTABEL, C.; KLARE, I.; NALLAPAREDDY, S. R.; HUANG, W. & MURRAY, B. E. A potential virulence gene, *hylEfm*, predominates in *Enterococcus faecium* of clinical origin. *The Journal of Infectious Diseases*, 187: 508–512, 2003.
- RÍOS-COVIÁN, D.; RUAS-MADIEDO, P.; MARGOLLES, A.; GUEIMONDE, M.; DE LOS REYES-GAVILÁN, C. G. & SALAZAR, N. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. *Frontiers in microbiology*, 7: 185, 2016.
- RIVA, A.; BORGIO, F.; LASSANDRO, C.; VERDUCI, E.; MORACE, G.; BORGHI, E. & BERRY, D. Pediatric obesity is associated with an altered gut microbiota and discordant shifts in Firmicutes populations. *Applied and Environmental Microbiology*, 19: 95–105, 2017.
- ROAGER, H. M.; VOGT, J. K.; KRISTENSEN, M.; HANSEN, L. B. S.; IBRUGGER, S.; MAERKEDAHN, R. B.; BAHL, M. I.; LIND, M. V.; NIELSEN, R. L. & FROKIAER, H. Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: A randomised cross-over trial. *Gut*, 68: 83–93, 2019.
- ROBERFROID, M. & SLAVIN, J. Nondigestible oligosaccharides. *Critical Reviews in Food Science and Nutrition*, 40(6): 461–480, 2000.
- SANDERS, M. E.; MERENSTEIN, D. J.; REID, G.; GIBSON, G. R. & RASTALL, R. A. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nature Reviews Gastroenterology & Hepatology*, 16(10): 605-616, 2019.
- SANGWAN, V.; TOMAR, S.; SINGH, R.; SINGH, A. & ALI, B. Galactooligosaccharides: novel components of designer foods. *Journal of Food Science*, 76(4): 103–111, 2011.
- SCAVUZZI, B. M.; HERNIQUE, F. C.; MIGLIORANZA, L. H. S.; SINMAO, A. N. C. & DICHI, I. Impact of prebiotics, probiotics and synbiotics on components of the metabolic syndrome. *Annals of Nutritional Disorders & Therapy*, 1(2): 1009, 2014.
- SCHMIDT, T. S. B.; RAES, J. & BORK, P. The Human Gut Microbiome: From Association to Modulation. *Cell*, 172: 1198–1215, 2018.
- SCHULZ, C.; GIEROK, P.; PETRUSCHKA, L.; LALK, M.; DER MÄ, U. & HAMMERSCHMIDT, S. Regulation of the arginine deiminase system by ArgR2 interferes with arginine metabolism and fitness of *Streptococcus pneumoniae*. *mBio*, 5: 185–195, 2014.

SEGERS, M. E. & LEBEER, S. Towards a better understanding of *Lactobacillus rhamnosus* GG host interactions. *Microbial Cell Factories*, 13: 7, 2014.

SEMEDO, T.; ALMEIDA SANTOS, M.; MARTINS, P.; SILVA LOPES, M. F.; FIGUEIREDO MARQUES, J. J.; TENREIRO, R. & BARRETO CRESPO M. T. Comparative study using type strains and clinical and food isolates to examine hemolytic activity and occurrence of the *cyl* operon in enterococci. *Journal of Clinical Microbiology*, 41: 2569–2576, 2003.

SENAN, S.; PRAJAPATI, J. B. & JOSHI, C. G. Whole-genome based validation of the adaptive properties of Indian origin probiotic *Lactobacillus helveticus* MTCC5463. *Journal of the Science of Food and Agriculture*, 95: 321-328, 2015.

SERBAN, D. E. Microbiota in inflammatory bowel disease pathogenesis and therapy: Is it all about diet? Nutrition in Clinical Practice: Official Publication of the American Society for Parenteral and Enteral Nutrition, 30(6): 760–779, 2015.

SILVA, M. F.; RIGO, D.; MOSSI, V.; GOLUNSKI, S.; DE OLIVEIRA KUHN, G.; DI LUCCIO, M.; DALLAGO, R.; DE OLIVEIRA, D.; OLIVEIRA, J. V. & TREICHEL, H. Enzymatic synthesis of fructooligosaccharides by inulinases from *Aspergillus niger* and *Kluyveromyces marxianus* NRRL Y-7571 in aqueous–organic medium. *Food Chemistry*, 138(1): 148–153, 2013.

SIMOVA, E. D.; BESHKOVA, D. B. & DIMITROV, P. Characterization and antimicrobial spectrum of bacteriocins produced by lactic acid bacteria isolated from traditional Bulgarian dairy products. *Journal of Applied Microbiology*, 106: 692-701, 2009.

SIMPSON, H. L. & CAMPBELL, B. J. Review article: dietary fibre-microbiota interactions. *Alimentary Pharmacology & Therapeutics*, 42: 158-179, 2015.

SKALKAM, M.L.; WIESE, M.; NIELSEN, D. S. & VAN ZANTEN, G. *In vitro* screening and evaluation of synbiotics. In: Probiotics, Prebiotics, and Synbiotics, Watson R.R., Preedy V.R. ed. Elsevier, London, p. 477-486, 2016.

SPLECHTNA, B.; NGUYEN, T. H.; STEINBOCK, M.; KULBE, K. D.; LORENZ, W. & HALTRICH, D. Production of prebiotic galactooligosaccharides from lactose using  $\beta$ -galactosidases from *Lactobacillus reuteri*. *Journal of Agricultural and Food Chemistry*, 54(14): 4999–5006, 2006.

SPRINZL, M. Elongation factor Tu: a regulatory GTPase with an integrated effector. *Trends in Biochemical Sciences*, 19: 245–250, 1994.

STADLBAUER, V.; LEBER, B.; LEMESCH, S.; TRAJANOSKI, S.; BASHIR, M.; HORVATH, A.; TAWDROUS, M.; STOJAKOVIC, T.; FAULER, G.; FICKERT, P.; HÖGENAUER, C.; KLYMIUK, I.; STIEGLER, P.; LAMPRECHT, M.; PIEBER, T. R.; TRIPOLT, N. J. & SOURIJ, H. *Lactobacillus casei* Shirota supplementation does not restore gut microbiota composition and gut barrier in metabolic syndrome: a randomized pilot study. *PLoS ONE*, 10: 14- 19, 2015.

- TAKAGI, R.; TSUJIKAWA, Y.; NOMOTO, R. & OSAWA, R. Comparison of the growth of *Lactobacillus delbrueckii*, *L. paracasei* and *L. plantarum* on inulin in co-culture systems. *Bioscience of Microbiota Food and Health*, 33: 139–146, 2014.
- TEJERO-SARINENA, S.; BARLOW, J.; COSTABILE, A.; GIBSON, G. R. & ROWLAND, I. Antipathogenic activity of probiotics against *Salmonella Typhimurium* and *Clostridium difficile* in anaerobic batch culture systems: is it due to synergies in probiotic mixtures or the specificity of single strains? *Anaerobe*, 24: 60-65, 2013.
- TEO, J. W.; KRISHNAN, P.; JUREEN, R. & LIN, R. T. Detection of an unusual van genotype in a vancomycin-resistant *Enterococcus faecium* hospital isolate. *Journal of Clinical Microbiology*, 49: 4297–4298, 2011.
- TJALSMA, H.; BOLHUIS, A.; JONGBLOED, J. D. H.; BRON, S. & VAN DIJL, J. M. Signal Peptide-Dependent Protein Transport in *Bacillus subtilis*: a Genome-Based Survey of the Secretome. *Microbiology and Molecular Biology Reviews*, 64(3): 515–547, 2000.
- TREMLET, H.; BAUER, K. C.; APPEL-CRESSWELL, S.; FINLAY, B. B. & WAUBANT, E. The gut microbiome in human neurological disease: A review. *Annals of Neurology*, 81: 369–382, 2017.
- TRINDER, M.; BISANZ, J. E.; BURTON, J. P. & REID, G. Bacteria need “sleep” too? Microbiome circadian rhythmicity, metabolic disease and beyond. *University of Toronto medical journal*, 92: 52-55, 2015.
- TSOLIS, K. C.; HAMED, M. B.; SIMOENS, K.; KOEPFF, J.; BUSCHE, T.; RÜCKERT, C.; OLDIGES, M.; KALINOWSKI, J.; ANNÉ, J.; KORMANEC, J.; BERNAERTS, K.; KARAMANOU, S. & ECONOMOU, A. Secretome dynamics in a Gram positive bacterial model. *Molecular & Cellular Proteomics*, 18(3): 423-436, 2018.
- TUFARELLI, V. & LAUDADIO, V. An overview on the functional food concept: perspectives and applied researches in probiotics, prebiotics and synbiotics. *Journal of Experimental Biology and Agricultural Sciences*, 4: 274-278, 2016.
- VAN LOO, J.; CLUNE, Y.; BENNETT, M. & COLLINS, J. K. The SYNCAN project: Goals, set-up, first results and settings of the human intervention study. *British Journal of Nutrition*, 93: 91–98, 2005.
- VAN ZYL, W.; DEANE, S. & DICKS, L. *Enterococcus mundtii* ST4SA and *Lactobacillus plantarum* 423 excludes *Listeria monocytogenes* from the GIT, as shown by bioluminescent studies in mice. *Beneficial Microbes*, 7: 227–235, 2016.
- VELASQUEZ-MANOFF, M. Gut microbiome: the peacekeepers. *Nature*, 518: 3–11, 2015.
- VERA, C.; CÓRDOVA, A.; ABURTO, C.; GUERRERO, C.; SUÁREZ, S. & ILLANES, A. Synthesis and purification of galactooligosaccharides: state of the art. *World Journal of Microbiology and Biotechnology*, 32(12): 197, 2016.

- VERDU, E. F.; BERCIK, P.; VERMA-GANDHU, M.; HUANG, X. X.; BLENNERHASSETT, P.; JACKSON, W.; MAO, Y.; WANG, L.; ROCHAT, F. & COLLINS, S. M. Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut*, 55: 182 – 190, 2006.
- VIMAL, A. & KUMAR, A. Biotechnological production and practical application of L-asparaginase enzyme. *Biotechnology & genetic engineering reviews*, 33(1): 1–22, 2017.
- WALKER, A. W.; INCE, J.; DUNCAN, S. H.; WEBSTER, L. M.; HOLTROP, G.; ZE, X.; BROWN, D.; STARES, M. D.; SCOTT, P.; BERGERAT, A.; LOUIS, P.; MCINTOSH, F.; JOHNSTONE, A. M.; LOBLEY, G. E.; PARKHILL, J. & FLINT, H. J. Dominant and diet responsive groups of bacteria within the human colonic microbiota. *ISME Journal*, 5: 220–30, 2011.
- WALTS, A. E.; MIROCHA, J. M.; LEONG, T.; ALBERTO, M. & MARCHEVSKY, M. D. Pathologic staging and survival of patients with synchronous bilateral lung carcinomas. *American Journal of Clinical Pathology*, 145: 244-250, 2016.
- WARANGKAR, S. C. & KHOBRADE, C. N. Screening, enrichment and media optimization for L-asparaginase production. *Journal of Cell and Tissue Research*, 9: 1963–1968, 2009.
- WERNER, G.; FLEIGE, C.; GERINGER, U.; VAN SCHAİK, W.; KLARE, I. & WITTE, W. IS element IS16 as a molecular screening tool to identify hospital-associated strains of *Enterococcus faecium*. *BMC Infectious Diseases*, 11: 80, 2011.
- WORLD HEALTH ORGANIZATION. Global report on diabetes. World Health Organization, 2016.
- WORLD HEALTH ORGANIZATION. Cancer fact sheet 2017. Switzerland: World Health Organization, 2017.
- XU, F.; ORUNA-CONCHA, M. J. & ELMORE, J. S. The use of asparaginase to reduce acrylamide levels in cooked food. *Food Chemistry*, 210: 163-171, 2016.
- YAN, F.; LIU, L.; DEMPSEY, P. J.; TSAI, Y. H.; RAINES, E. W.; WILSON, C. L.; CAO, H.; CAO, Z.; LIU, L. & POLK, D. B. A *Lactobacillus rhamnosus* GG- derived soluble protein, p40, stimulates ligand release from intestinal epithelial cells to transactivate epidermal growth factor receptor. *Journal of Biological Chemistry*, 288: 30742–30751, 2013.
- YERLIKAYA, O. & AKBULUT, N. Potential use of probiotic *Enterococcus faecium* and *Enterococcus durans* strains in Izmir Tulum cheese as adjunct culture. *Journal of Food Science and Technology*, 56(4): 2175-2185, 2019.
- YOUNIS, K.; AHMAD, S. & JAHAN, K. Health benefits and application of prebiotics in foods. *Journal of Food Processing and Technology*, 6(433): 2, 2015.
- ZHAO, L.; ZHANG, F.; DING, X.; WU, G.; LAM, Y. Y.; WANG, X.; FU, H.; XUE, X.; LU, C. & MA, J. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science*, 359: 1151–1156, 2018.

ZÚÑIGA, M.; PÉREZ, G. & GONZÁLEZ-CANDELAS, F. Evolution of arginine deiminase (ADI) pathway genes. *Molecular Phylogenetics and Evolution*, 25: 429–444, 2002.

## 9. APÊNDICES

### **Manuscrito submetido à revista Archives of Microbiology:**

Re: "Genomic analysis of Enterococcus durans LAB18S, a potential probiotic strain isolated from cheese"

Full author list: Carolina Baldisserotto Comerlato; Janira Prichula; Francieli Maboni Siqueira; Ana Carolina Ritter; Ana Paula Mutterle Varela; Fabiana Quoos Mayer; Adriano Brandelli

Dear Dr Comerlato,

We have received the submission entitled: "Genomic analysis of Enterococcus durans LAB18S, a potential probiotic strain isolated from cheese" for possible publication in Archives of Microbiology, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. Prof. Adriano Brandelli who will be able to track the status of the paper through his/her login.

If you have any objections, please contact the editorial office as soon as possible. If we do not hear back from you, we will assume you agree with your co-authorship.

Thank you very much.

With kind regards,

Springer Journals Editorial Office  
Archives of Microbiology

Manuscrito aceito para publicação na revista Food Microbiology:

<https://doi.org/10.1016/j.fm.2020.103430>



Food Microbiology

Available online 14 January 2020, 103430

In Press, Journal Pre-proof



Short communication

## Proteomic study of *Enterococcus durans* LAB18S growing on prebiotic oligosaccharides

Carolina Baldisserotto Comerlato, Ana Carolina Ritter, Kendi Nishino Miyamoto, Adriano Brandelli  

 Show more

<https://doi.org/10.1016/j.fm.2020.103430>

[Get rights and content](#)

### Highlights

- *Enterococcus durans* LAB18S was grown on prebiotic oligosaccharides.
- Proteomic analysis revealed differential protein expression in FOS and GOS.
- Proteins related to carbohydrate and nitrogen metabolism, adhesion and stress response were upregulated.
- Proteomics as useful tool to study the mechanisms of microbial response to prebiotics.

**Manuscrito submetido à revista Journal of Proteomics:**

Dear Dr. Carolina Comerlato,

You have been listed as a Co-Author of the following submission:

Journal: Journal of Proteomics

Title: Comparative proteomic analysis reveals metabolic variability of probiotic *Enterococcus durans* during aerobic and anaerobic cultivation

Corresponding Author: Adriano Brandelli

Co-Authors: Carolina Comerlato; Xu Zhang; Krystal Walker; Daniel Figeys

To be kept informed of the status of your submission, register or log in (if you already have an Elsevier profile).

Register

here: <https://ees.elsevier.com/jprot/default.asp?acw=&pg=preRegistration.asp&user=coauthor&fname=Carolina&lname=Comerlato&email=carolinabcomerlato@gmail.com>

Or log

in: <https://ees.elsevier.com/jprot/default.asp?acw=&pg=login.asp&email=carolinabcomerlato@gmail.com>

If you did not co-author this submission, please do not follow the above link but instead contact the Corresponding Author of this submission at [abrand@ufrgs.br](mailto:abrand@ufrgs.br).

Thank you,

Journal of Proteomics



## 10. CURRICULUM VITÆ resumido

**COMERLATO, C. B.**

### 1. DADOS PESSOAIS

**Nome:** Carolina Baldisserotto Comerlato

**Local e data de nascimento:** Caxias do Sul, RS, Brasil, 29/06/1985

**Endereço profissional:** Rua Ramiro Barcelos, 630, Moinhos de Vento, Porto Alegre, RS.

**Telefone profissional:** (51) 3314-3434 Ramal: 8076

**E-mail:** [carolina.comerlato@hmv.org.br](mailto:carolina.comerlato@hmv.org.br)

### 2. FORMAÇÃO:

Graduação em Biomedicina (UFCSPA, 2008 - 2012); Mestrado em Microbiologia Agrícola e do Ambiente (UFRGS, 2013 - 2015); Aluna de doutorado em Biologia Celular e Molecular (UFRGS, 2015 - 2019); Doutorado sanduíche (uOttawa, 2018 – 2019).

Outros cursos: Curso EAD Introduction to Systematic Review and Meta-Analysis (Johns Hopkins University, USA, 36 h); CBAB - Proteomics as an analytical tool for biosafety (UFSC, 80h).

### 3. ESTÁGIOS:

**Monitoria** (Março 2011 – Novembro 2011; Programa de Educação para o Trabalho em Saúde – PET-Saúde; Monitora; responsável Professora Cláudia Bicca; 300 h). Participei como monitora do PET-Saúde desenvolvendo um projeto sobre sexualidade levando informação em palestras na UBS e escolas da região.

**Estágio Curricular** (Janeiro 2012 – julho de 2012; Fundação Estadual de Produção e Pesquisa em Saúde – FEPPS; 500 h). Realizei análises microbiológicas em alimentos e em água que eram recebidos de todo Rio Grande do Sul.

**Estágio Curricular** (Julho 2012 – Novembro 2012; Hospital Materno Infantil Presidente Vargas; 500 h). Realizei um estágio na área de análises clínicas passando pelas áreas de hematologia, bioquímica, triagem das amostras neonatal e microbiologia.

**Iniciação científica remunerada** (2008 – 2009; Hospital de Clínicas de Porto Alegre, PIBIC CNPq; Dra Joíza Camargo). Participei de um projeto na área de pesquisa clínica para utilização do teste de hemoglobina glicada para uso diagnóstico da diabetes. Realizei a triagem dos pacientes, entrevistas, coleta de dados e triagem inicial de urina e sangue.

**Iniciação Científica não remunerada** (2010 – 2012; Laboratório de cocos Gram positivos, UFCSPA; não remunerado; Professor Pedro d’Azevedo).

Realizei um estágio de iniciação científica na área de Microbiologia Clínica onde aprendi técnicas de Microbiologia básica e Biologia Molecular. Realizei nesse laboratório meu trabalho de conclusão de curso com publicação na revista Memórias do Instituto Oswaldo Cruz.

### 4. PRÊMIOS E DISTINÇÕES

2011 - Destaque na categoria de apresentação oral do Programa de Educação pelo Trabalho para a saúde (PET-SAÚDE) da IV Semana Científica da UFCSPA e I Semana de Tecnologia e Inovação, UFCSPA.

2009 - Destaque em sessão de apresentação oral do trabalho "Diagnóstico dos estágios de hiperglicemia: Glicemia de jejum ou teste oral de tolerância glicose", Centro Universitário Feevale, II Congresso Internacional de Bioanálises.

### 5. EXPERIÊNCIA PROFISSIONAL OU DIDÁTICA ANTERIOR

2019 – atual: Hospital Moinhos de Vento, PROADI-SUS; Pesquisadora nível III, projeto HTLV.

2019: UFCSPA, Aula ministrada para o PPG Ciências da Saúde: Técnicas para análise das comunidades microbianas associadas ao corpo humano; 4 h.

UFCSPA, Aula ministrada para o PPG Ciências da Saúde: Microbiota como tema de pesquisa e perspectivas biotecnológicas; 4 h.

2018: Palestra ministrada com o título: “Proteômica: metodologias e aplicações no estudo da ciência de alimentos”. I Workshop em Ciência e Tecnologia de Alimentos do PPGCTA, 2018.

## 6. ARTIGOS COMPLETOS PUBLICADOS

COMERLATO; C.B.; RITTER; A. C.; MIYAMOTO; K. N.;BRANDELLI; A. Proteomic study of *Enterococcus durans* LAB18S growing on prebiotic oligosaccharides. *Food Microbiology*, 2020.

COMERLATO, C.B.; MOTTA, A. S.; FRAZZON, A. P. G.; SANTESTEVAN, N. A. & BUBOLTZ, R. Antimicrobial compounds produced by *Enterococcus* spp. isolates from fecal samples of wild South American fur seals. *Journal of Microbiology and Antimicrobials*, 8: 14-21, 2016.

COMERLATO, C. B.; RESENDE, M. C. C.; CAIERAO, J. & DAZEVEDO, P. A. Presence of virulence factors in *Enterococcus faecalis* and *Enterococcus faecium* susceptible and resistant to vancomycin. *Memórias do Instituto Oswaldo Cruz*, 108: 590-595, 2013.

CAVAGNOLLI, G.; COMERLATO, C. B.; RENZ, P. B.; COMERLATO, J.;CAMARGO, J. L. & GROSS, J. L. HbA1c measurement for the diagnosis of diabetes: is it enough?. *Diabetic Medicine*, 28: 31-35, 2011.

## 7. RESUMOS E TRABALHOS APRESENTADOS EM CONGRESSOS

COMERLATO, C. B.; MOURA, T. M.; BUBOLTZ, J. R.; SANTESTEVAN, N. A. & FRAZZON, A. P. G. Potencial biopreservante da munditcina KS produzida por *Enterococcus mundtii* de fezes de lobo-marinho no sul do Brasil. Anais do Simpósio Latino Americano de Ciência de Alimentos, 2015.

RESENDE, M. C. C.; COMERLATO, C. B.; DAZEVEDO, P. A. Perfil de Resistência de *Enterococcus* Provenientes de Hospitais de Porto Alegre In: II Congresso Latino Americano de Resistência Microbiana, Gramado, 55: 01-93, 2011.

CAVAGNOLLI, G.; COMERLATO, J.; COMERLATO, C. B.; GROSS, J. L. & CAMARGO, J.L. O teste A1C no diagnóstico de diabetes: qual o melhor ponto de corte? 44 Congresso Brasileiro de Patologia Clínica e Medicina Laboratorial, Rio de Janeiro. *Jornal Brasileiro de Patologia e Medicina Laboratorial*. 46, 2010.

CAVAGNOLLI, G; COMERLATO, J; COMERLATO, C. B; GROSS, J. L. & CAMARGO, J.L Diagnóstico dos estágios de hiperglicemia: Glicemia de jejum ou Teste Oral de Tolerância a Glicose. 43 Congresso Brasileiro de Patologia Clínica e Medicina Laboratorial, 2009, Belo Horizonte. *Jornal Brasileiro de Patologia e Medicina Laboratorial*, 42:128, 2009.

CAVAGNOLLI, G.; COMERLATO, J.; COMERLATO, C. B.; GROSS, J. L. & CAMARGO, J.L Diagnóstico dos Estágios de Hiperglicemia: Glicemia de Jejum ou Teste Oral de Tolerância à glicose. In: 43 Congresso Brasileiro de Patologia Clínica e Medicina Laboratoria, Belo Horizonte. *Jornal Brasileiro de Patologia e Medicina Laboratorial*. , 45:128, 2008.