Brazilian Journal of Microbiology 45, 3, 1089-1094 (2014)
ISSN 1678-4405
Copyright © 2014, Sociedade Brasileira de Microbiologia
www.sbmicrobiologia.org.br

Research Paper

Antiviral activity of a *Bacillus* sp. P34 peptide against pathogenic viruses of domestic animals

Débora Scopel e Silva[1*], Clarissa Caetano de Castro[1], Fábio da Silva e Silva[1], Voltaire Sant'anna[2], Gilberto D'Avila Vargas[1], Marcelo de Lima[1], Geferson Fischer[1], Adriano Brandelli[3], Amanda de Souza da Motta[4], Silvia de Oliveira Hübner[1]

[1]Laboratório de Virologia e Imunologia Animal, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, RS, Brazil.
[2]Departamento de Ciência e Tecnologia de Alimentos, Universidade Estadual do Rio Grande do Sul, Encantado, RS, Brazil.
[3]Laboratório de Bioquímica e Microbiologia Aplicada, Departamento de Ciência de Alimentos, Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
[4]Instituto de Ciências Básicas da Saúde, Departamento de Microbiologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

Submitted: August 7, 2013; Approved: March 14, 2014.

Abstract

P34 is an antimicrobial peptide produced by a *Bacillus* sp. strain isolated from the intestinal contents of a fish in the Brazilian Amazon basin with reported antibacterial activity. The aim of this work was to evaluate the peptide P34 for its *in vitro* antiviral properties against canine adenovirus type 2 (CAV-2), canine coronavirus (CCoV), canine distemper virus (CDV), canine parvovirus type 2 (CPV-2), equine arteritis virus (EAV), equine influenza virus (EIV), feline calicivirus (FCV) and feline herpesvirus type 1 (FHV-1). The results showed that the peptide P34 exhibited antiviral activity against EAV and FHV-1. The peptide P34 inhibited the replication of EAV by 99.9% and FHV-1 by 94.4%. Virucidal activity was detected only against EAV. When P34 and EAV were incubated for 6 h at 37 °C the viral titer reduced from $10^{4.5}$ $TCID_{50}$ to $10^{2.75}$ $TCID_{50}$, showing a percent of inhibition of 98.6%. In conclusion, our results demonstrated that P34 inhibited EAV and FHV-1 replication in infected cell cultures and it showed virucidal activity against EAV. Since there is documented resistance to the current drugs used against herpesviruses and there is no treatment for equine viral arteritis, it is advisable to search for new antiviral compounds to overcome these infections.

Key words: antimicrobial peptides, antiviral activity, herpesvirus, equine viral arteritis.

Introduction

The impact of the increasing resistance of microorganisms to drugs and specific antimicrobial substances has motivated several research groups. Since their discovery, the antimicrobial peptides (AMPs) are conquering special attention as important therapeutic alternatives for the prevention and treatment of infections caused by a large number of microorganisms (Oyston *et al.*, 2009). AMPs are universal features of the defense systems of all forms of life, with representatives found in organisms ranging from bacteria, plants, invertebrate and vertebrate species, including mammals (Jenssen *et al.*, 2006). Studies about antiviral compounds date from 1950 (Felipe *et al.*, 2006), but for several reasons such as serious side effects, just a few drugs were approved for clinical use (De Clercq, 2004).

Antimicrobial activity was reported among several bacteria isolated from the aquatic environments of Brazilian Amazon basin (Motta *et al.*, 2004). Among them, a species of *Bacillus* producing an antimicrobial peptide was isolated from the intestinal contents of the fish Piau-com-

Send correspondence to D.S. Silva. Rua Santo Ângelo 1685, 96090-650 Pelotas, RS, Brazil. E-mail: scopeldebora@yahoo.com.br.

pinta (*Leporinus* sp.) (Motta *et al.*, 2007b). This peptide was purified and named P34 and its antimicrobial activity was characterized as a fengycin-like substance (Motta *et al.*, 2007a). Its inhibitory activity was detected against Gram-positive bacteria, like *Listeria monocytogenes* and *Bacillus cereus* (Motta *et al.*, 2007b), and Gram-negative bacteria like *Escherichia coli* and *Salmonella enteritidis* (Motta *et al.*, 2008). While some studies on P34 have shown its importance as a potential food preservative (Motta *et al.*, 2007a), little attention has been addressed to its application as an antimicrobial substance in clinical studies.

Since there is no data regarding the antiviral activity of this peptide, the aim of the present work was to evaluate the activity exerted by the peptide P34 against canine adenovirus (CAV-2), canine coronavirus (CCoV), canine distemper virus (CDV), canine parvovirus type 2 (CPV-2), equine arteritis virus (EAV), equine influenza virus (EIV), feline calicivirus (FCV) and feline herpesvirus type 1 (FHV-1).

Materials and Methods

Antimicrobial peptide (P34), cells and viruses

The peptide P34 was produced as described elsewhere (Motta *et al.*, 2007b). After purification, total protein concentration was measured in triplicate by the Lowry method according to the manufacturer's protocol (Total Protein Kit, Micro Lowry, Peterson's Modification - Sigma Aldrich, USA). The purified peptide was analyzed by mass spectrometry (Stein, 2008) (Ettan MALDI-TOF Pro-System, Amersham Biosciences, Sweden) operating in reflectron mode with positive ionization at 20 kV and using a matrix of α-ciano-4-hydroxycinnamic acid (Sigma-Aldrich, USA). The peptide was stored at -20 °C until used for antiviral assays.

Madin-Darby Canine Kidney (MDCK - ATCC® Number: CCL-34™, USA), Crandell-Rees Feline Kidney (CRFK - ATCC® Number: CCL-94™, USA) and Rabbit Kidney (RK13 - ATCC® Number: CCL-37™, USA) cells were cultivated in Eagle's minimum essential medium (E-MEM - Sigma Aldrich, USA) supplemented with 10% of bovine fetal serum (BFS, Gibco, USA), penicillin (Sigma-Aldrich, USA), streptomycin (Vetec, Brasil), amphotericin B (Cristália, Brasil) and enrofloxacin (Bayer, Brasil), in an incubator at 37 °C.

The antiviral activity of the AMP P34 was evaluated against viruses with different phenotypic and genotypic features. FCV (Weiblen *et al.*, 1988), CCoV (MAV 795 strain), EAV (Bucyrus strain) and EIV (local isolate) were kindly provided by the Virology Laboratory of the Federal University of Santa Maria (UFSM). CAV-2 (Toronto A26/61 strain), CDV (Lederle VR128 strain), CPV-2 (Cornell strain) and FHV-1 (B927 strain) were kindly provided by Desidério Finamor Veterinary Research Institute (IPVDF). These viruses were propagated on MDCK, CRFK or RK13 cell cultures.

Cytotoxicity assays

MDCK, CRFK and RK13 cells grown in microplates (TPP, Switzerland) were incubated with different concentrations of P34 (from 0,23 μg/mL to 6,87 μg/mL) for 72 h at 37 °C and 5% CO_2. Cell viability was measured by the neutral red dye uptake (NRU, Vetec, Brasil) assay (Borenfreund and Puerner, 1984) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, USA) procedure (Mosmann, 1983). The percentage of cell viability (CV) was calculated as: CV = AT/ AC x 100, where AT and AC were the absorbances of treated and control cells, respectively (Vaucher *et al.*, 2010). The cytotoxicity of P34 was expressed as the concentration at which 50% cytotoxicity was observed (CC_{50}).

Antiviral assays

Cytopathic effect inhibition (CPE) assay

The inhibition of CPE assays were performed on confluent MDCK, CRFK and RK13 cell monolayers, in the presence or absence of P34 in its non-cytotoxic concentration for each cell lineage, described in the results. Endpoint titrations were performed as described elsewhere (Mahy and Kangro, 1996) and titers were expressed in tissue culture infective dose 50% ($TCID_{50}$/100 μL). The cells were kept in an incubator at 37 °C and observed for CPE after 72 h.

The viral percents of inhibition (PI) were calculated by PI = [1- (Titer of treated/ Titer of controls)] x 100, adapted from Felipe *et al.* (2006).

P34 virucidal effect

Virus strains were incubated at 37 °C for 6 h with E-MEM in the presence or absence of P34 (in non-cytotoxic concentrations for each cell lineage). After the incubation period, the infectivity was immediately determined by virus titrations on cell cultures.

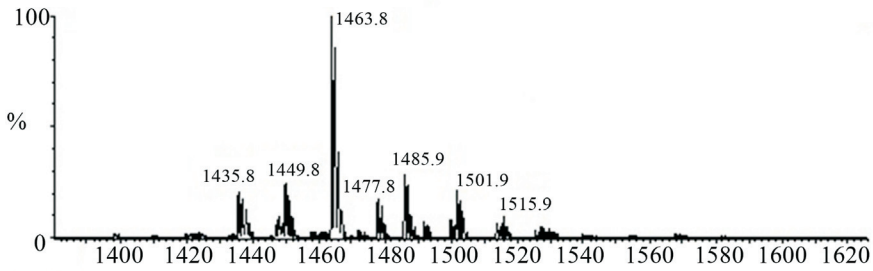
Statistical analysis

All assays were performed in triplicate. Statistical analysis were performed using a two-tailed Student's t-test and values were considered significant when $p < 0.05$.

Results

Peptide P34

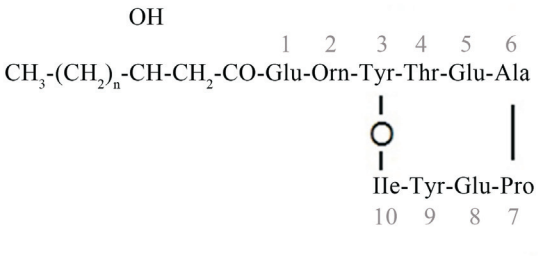
The mass spectrum of the purified peptide P34 revealed typical *m/z* peaks of the lipopeptide fengycin (Figure 1). The *m/z* peaks at 1449.8, 1463.8 and 1477.8 differed by 14 Da, equivalent to a CH_2 group. These peaks were assigned to C15, C16 and C17 forms of Ala-6-fengycin. Other peaks corresponding to Na^+ and K^+ adducts of fengycin were also observed.



(A)

m/z	Structure
1449.8	Ala-6-C15-fengycin [M+H]+
1463.8/1485.9/1501.9	Ala-6-C16-fengycin [M+H/Na/K]+
1477.8/1515.9	Ala-6-C17-fengycin [M+H/K]+

(B)



(C)

Figure 1 - (A) Mass spectrum of the purified peptide P34. (B) Assignments of the *m/z* peaks with isoforms of fengycin, according to Stein (2008). (C) Structure of Ala-6-fengycin.

P34 cytotoxicity

In order to distinguish selective antiviral activity from cytotoxicity, the peptide was evaluated on MDCK, CRFK and RK13 cells by the NRU and MTT assays. CC_{50} was quite similar in both NRU and MTT tests for each cell lineage. CC_{50} values were 2.11 μg/mL, 2.5 μg/mL and 3.92 μg/mL for MDCK, CRFK and RK13 cells, respectively. Cytotoxicity was not observed at 1.37 μg/mL of the peptide P34 for MDCK, 0.92 μg/mL for CRFK and 2.29 μg/mL for RK13 cell cultures. These concentrations were then used in all the subsequent assays.

Antiviral assays

Titrations showed that the presence of the peptide P34 had no statistically significant effect ($p > 0.05$) against the production of viral particles of CAV-2, CCoV, CDV, CPV-2, EIV and FCV. However, a significant reduction on viral titers occurred when P34 (2.29 μg/mL and 0.92 μg/mL, respectively) was incubated with EAV and FHV-1 (Table 1). The titer of EAV was expressively reduced from 10^7 $TCID_{50}$ to $10^{1,75}$ $TCID_{50}$ in the presence of P34, presenting a PI of 99.9%. The titer of FHV-1 was $10^{4,5}$ $TCID_{50}$ in the presence of P34 and $10^{5,75}$ $TCID_{50}$ in its absence, resulting in a PI of 94.4%. The peptide P34 had only a direct inactivating effect against EAV infectious particles. A potent virucidal effect was observed and EAV infectivity was reduced by 98.6%. After 6 h of incubation, EAV titer was reduced from $10^{4.5}$ $TCID_{50}$ to $10^{2.75}$ $TCID_{50}$ in the presence of P34 ($p < 0.05$).

Discussion

A great number of peptides isolated from different sources have been studied for antiviral activities (Andreu and Rivas, 1998; Antimicrobial Peptide Database/ APD: http://aps.unmc.edu/AP/main.html). Several AMPs have been tested, but just a few of them have reached the clinical routine (Oevermann *et al.*, 2003, Wachsman *et al.*, 2003).

Ideally, to be the most useful, any antimicrobial agent has to exhibit a broad-spectrum antimicrobial activity (Mohan *et al.*, 2010). P34 is an anionic, thermostable, hydrophobic, lipidic, bacteriocin-like substance produced by a *Bacillus* sp. with antimicrobial properties against bacteria (Motta *et al.*, 2007b, 2008) and viruses, according to the present study. However, anionic antimicrobial peptides are

Table 1 - Antiviral activity of the peptide P34 against some viruses with different genotipic and fenotipic features.

Virus	Genotipic and fenotipic features	P34 antiviral activity
	DNA	
CAV-2	Non-enveloped	Absent
CPV-2	Non-enveloped	Absent
FHV-1	Enveloped	Present
	RNA	
CCoV	Enveloped	Absent
CDV	Enveloped	Absent
EAV	Enveloped	Present
EIV	Enveloped	Absent
FCV	Non-enveloped	Absent

very rare (Paulmann *et al.*, 2002) and it is thought that these peptides were developed in response to the resistance mechanisms toward cationic antimicrobial peptides (Lai *et al.*, 2002), which are found in all species and are potential broad-spectrum antiviral agents (Albiol-Matanic and Castilla, 2004).

In order to evaluate the peptide P34 as an antiviral substance *in vitro*, CAV-2, CPV-2 and FHV-1 were exposed to the AMP, being all DNA viruses, only FHV-1 having an envelope (Felipe *et al.*, 2006; Decaro and Buonavoglia, 2012, San Martín, 2012). The RNA viruses tested were CCoV, CDV, EAV, EIV and FCV, all enveloped viruses except for FCV (Seki *et al.*, 2003; Abd-Eldaim *et al.*, 2005; Diel *et al.*, 2006; Gorbalenya *et al.*, 2006; Decaro *et al.*, 2007). According to the assays performed it seems that the peptide P34 does not have a broad antiviral activity, since it only inhibited EAV and FHV-1.

Some peptides have demonstrated their ability to kill rapidly a broad range of microorganisms including multidrug resistant bacteria, fungi and viruses by their lytic membrane properties (Reddy *et al.*, 2004). AMPs like surfactin, magainin, mellitin and cecropin are known for their ability to interact with lipid membranes resulting in destabilization, translocation, pore formation or lysis (Vollenbroich *et al.*, 1997; Sitaram and Nagaraj, 1999). It is possible that the peptide P34 interferes with the adsorption, penetration or viral replication, or even exerts a competition with the viral particles for the cellular receptors used for EAV and FHV-1 infections. Blocking viral entry may occur by specific interactions with cellular receptors or viral envelope compounds, apart from viral glycoproteins (Jenssen *et al.*, 2006). A possible mechanism proposed to explain the activity of P34 against FHV-1 would be its interaction with cellular receptors like heparan sulfate, or even the blocking of certain viral glycoproteins. Heparan sulfate is the most important glycosaminoglycan molecule associated with to herpesvirus attachment to host cells (Spillmann, 2001; Luganini *et al.*, 2010), consequently, any interference with heparan sulfate can reduce the viral infection (Shieh *et al.*, 1992).

The virucidal activity of P34 may be due to a physicochemical interaction of the membrane-active surfactant with the virus lipid membrane, similarly to fengycin (Steller *et al.*, 1999) or, alternatively the peptide P34 is EAV-specific, as no viral inactivation was detected against all the other enveloped viruses tested. We hypothesize that this peptide inactivates the virus through an interaction with a non-lipidic structural component.

EAV is a member of the family *Arteriviridae* and belongs to the order *Nidovirales*, along with porcine respiratory and reproductive syndrome virus, simian hemorrhagic fever virus and lactate dehydrogenase elevating virus (de Vries *et al.*, 1997; Gorbalenya *et al.*, 2006). Although equine viral arteritis causes severe economic losses to the equine industry, there is no specific treatment (Timoney and McCollum, 1993). Thus, there is a need for the development of antiviral drugs for the treatment of the disease. Herpesviruses are cosmopolite agents causing several infections to humans and animals, especially in immunocompromised individuals (Felipe *et al.*, 2006). A remarkable feature of the members of this family is their ability to cause and reactivate latent infections in their hosts, and this is important for the control of the disease (Hübner *et al.*, 2005). Among the drugs that possess inhibitory action against herpesvirus replication, the most used in the human medicine are the nucleoside analogues (De Clercq, 2012) and there is evidence of resistance to some of them (De Clercq, 2004). Likewise it is necessary to search for new compounds with alternative mechanisms of action.

Effective antiviral agents are lacking, specifically those which target RNA viruses (Li *et al.*, 2011). The current antiviral drug armamentarium comprises about 40 compounds that have been officially approved for clinical use; however, most of the approved drugs are used for the treatment of human immunodeficiency virus infections (Felipe *et al.*, 2006). The fast and increased pathogen dissemination and resistance to drugs have forced the scientists to consider alternative methods to overcome infections (Motta *et al.*, 2007a), mainly the emerging and re-emerging viral infections (Li *et al.*, 2011). Therefore, as many AMPs are produced in nature, they may become an alternative to control specific pathogen infections (Riley and Wertz, 2002) and, according to the present study, the peptide P34 may be an interesting therapeutic prospection for the treatment of horses and cats affected by EAV and FHV-1, respectively. However, more detailed studies *in vitro* and *in vivo* must be performed to elucidate the specific mechanism of action of this peptide against viruses.

In summary, our results have indicated that the peptide P34 showed antiviral activity against EAV and FHV-1, with virucidal properties only against EAV. Nevertheless no antiviral activity was detected against CAV-2, CCoV, CDV, CPV-2, EIV and FCV.

Acknowledgments

Authors thank CNPq and CAPES for the financial support.

References

Abd-Eldaim M, Potgieter L, Kennedy M (2005) Genetic analysis of feline caliciviruses associated with a hemorrhagic-like disease. J Vet Diag Invest 17:420-429.

Albiol Matanic VC, Castilla V (2004) Antiviral activity of antimicrobial cationic peptides against Junin virus and herpes simplex virus. Int J Antimicrob Agents 23:382-389.

Andreu D, Rivas L (1998) Animal antimicrobial peptides: An overview. Biopolymers (Peptide Science) 47:415-433.

Borenfreund E, Puerner JA (1984) A simple quantitative procedure using monolayer culture for toxicity assays. J Tissue Cult Meth 9:7-9.

Decaro N, Buonavoglia C (2012) Canine parvovirus - a review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet Microbiol 155:1-12.

Decaro N, Martella V, Elia G, Campolo M, Desario C, Cirone F, Tempesta M, Buonavoglia C (2007) Molecular characterisation of the virulent canine coronavirus CB/05 strain. Virus Res 125:54-60.

De Clercq E (2004) Antiviral drugs in current clinical use. J Clin Virol 30:115-133.

De Clercq E (2012) Milestones in the discovery of antiviral agents: Nucleosides and nucleotides. Acta Pharm Sin B 2:535-548.

de Vries AAF, Horzinek MC, Rottier PJM, de Groot RJ (1997) The genome organization of the *Nidovirales*: similarities and differences between arteri-, toro-, and coronaviruses. Semin Virol 8:33-47.

Diel DG, Almeida SR, Weiblen R, Frandoloso R, Anziliero D, Kreutz LC, Groff FHS, Flores EF (2006) Prevalência de anticorpos contra os vírus da influenza, da arterite viral e herpesvírus em equinos do estado do Rio Grande do Sul, Brasil. Cienc Rural 36:1467-1473.

Felipe AMM, Rincão VP, Benati FJ, Linhares REC, Galina KJ, Toledo CE, Lopes GC, Mello JC, Nozawa C (2006) Antiviral effect of *Guazuma ulmifolia* and *Stryphnodendron adstringens* on poliovirus and bovine herpesvirus. Biol Pharm Bull 29:1092-1096.

Gorbalenya AE, Enjuanes L, Ziebuhr J, Snijder EJ (2006) *Nidovirales*: evolving the largest RNA virus genome. Virus Res 117:17-37.

Hübner SO, Oliveira AP, Franco AC, Esteves PA, Silva AD, Spilki FR, Rijsewijk FA, Roehe PM (2005) Experimental infection of calves with a gI, gE, US9 negative bovine herpesvirus type 5. Comp Immunol Microb 28:187-196.

Jenssen H, Hamill P, Hancock REW (2006) Peptide antimicrobial agents. Clin Microbiol Rev 19:491-511.

Lai R, Liu H, Hui Lee W, Zhang Y (2002) An anionic antimicrobial peptide from toad *Bombina maxima*. Biochem Biophys Res Commun 295:796-799.

Li Q, Zhao Z, Zhou D, Chen Y, Hong W, Cao L, Yang J, Zhang Y, Shi W, Cao Z, Wu Y, Yan H, Li W (2011) Virucidal activity of a scorpion venom peptide variant mucroporin-M1 agains measles, SARS-CoV, and influenza H5N1 viruses. Peptides 32:1518-1525.

Luganini A, Giuliani A, Pirri G, Pizzuto L, Landolfo S, Gribaudo G (2010) Peptide-derivatized dendrimers inhibit human cytomegalovirus infection by blocking virus binding to cell surface heparan sulphate. Antiviral Res 85:532-540.

Mahy BWJ, Kangro HO (1996) Virology Methods Manual. 1st ed. Harcourt Brace & Company, London.

Mohan KVK, Shilpakala SR, Chintamani DA (2010) Antiviral activity of selected antimicrobial peptides against vaccinia virus. Antiviral Res 86:306-311.

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55-63.

Motta AS, Cannavan FS, Tsai SM, Brandelli A (2007a) Characterization of a broad range antibacterial substance from a new *Bacillus* species isolated from Amazon basin. Arch Microbiol 188:367-375.

Motta AS, Cladera-Olivera F, Brandelli A (2004) Screening for antimicrobial activity among bacteria isolated from the Amazon basin. Braz J Microbiol 35:307-310.

Motta AS, Flores FS, Souto AA, Brandelli A (2008) Antibacterial activity of a bacteriocin-like substance produced by *Bacillus* sp. P34 that targets the bacterial cell envelope. Antonie Leeuwenhoek 93:275-284.

Motta AS, Lorenzini DM, Brandelli A (2007b) Purification and partial characterization of an antimicrobial peptide produced by a novel *Bacillus* sp. isolated from the Amazon Basin. Curr Microbiol 54:282-286.

Oevermann A, Engels M, Thomas U, Pellegrini A (2003) The antiviral activity of naturally occurring proteins and their peptide fragments after chemical modification. Antiviral Res 59:23-33.

Oyston PC, Fox MA, Richards SJ, Clark GC (2009) Novel peptide therapeutics for treatment of infections. J Med Microbiol 58:977-987.

Paulmann M, Arnold T, Linke D, Özdirekcan S, Kopp A, Gutsmann T, Kalbacher H, Wanke I, Schuenemann VJ, Habeck M, Bürck J, Ulrich AS, Schittek B (2002) Structure-activity analysis of the dermcidin-derived peptide DCD-1L, an anionic antimicrobial peptide present in human sweat. J Biol Chem 287:8434-8443.

Reddy KVR, Yedery RD, Aranha C (2004) Antimicrobial peptides: premises and promises. Int J Antimicrob Ag 24:536-547.

Riley MA, Wertz JE (2002) Bacteriocins: evolution, ecology and application. Annu Rev Microbiol 56:117-137.

San Martín C (2012) Latest insights on adenovirus structure and assembly. Viruses 4:847-877.

Seki F, Ono N, Yamaguchi R, Yanagi Y (2003) Efficient isolation of wild strains of canine distemper virus in vero cells expressing canine SLAM (CD150) and their adaptability to marmoset B95a cells. J Virol 77:9943-9950.

Shieh MT, Wudunn D, Montgomery RI, Esko JD, Spear PG (1992) Cell surface receptors for herpes simplex virus are heparan sulfate proteoglycans. J Cell Biol 116:1273-1281.

Sitaram N, Nagaraj R (1999) Interaction of antimicrobial peptides with biological and model membranes: structural and change requirements for activity. Biochim Biophys Acta 1462:29-54.

Spillmann D (2001) Heparan sulfate: anchor for viral intruders? Biochimie 83:811-817.

Stein T (2008) Whole-cell matrix-assisted laser desorption/ionization mass spectrometry for rapid identification of bacteriocin/lanthibiotic-producing bacteria. Rapid Commun Mass Spectrom 22:1146-1152.

Steller S, Vollenbroich D, Leenders F, Stein T, Conrad B, Hofemeister J, Jaques P, Thonart P, Valter J (1999) Structural and functional organization of the fengycin synthetase multienzyme system from *Bacillus subtilis* b213 and A1/3. Chem Biol 6:31-41.

Timoney PJ, McCollum WH (1993) Equine viral arteritis. Vet. Clin. North Am. Equine Prac 9:295-309.

Vaucher RA, Teixeira ML, Brandelli A (2010) Investigation of the cytotoxicity of antimicrobial peptide P40 on eukaryotic cells. Curr Microbiol 60:1-5.

Vollenbroich D, Pauli G, Ozel M, Valter J (1997) Antimycoplasm properties and application in cell culture of surfactin, a lipopeptide antibiotic from *Bacillus subtilis*. Appl and Environ Microbiol 63:44-49.

Wachsman MB, Castilla V, Holgado APR, Torres RA, Sesma F, Coto CE (2003) Enterocin CRL35 inhibits late stages of HSV-1 and HSV-2 replication *in vitro*. Antiviral Res 58:17-24.

Weiblen R, Raiser AG, Rahal SC, Canabarro TF (1988) Isolation of feline calicivirus from cats in Brazil. Vet Rec 122:94-95.

List of Abbreviations

AMP - antimicrobial peptide
CAV-2 - canine adenovirus type 2
CC_{50} - Cytotoxic concentration 50%
CCoV - canine coronavirus
CDV - canine distemper virus
CPE - cytopathic effect
CPV-2 - canine parvovirus type 2
CRFK - Crandell-Rees Feline Kidney
EAV - equine arteritis virus
EIV - equine influenza virus
E-MEM - Eagle's minimum essential medium
FCV - feline calicivirus
FHV-1 - feline herpesvirus type 1
MDCK - Madin-Darby Canine Kidney
MTT - diphenyltetrazolium bromide
NRU - neutral red uptake
P34 - peptide P34
PI - percent of inhibition
RK13 - Rabbit Kidney Cells
$TCID_{50}$ - tissue culture infective dose 50%

All the content of the journal, except where otherwise noted, is licensed under a Creative Commons License CC BY-NC.