UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE FARMÁCIA

TRABALHO DE CONCLUSÃO DE CURSO DE FARMÁCIA

SOUTH BRAZILIAN BROMELIADS-ASSOCIATED MICROORGANISMS AS SOURCE OF ANTIBIOFILM AND ANTIBIOTIC COMPOUNDS

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Trabalho de Conclusão de Curso apresentado ao Curso de Farmácia da Universidade Federal do Rio grande do Sul como requisito à obtenção do título de grau de Farmacêutico.

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Coorientador: Rodrigo Campos da Silva

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Dedico este trabalho a todos que contribuem para o avanço da Ciência no Brasil.

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APRESENTAÇÃO

Esse Trabalho de Conclusão de Curso foi redigido sob a forma de artigo ao qual foi elaborado segundo as normas da revista Microbial Pathogenesis apresentadas em anexo.

BROMELIADS-ASSOCIATED MICROORGANISMS AS SOURCE OF ANTIBIOFILM AND ANTIBIOTIC COMPOUNDS

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Abstract

The majority of the World's biodiversity remains to be rationally explored, especially the remaining Atlantic Forest in Brazil and preeminently the abounding plants bromeliads. Bromeliad tanks (phytotelma) act as reservoirs throughout the year, maintaining a complex and high microbial biodiversity. In this context, microbial metabolites may offer many biological activities. Studies on the microbial activity associating bromeliad are rare, hence, the present work studied the bioprospection of antibiotic and antibiofilm microbial metabolites. Six water tank samples were collected from Ilópolis in the Park of Ibama (Rio Grande do Sul, Brazil) and aliquots were inoculated in different culture media. Ninety-three microorganisms were isolated after primarily selection. Seven microorganisms were chosen to follow the process of fermentation and tested as antibiotic and antibiofilm metabolites against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*., 35% and 64% fractions have produced antibacterial activity and antibiofilm activity against *S. aureus*, presenting good preliminary results and demonstrating that the bromeliad are an important niche to be explored.

Highlights

Water tank bromeliads as a richness microbial biodiversity with potential compounds with activity against microorganisms and biofilm.

Keywords: Bromeliads, phylloplane, microoraganisms, antibiofilm, antibiotic.

1. Introduction

Search for natural products is predominant to enabling discovery and development of new therapeutic agents. The list of breakthrough medicines originated from natural resources is long and their impact are widespread as antitumor, anticoagulant and anti-infective drugs. [1–4]. Two important milestones in the history of antibiotics were the discovery of penicillin and streptomycin, starting the Golden Age for Antibiotics (1940-1960). This process requires sample collection of microorganisms, fermentation, product isolation and testing against target organisms. Nearly 47% of the microbial metabolites exhibit some biological activity [3,5].

The gradual decline in the discovery and development of antibiotic linked to the evolution of antimicrobial resistance (AMR), including excessive and inappropriate antimicrobial prescribing during the COVID-19 pandemic have been of great concern to researchers [6,7]. The global threat of AMR will persist beyond the COVID-19 and bacterial infections unsuccessfully treated due to AMR claim at least 700000 lives per year, projected to be associated with the deaths of 10 million people per year by 2050, surpassing even deaths caused by cancer[8,9].

Biofilms are an important element to bacterial virulence; it is structurally complex, embedded with extracellular polymeric substances (EPS) that confer adaptive resistance and barrier protection [10–12]. Eighty percent of bacterial chronic infections are associated to biofilm formation [13]. Antibiotic therapies against biofilms frequently require the use of high doses for prolonged time, but often fail to combat persistent infections associated with biofilms. Consequently, increasing the cost per hospitalized patient with chronic infections and high mortality and morbidity rates [11,14,15]. The most common forms of pathogenic bacterial *Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa* and *Enterobacter* spp. [16].

Hence, there is an urgent need for alternatives to antibiotics and the development of antibiofilm compounds for infection treatment control. Great part of the world's biodiversity remains to be explored and most known species have never been tested for any kind of bioactivity, mainly species in oceans and rainforests [5]. The Atlantic Forest is – one of the 34 hotspots in the world and the richest in terms of biodiversity – preeminently the bromeliads which are abounding plants in the Atlantic Forest, containing about 58 genera with 3140 species [14,15]. Tankforming and bulb-producing epiphytic bromeliads engage in beneficial exchanges and provide important microhabitats, originating environment for the development of a complex microbial community, as a result of the water containing a high nutrient concentration [19,20]. However, only 13% remains of the Atlantic Forest in Brazil and few studies about the great biodiversity and bioprospection are performed, especially with water tank (phytotelma) [21–30].

Ruivo et al. 2005 and Carmo et al. 2014 has shown great biodiversity in water tank. New yeast species *Candida bromeliacearum* sp. nov. and *Candida ubatubensis* sp. nov. were isolated from the water tanks of

Canistropsis seidelii. 90% of bacteria isolated from the water held in bromeliads tank presented enzymatic activity.

Therefore, as it was possible to observe the lack of studies related to antibiotics and antibiofilm activity, the present work aimed to enrich the area providing more knowledge to the subject, as well as the importance of the Atlantic Forest, which provides a huge diversity of microorganisms that can lead us to more natural products.

2. Material and Methods

2.1 Sampling and isolation

Sampling was conducted collecting water with sterile syringe from bromeliad tanks. Three distinct species of bromelias were sampled: *Aechmea calyculata* (E.Morren) Baker, *Vriesea friburgensis* Mez *and Vriesea platynema* Gaudich, which were collected from Ilópolis in the Park of Ibama (28°55'59.5"S 52°08'04.0"O / Rio Grande do Sul state, Brazil). Registered in the Brazilian Environmental Ministry—platform SISGEN #AD3A66E. Two hundred microliters of water samples were added to agar plates and spread with the aid of Drigalsky's handle on the following media: actinomycetes (ACM), potato dextrose agar (PDA), cellulose (CEL), fermentation medium (FM), Luria Bertani agar (LB), milk (M), M1D, R2A, R2AM, sabouraud (SB) and yeast extract peptone dextrose (YPD) according to appendix A. The incubation was at 25°C for 3-7 days. The isolated was stored in skim milk and 10% glycerol in freezer at -20°C.

2.2 Metabolites production

Seven isolated microorganisms were chosen and grown according to Table 1. 1000 uL of bacterial inoculum (OD 500nm) was added to Erlenmeyer flasks containing 100 mL of liquid broth. They were cultivated at 28 °C under incubator shaker series (New Brunswick Scientific ®) at 150 rpm for 5 days. After this period, all cultures were centrifuged (Molecular Devices®**)** at 10.000 rpm for 10 minutes. The pallet was removed and the liquid were filtered in 0.22 µm filter. After this period, liquid–liquid extraction was performed with ethyl acetate generating two phases, the phases were divided into two portions: (i) ethyl acetate extract (EAE), the organic phase; (ii) residual aqueous phase (AqE). The organic phase extracts were concentrated by rotary evaporation. The fraction extracts were frozen and dried by lyophilization (Edwards do Brasil®).

TABLE 1 - Microorganisms isolated from the bromeliad water tank

Bromeliad species	**ID**	**Microorganisms isolated**	**Grown cultivation**
Aechmea calyculata (E.Morren) Baker (n_1)	A7S3	Yeast	Sabouraud
Aechmea calyculata (E.Morren) Baker (n_2)	A11A3	Bacteria	ACM
Aechmea calyculata (E.Morren) Baker (n_2)	A11RM1	Bacteria	RM
Vriesea friburgensis Mez (n_1)	A1M3	Bacteria	M1D
Vriesea friburgensis Mez (n_2)	A8A4	Bacteria	ACM

Vriesea platynema Gaudich (n_1)	A10A2	Yeast	ACM
Vriesea platynema Gaudich (n_2)	A3S3	Yeast	Sabouraud

2.3 Bacterial growth and biofilm formation assays

Staphylococcus aureus Newman *(*ATCC 25904), *Staphylococcus epidermids* (ATCC 35984) and *Pseudomonas aeruginosa* (ATCC 27853) were cultivated on Muller Hinton agar (Merck®**)** at 37º C overnight. Several colonies were used to make the bacterial suspension in sterile sodium chloride 0.9% to achieve an optical density of 0.150 ± 0.010 at $OD_{600\ nm}$. (Spectramax m2) (Dos Reis, S. V et al. 2020). *S. aureus*, *S. epidermids* and *P. aeruginosa* bacterial growth and biofilm formation were evaluated in 96-well microtiter. Control of sterility, 40 µL of culture media, tryptic soy broth (TSB) or brain heart infusion (BHI) for *S. aureus*, + 80 uL of saline and 80 uL of sterile water. Solution test: 40 µL of culture media, 4 µL of fraction test (1mg/mL), 76 µL of sterile water, and 80 uL of bacterial suspension. Positive control activity: 80uL antibiotic (gentamicin or vancomycin 20 ug/mL) + 80 uL bacteria suspension + 40 uL media. Plates were incubated for 24 h period at 37 °C and measured at $OD_{600\ nm}$. After the incubation period, the content of wells was gently removed and washed 3 times with 0.9 % saline. Subsequential heat to fix the biofilm in an oven for 1h at 60°C. 200 uL of Crystal violet (0.4%) was used to stain the bacteria for 15 min. The biofilm was eluted with 200 uL of ethanol (99.5%) for 30 min without shaking. Absorbance was measured at $OD_{570\ nm}$. The sterility control of was used as spectrophotometric blank [31].

3. Statistical analysis

All statistical analyzes were performed using GraphPad Prism. The data concerning the bacterial growth and biofilm assays were analyzed by one-way analysis of variance (ANOVA) with Dunnett multiple comparison test, with a 5% significance level.

4. Results

4.1 Sample collection and isolation

Ninety and three microorganisms were isolated both bacteria and fungi (Table 2). 38% of the isolates were collected from the plant *Vriesea friburgensis* Mez, 34% *Aechmea calyculata* (E.Morren) Baker and 28% *Vriesea platynema* Gaudich.

TABLE 2 -Frequency of the microorganisms isolated from the bromeliad water tank

	Bromeliad		
Culture media used	***Aechmea calyculata* (E.Morren) Baker (n= 2)**	***Vriesea friburgensis* Mez (n= 2)**	***Vriesea platynema* Gaudich (n= 2)**
Actinomycetes media	4	3	2
Cellulose media	6	4	3
Fermentation medium	2	6	2
Miller's LB Broth Base	3	3	2
Milk agar	2	3	2
M1D media	1	3	7

Potato agar media	1	1	0
R2A media	1	2	0
R2AM media	6	1	2
Sabouraud media	5	4	3
Yeast extract peptone dextrose	1	5	3
Total number of isolates	32	35	26

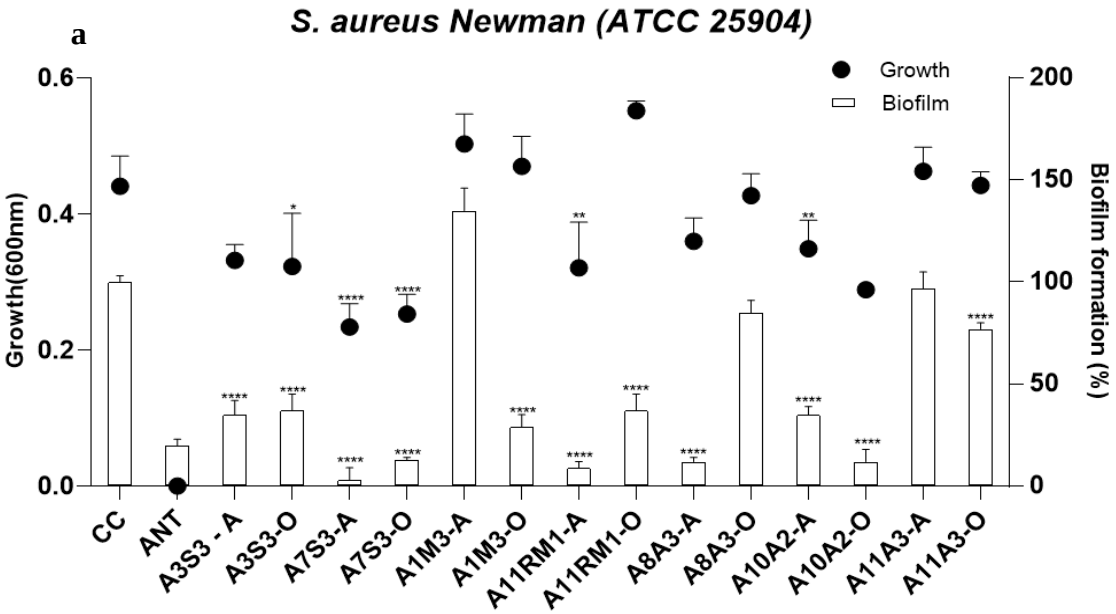
The criteria selection follows of seven microorganisms: a) selective actinomycetes b) growth in 24 hours and c) different morphologically.

4.2 Antibacterial and antibiofilm activities test

Fraction (1 mg/mL) were evaluated concerning their antibiofilm and antibacterial activities against *S. aureus* and *S. epidermidis* and thirteen fraction extracts were test against *P. aeruginosa*. Fractions produced antibiotic activity against *Staphylococcus aureus*, 27% A3S3-O ($p= 0,0157$), 47% A7S3-A ($p<0,0001$), 43% A7S3-O ($p<0,0001$) 27% A11RM1-A ($p=0,0016$) and 21% A10A2-O ($p=0,0032$) bacterial growth. Three fractions produced antibiofilm activity without inhibiting bacterial growth displaying an inhibition of 71% for A1M3-O ($p<0,0001$), 63% for A11RM1-O ($p<0,0001$) and 23% for A11A3-O ($p<0,0001$) upon biofilm formation of *S. aureus* (Fig.1a).

Six fraction extracts produced antibiotic against *S. epidermids* with inhibitions of 49% for A3S3-A, 37% for A3S3-O, 56% for A7S3-A, 55% for A7S3-O, for A8A3 38% and 49% for A11A3-O with a significant difference in relation to the bacterial growth control ($p < 0.05$). No significant difference in relation biofilm formation was observed. (Fig.1b).

One extract demonstrated antibiotic activity against *P. aeruginosa* A3S3-O and reduced 19% of bacterial growth ($p < 0.05$). A10A2-A inhibited 22% ($p<0.05$) of biofilm formation. (Fig.1c).



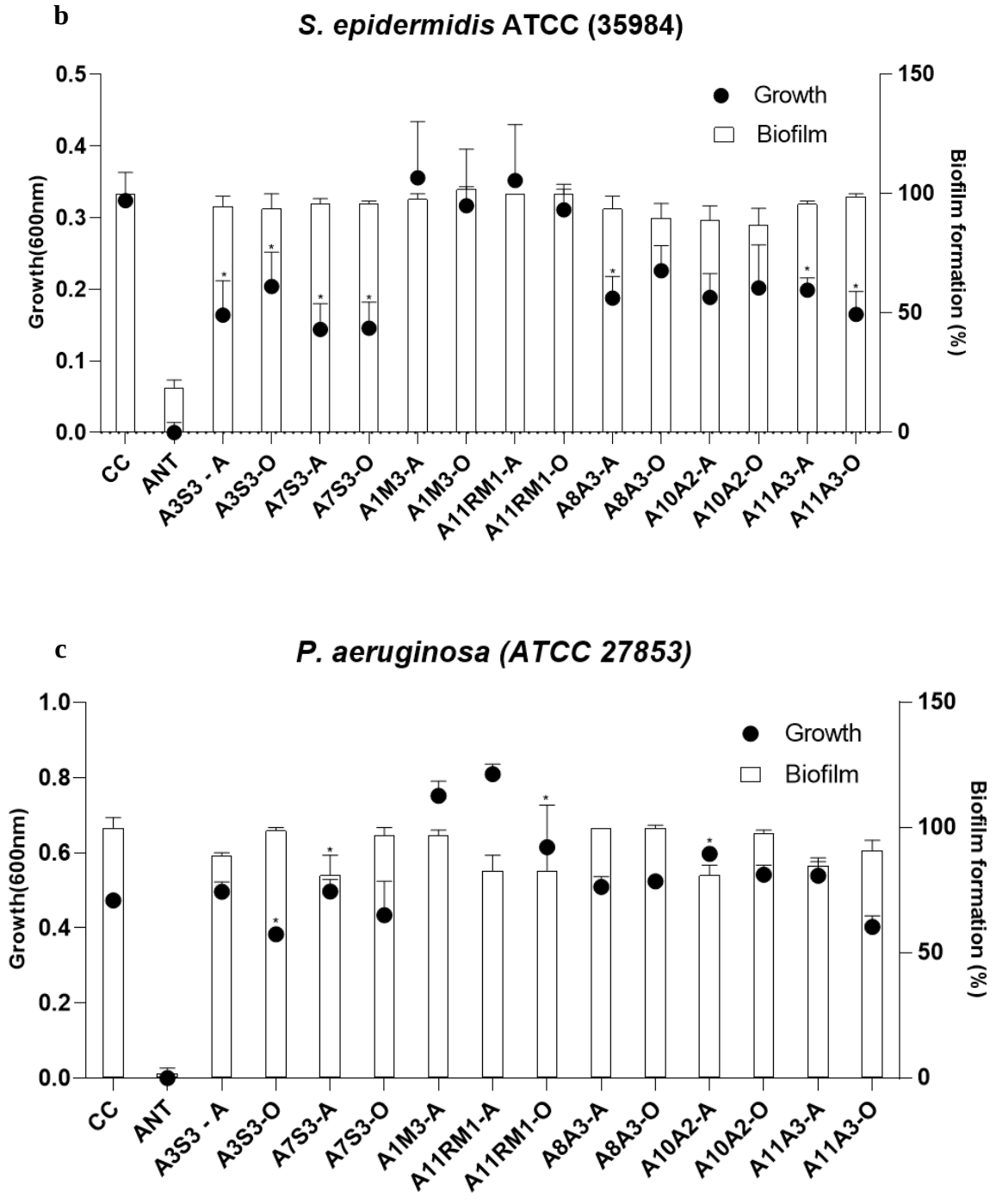


Figure 1. In vitro antibiofilm and antibacterial activity of *Staphylococcus aureus* Newman (a), *Staphylococcus epidermidis* (b) and *Pseudomonas aeruginosa* (c). Bars represent the mean ± SD. The data concerning the bacterial growth and biofilm assays were analyzed by one-way analysis of variance (ANOVA) with Dunnett multiple comparison test. ****Represents statistical significance $p \leq 0.0001$. ** Represents statistical significance p≤,0,01. * Represents statistical significance p≤0,05.

5. Discussion

Herein we presented the isolation of bromelia-associated microorganisms and the successful metabolite production aiming antibiotic and antibiofilm activity against pathogenic bacteria. The approach used to isolate microorganisms retrieves a high number of isolates corroborating with other biodiversity studies associated to bromeliads[22,23,29,32]. It is important to emphasize the microbiological importance of this ecosystem, since over the last 14 years, 22 new yeast species were discovered in association with bromeliads

[21]. Our results showed a high biodiversity, including bacteria, yeasts and fungi. After a preliminary selection (selective actinomycetes, growth in 24 hours and different morphologically) and cultivation of seven microorganisms, we achieve significant results for six isolates, particularly against the important pathogen *S. aureus*. This high activity is related to environmental challenges these microorganisms face in nature, *i. e.* variable water condition (dry and wet climate seasons) [33], attack of other microbial enemies [34] and synergetic interactions with the bromelia plant [35]. Altogether, these challenges impose an evolutionary pressure on these microorganisms forcing them to produce particular metabolites of all classes. Previous report showed that 80% of the isolated yeast possesses at least one enzymatic activity, especially, protease activity is predominant, followed by xylanase, amylase, pectinase and cellulase activities[22]. Moreover, metagenomics studies had identified *Actinobacteria* (12%) and the genus *Streptomyces* both in water tanks and other isolates with great versatility in the production of enzymes. [19,23, [36], further studies are required, as 60% of new antibiotic were isolated from actinobacteria [4]. Consequently, phytotelma is rich in biodiversity and has biotechnological potential, microorganisms present in the water tank can be an applicable resource for bioprospection and pharmacological prospective antibiotic agents. Resistant bacteria are one of the major problems that challenges the public health of the 21st century. Therefore, the discovery of new antibacterial and antibiofilm substances are extremely necessary.

The results herein obtained has shown 35% antibacterial activity against *S. aureus.* The best result obtained decreased bacterial growth by half (A7S3-A and A7S3-O), this great preliminary result can be enhanced after performing other extraction methods, such as chromatography column. The antibacterial activity corroborates with the study of Araujo et al [19] that 4/100 strains produce strong suppression against *S. aureus.* Furthermore, the extract A7S3 both the aqueous and the organic phase obtained an antibacterial activity against *S. epidermidis*. However, A7S3 has obtained similar results in both aqueous and organic phase, so it will be necessary to use other solvents for liquid-liquid separation.

As far we know, this work is the first to test the antibiofilm activity of the secondary metabolites produced by microorganisms in water tank from bromeliad. Good preliminary results were obtained, 64% fractions have produced antibiofilm activity against *S. aureus*, the range up 60% inhibit biofilm formation, considered between a high and good range in accordance with Yuyama et al [24] who acknowledge the range of 100–70% as high, 69–40% as good and 39–20% as moderate for biofilm inhibition. The resulted fraction A1M3-O, A11RM1-O and A11A3-O were capable to inhibit *S. aureus* Newman biofilm without interfering in bacterial growth.

The isolates A3S3 and A7S3 were phenotypically characterized by means of macro/micro-morphological are similar genus *Rhodotorula*, this result collaborates with Patricia Valente et al [23], who found ten different species of this genus, the isolates were not found in the water accumulated inside the bromeliad tank, but endophytic isolates and bromeliad flower.

Water tank bromeliads has a richness microbial biodiversity with potential biotechnology. It is important to perform molecular identification of the isolates, antibacterial and antibiofilm activity, considering that just 7.5% of isolates was tested. We emphasize the importance of performing fractionation of the extracts in the future for possible characterization of secondary metabolites.

6. Conclusion

One of the main tasks of applied microbiology to biotechnology is to develop procedures to discover new microbial metabolites within a wide diversity, the correlation between biodiversity and biotechnology which in this context can be seen as a vast reservoir of active metabolites against pathogenic microbial, where biofilm activity showed to be the most promise. Especially acting in inhibiting biofilm growth against *S.aureus.* This work obtained great preliminary results and encouragement to test antimicrobial activities using the isolated microorganisms from the bromeliad water tank. It also highlights the richness of the microorganisms that inhabit the Atlantic Forest and the importance of nature preservation.

Credit authorship contribution statement

Ana Luiza de Azevedo Gomes: Methodology, Data curation, Investigation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Rodrigo Campo da Silva:** Conceptualization, Investigation Writing - original draft, Writing - review & editing**. Marco Vinicius Vizioli Klaus:** Conceptualization, Investigation Writing - original draft, Writing - review & editing**. Elisete Maria de Freitas:** Conceptualization, Investigation. **Alexandre José Macedo:** Conceptualization, Investigation, Funding acquisition, Writing - original draft, Supervision, Writing - review & editing.

7. Conflict of interest statement

The authors declare that they have no conflict of interest.

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Appendix. Supplementary data

Media	Broth agar		trademark
actinomycetes (ACM)	actinomycete agar	22g/L	Difco ®
	sodium chloride	24g/L	Nuclear®
	magnesium chloride	11g/L	Synth®
	glycerol	5g/L	Synth®
potato dextrose (P)		35 g/L	Merck ®
cellulose (CEL)	sodium sulphate	3g/L	Dinâmica®
	sulphate magensium	1g/L	Dinâmica®
	monopotassium phosphate	1g/L	Dinâmica®
	carboxymethylcellulose	5g/L	Dinâmica®
	yeast extract	0,6 g	Hymedia®
	sodium chloride	24 g	Hymedia®
	magnesium chloride	11g	Synth®
fermentation medium (FM)	amide	3g/L	Hymedia®
	glucose	1g/L	Hymedia®
	peptone	1g/L	Hymedia®
	sodium chloride	24g/L	Nuclear®
	magnesium chloride	11g/L	Synth®
	agar	17g/L	Merck ®
Miller's LB Broth Base (LB)		35 g/L	Merck ®
milk agar	milk	10g/L	Nestlé ®
	agar	17g/L	Merck ®
M1D	sulphate magensium	5g/L	Dinâmica®
	yeast extract	1g/L	Hymedia®
	peptone	1g/L	Hymedia®
	sodium chloride	24g/L	Nuclear®
	magnesium chloride	11g/L	Synth®
	agar	20g/L	Merck ®

R2A		18,2 g /L	Merck ®
R2AM	R2A agar	18,2 g/L	Merck ®
	sodium chloride	24g/L	Nuclear®
	magnesium chloride	11g/L	Synth®
	glycerol	5g/L	Synth®
sabouraud (S)	dextrose	40g/L	Hymedia®
	peptone	10g/L	Hymedia®
	agar	20g/L	Merck ®
yeast extract peptone dextrose (YPD)	yeast extract	10g/L	Hymedia®
	peptone	20g/L	Hymedia®
	glucose	20g/L	Hymedia®
	agar	17g/L	Merck ®

References

[1] D.J. Newman, G.M. Cragg, Natural Products as Sources of New Drugs from 1981 to 2014, Journal of Natural Products. 79 (2016) 629–661. https://doi.org/10.1021/acs.jnatprod.5b01055.

[2] S.B. Singh, F. Pelaez, Biodiversity, chemical diversity and drug discovery, 2008. 10.1007/978-3-7643-8117-2_4

[3] L. Katz, R.H. Baltz, Natural product discovery: past, present, and future, Journal of Industrial Microbiology and Biotechnology. 43 (2016) 155–176. https://doi.org/10.1007/s10295-015-1723-5.

[4] K. Jakubiec-Krzesniak, A. Rajnisz-Mateusiak, A. Guspiel, J. Ziemska, J. Solecka, Secondary metabolites of actinomycetes and their antibacterial, antifungal and antiviral properties, Polish Journal of Microbiology. 67 (2018) 259–272. https://doi.org/10.21307/pjm-2018-048.

[5] J. Bérdy, Thoughts and facts about antibiotics: Where we are now and where we are heading, Journal of Antibiotics. 65 (2012) 385–395. https://doi.org/10.1038/ja.2012.27.

[6] M. Hutchings, A. Truman, B. Wilkinson, Antibiotics: past, present and future, Current Opinion in Microbiology. 51 (2019) 72–80. https://doi.org/10.1016/j.mib.2019.10.008.

[7] Hassan, M. Z., Basnyat, B., & Meeyai, A. (2021). ISARIC (International Severe Acute Respiratory and Emerging Infections Consortium).

[8] S. Ghosh, C. Bornman, M.M. Zafer, Antimicrobial Resistance Threats in the emerging COVID-19 pandemic: Where do we stand?, Journal of Infection and Public Health. 14 (2021) 555–560. https://doi.org/10.1016/j.jiph.2021.02.011.

[9] O'neill, J. (2014). Antimicrobial resistance. Tackling a crisis for the health and wealth of nations.

[10] C.W. Hall, T.F. Mah, Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria, FEMS Microbiology Reviews. 41 (2017) 276–301. https://doi.org/10.1093/femsre/fux010.

[11] J.W. Costerton, P.S. Stewart, E.P. Greenberg, Bacterial Biofilms: A Common Cause of Persistent Infections, 1999.

[12] R.A.G. da Silva, I. Afonina, K.A. Kline, Eradicating biofilm infections: an update on current and prospective approaches, Current Opinion in Microbiology. 63 (2021) 117–125. https://doi.org/10.1016/j.mib.2021.07.001.

[13] A. Singh, A. Amod, P. Pandey, P. Bose, M.S. Pingali, S. Shivalkar, P.K. Varadwaj, A.K. Sahoo, S.K. Samanta, Bacterial biofilm infections, their resistance to antibiotics therapy and current treatment strategies, Biomedical Materials (Bristol). 17 (2022). https://doi.org/10.1088/1748-605X/ac50f6.

[14] A.J. Macedo, W.-R. Abraham, Can Infectious Biofilm be Controlled by Blocking Bacterial Communication?, 2009.

[15] E.F. Berbari, A.D. Hanssen, M.C. Duffy, J.M. Steckelberg, D.M. Ilstrup, W.S. Harmsen, D.R. Osmon, Risk Factors for Prosthetic Joint Infection: Case-Control Study, n.d. https://academic.oup.com/cid/article/27/5/1247/480445.

[16] N. Rabin, Y. Zheng, C. Opoku-Temeng, Y. Du, E. Bonsu, H.O. Sintim, Biofilm formation mechanisms and targets for developing antibiofilm agents, Future Medicinal Chemistry. 7 (2015) 493–512. https://doi.org/10.4155/fmc.15.6.

[17] C.L. Rezende, F.R. Scarano, E.D. Assad, C.A. Joly, J.P. Metzger, B.B.N. Strassburg, M. Tabarelli, G.A. Fonseca, R.A. Mittermeier, From hotspot to hopespot: An opportunity for the Brazilian Atlantic Forest, Perspectives in Ecology and Conservation. 16 (2018) 208–214. https://doi.org/10.1016/j.pecon.2018.10.002.

[18] T.J. Givnish, M.H.J. Barfuss, B. van Ee, R. Riina, K. Schulte, R. Horres, P.A. Gonsiska, R.S. Jabaily, D.M. Crayn, J.A.C. Smith, K. Winter, G.K. Brown, T.M. Evans, B.K. Holst, H. Luther, W. Till, G. Zizka, P.E. Berry, K.J. Sytsma, Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny, American Journal of Botany. 98 (2011) 872–895. https://doi.org/10.3732/ajb.1000059.

[19] S.K. Goffredi, A.H. Kantor, W.T. Woodside, Aquatic Microbial Habitats Within a Neotropical Rainforest: Bromeliads and pH-Associated Trends in Bacterial Diversity and Composition, Microbial Ecology. 61 (2011) 529–542. https://doi.org/10.1007/s00248-010-9781-8.

[20] B. Aechmea Bracteata Author, D.H. Benzing, J.A. Derr, J.E. Titus, The Water Chemistry of Microcosms Associated with the The Water Chemistry of Microcosms Associated with the Bromeliad Aechmea bracteata, 1972.

[21] Atlas dos Remanescentes Florestais da Mata Atlântica-ano base 2011 FUNDAÇÃO SOS MATA ATLÂNTICA INSTITUTO NACIONAL DE PESQUISAS ESPACIAIS-INPE ATLAS DOS MUNICÍPIOS DA MATA ATLÂNTICA Ano base 2011, n.d. http://mapas.sosma.org.br.

[22] F.L. Carmo, H.F. Santos, R.S. Peixoto, A.S. Rosado, F. v Araujo, Tank bromeliad water: Similar or distinct environments for research of bacterial bioactives?, 2014. www.sbmicrobiologia.org.br.

[23] M. Fontes Landell, J. Nunes Mautone, P. Valente, BIODIVERSITY OF YEASTS ASSOCIATED TO BROMELIADS IN ITAPUÃ PARK, VIAMÃO/RS, 1999.

[24] M.F. Landell, J. Inácio, Á. Fonseca, M.H. Vainstein, P. Valente, Cryptococcus bromeliarum sp. nov., an orange-coloured basidiomycetous yeast isolated from bromeliads in Brazil, International Journal of Systematic and Evolutionary Microbiology. 59 (2009) 910–913. https://doi.org/10.1099/ijs.0.005652-0.

[25] M.F. Landell, J. Salton, K. Caumo, L. Broetto, M.B. Rott, Isolation and genotyping of free-living environmental isolates of Acanthamoeba spp. from bromeliads in Southern Brazil, Experimental Parasitology. 134 (2013) 290–294. https://doi.org/10.1016/j.exppara.2013.03.028.

[26] M.F. Landell, R. Billodre, J.P. Ramos, O. Leoncini, M.H. Vainstein, P. Valente, Candida aechmeae sp. nov. and Candida vrieseae sp. nov., novel yeast species isolated from the phylloplane of bromeliads in Southern Brazil, International Journal of Systematic and Evolutionary Microbiology. 60 (2010) 244–248. https://doi.org/10.1099/ijs.0.011577-0.

[27] J.D.P. Bezerra, H.M.C. Navarro, J.H. Almeida, C.R. Félix, M.F. Landell, Valentiella maceioensis gen. et sp. nov. (Herpotrichiellaceae, Chaetothyriales), a new black yeast-like fungus isolated from bromeliads in Brazil, Mycological Progress. 21 (2022) 30. https://doi.org/10.1007/s11557-022-01783-3.

[28] H.M. Casanova Navarro, C.R. Félix, G.V.B. Paulino, J.H. Almeida, P. Valente, M.F. Landell, Richness and biotechnological potential of the yeast community associated with the bromeliad phylloplane in the Brazilian Neotropical Forest, Mycological Progress. 19 (2020) 1387–1401. https://doi.org/10.1007/s11557-020-01631-2.

[29] M.F. Landell, L.R. Brandão, S.V.B. Safar, F.C.O. Gomes, C.R. Fèlix, A.R.O. Santos, D.M. Pagani, J.P. Ramos, L. Broetto, T. Mott, M.H. Vainstein, P. Valente, C.A. Rosa, Bullera vrieseae sp. Nov., a tremellaceous yeast species isolated from bromeliads, International Journal of Systematic and Evolutionary Microbiology. 65 (2015) 2466–2471. https://doi.org/10.1099/ijs.0.000285.

[30] D.D.S. Trentin, R.B. Giordani, K.R. Zimmer, A.G. da Silva, M.V. da Silva, M.T.D.S. Correia, I.J.R. Baumvol, A.J. MacEdo, Potential of medicinal plants from the Brazilian semi-arid region (Caatinga) against Staphylococcus epidermidis planktonic and biofilm lifestyles, Journal of Ethnopharmacology. 137 (2011) 327–335. https://doi.org/10.1016/j.jep.2011.05.030.

[31] T. Simone, C. Reginatto, UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE PROGRAMA DE PÓS-GRADUAÇÃO EM MICROBIOLOGIA AGRÍCOLA E DO AMBIENTE DIVERSIDADE DE BACTÉRIAS ASSOCIADAS A BROMÉLIAS DO PARQUE ESTADUAL DE ITAPUÃ/RS, 2008.

[32] S. Tiwari, S. Moghe, W.B. Gurnule, D.S. Bhagat, A. Gunjal, Habitat-specific microbial community associated with the biodiversity hotspot, Microbial Diversity in Hotspots. (2022) 25–43. https://doi.org/10.1016/B978-0-323-90148-2.00018-3.

[33] S. Shinde, P. Munot, Y. Hivarkar, S. Patil, A. Patwardhan, Exploration of microbial ecology and diversity in hotspots, Microbial Diversity in Hotspots. (2022) 1–23. https://doi.org/10.1016/B978-0-323-90148-2.00015-8.

[34] F.C.O. Gomes, S.V.B. Safar, A.R. Marques, A.O. Medeiros, A.R.O. Santos, C. Carvalho, M.A. Lachance, J.P. Sampaio, C.A. Rosa, The diversity and extracellular enzymatic activities of yeasts isolated from water tanks of Vriesea minarum, an endangered bromeliad species in Brazil, and the description of Occultifur brasiliensis f.a., sp. nov, Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology. 107 (2015) 597–611. https://doi.org/10.1007/s10482-014-0356-4.

[35] K.M. Rodriguez-Nuñez, J.M. Rullan-Cardec, C. Rios-Velazquez, The metagenome of bromeliads phytotelma in Puerto Rico, Data in Brief. 16 (2018) 19–22. https://doi.org/10.1016/j.dib.2017.10.065.

MICROBIAL PATHOGENESIS

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[2] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
[3] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
Reference to a chapter in an edited book:
[4] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.
Reference to a website:
[5] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13 March 2003).
Reference to a dataset:
[dataset] [6] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. https://doi.org/10.17632/xwj98nb39r.1.
Reference to software:
[7] E. Coon, M. Berndt, A. Jan, D. Svyatsky, A. Atchley, E. Kikinzon, D. Harp, G. Manzini, E. Shelef, K. Lipnikov, R. Garimella, C. Xu, D. Moulton, S. Karra, S. Painter, E. Jafarov, S. Molins, Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88), Zenodo, March 25, 2020. https://doi.org/10.5281/zenodo.3727209.

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