I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ·· XV SHEB ·· XV ENZITEC FLORIANÓPOLIS ·· 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil

ANTIMICROBIAL SUBSTANCES PRODUCTION BY MICROBIOTA OF PIRANHA (*Serrasalmus rhombeus*) AND TUCUNARÉ (*Cichla mirianae*) POTENTIALS AND CHALLENGES.

Luan S. M. Almeida[1], Sheila da Silva [1,2] João Ricardo V. Amaral[1,2], Andrew Whiteley[3], Andrew Macrae [1,2] and Selma S. de Oliveira [1,2*]

[1] Laboratório de Biotecnologia Sustentável e Bioinformática Microbiana, Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes, UFRJ, Rio de Janeiro Brazil
[2]Programa de Biotecnologia Vegetal e Bioprocessos, UFRJ, Rio de Janeiro , Brazil
[2] Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia

** Corresponding author's email address:selma@micro.ufrj.br*

ABSTRACT

There is a consensus among health organizations that antimicrobial resistance is one of the greatest global threats to public health. From the indiscriminate use to the inadequate disposal of antibiotics, several factors contribute to the intensification of this problem. In face of this challenge, there are alternatives, such as bioprospecting for antimicrobial substances. Bacteria have the ability to produce antimicrobial compounds, such as bacteriocins, which are antimicrobial peptides produced at the ribosomal level. This project aims to bioprospect *Serrasalmus rhombeus* ("Piranha-preta") and *Cichla mirianae* ("Tucunaré-Fogo") fish samples for bacteria that produce antimicrobial substances, which are capable of combating antibiotic-resistant hospital pathogens. For this, five fish individuals of each species were collected in the São Benedito River, Pará. The samples were collected using swabs in three different areas of the fish: mouth (A), gills (B) and anus (C). For isolation, the samples were inoculated in 4 mL of nutrient broth under stirring for 48 h at 28ºC and then the streak plate technique was performed to obtain isolated colonies. The bacteria were preliminarily identified by the matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry technique. Antimicrobial activity was detected by agar well diffusion assay methodology. For this, 50 µL of the pathogenic bacteria culture was inoculated in 4 mL of semi-solid medium in a liquid state stabilized at 45°C, then poured onto a plate containing nutrient agar medium, forming a mat. Then, 6 mm wells were made in the agar and 50 µL of the isolated bacteria cultures were added in order to verify the production of antimicrobial substances. The plates were incubated at 28ºC for 24 hours and after this period, the presence or absence of the inhibition halo around the wells with the tested bacteria was verified. To date, bacterial isolation from samples of individuals of the species *S. rhombeus* has been completed and the bacterial isolation of individuals of the species *C. mirianae* is still in progress.As a result, of the 71 bacteria isolated from samples of the fish *S. rhombeu*s, 48 were identified by Maldi-tof, belonging to 14 different species. For the bacteria isolated from this fish, it was possible to verify antagonistic activity in 45 strains. For the *C. mirianae* fish samples, bacteria from regions A and B have been isolated to date, from which it was possible to isolate 40 bacteria, among which 22 were identified using Maldi-tof and 7 strains were antagonistic against Klebsiella pneumoniae and Enterococcus faecalis."

Keywords: bacteriocins, antagonism, fish, river

1 INTRODUCTION

Infections caused by multidrug-resistant bacteria are one of the biggest threats to global public health. Bioprospecting for novel antimicrobial substances with the potential to control these multidrug-resistant pathogens appears as a possibility to circumvent the problem. Microorganisms produce a variety of different compounds and enzymes that can be used as biotechnological products. Bacteria, for instance, have the ability to synthesize antimicrobial compounds, such as bacteriocins, which are peptides produced at the ribosomal level that emerge as an alternative to existing antibiotics.

2 MATERIAL & METHODS

The coletes were performed In the São Benedito river, in Pará state, Brazil. Five individuals of each species of fish (*S. rhombeus* and *C.mirianae*) were used to collect swab samples (mouth, scales and mouth). The swabs were maintained in microtubes containing 1000 uL of Alkaline Peptone Water medium and and 20% glycerol, and were kept at a temperature of -80ºC (Silva, 2023).

For bacterial isolation, 100 uL of each microtube sample were inoculated in 4mL of Nutrient Broth and then kept under agitation for 48h at 28ºC. Subsequently, depletion was carried out on nutrient agar plates in order to obtain isolated colonies. The Gram staining technique was used to observe the morphology and arrangement of the cells, in addition to allowing visualization of the purity of the isolation and comparison of images with the literature. Preliminary identification was performed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry.

Antimicrobial activity was observed using the agar well diffusion assay methodology. All isolated strains were tested for inhibition activity. For the tests using *S. rhombeus (piranha)* strains, as potential producers, 12 pathogenic bacteria were used: *Acinetobacter baumannii, Escherichia coli E*SBL, *Enterococcus cloacae* NDM, *Klebsiella pneumoniae* KPC, *K. pneumoniae* ESBL, *Pseudomonas aeruginosa* SPM, *P. aeruginosa, Salmonella* Typhimurium ATCC 13311, *Staphylococcus aureus* ATCC 6538, *P. aeruginosa* ATCC 15422, *Enterococcus faecalis* 5ae, *Staphylococcus epidermidis.* The strains isolated from *C. mirianae* (Tucunaré) were tested against *K. pneumoniae* 19 ae, *E. faecalis* ATCC29212, *P. aeruginosa* ATCC15442 and *S.* Typhimurium ATCC8394.

3 RESULTS & DISCUSSION

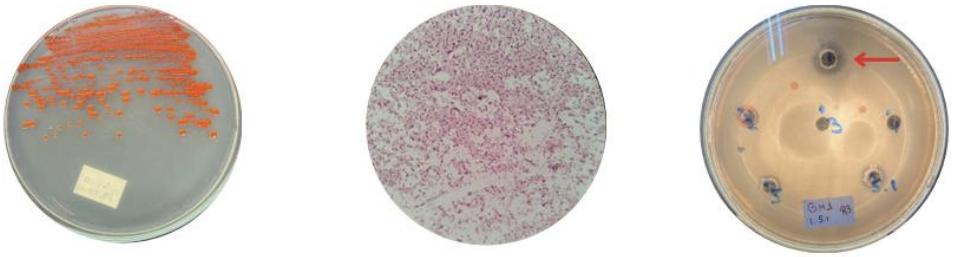
Table 1 shows the main preliminary results obtained thus far The assays performed with isolates from *S. rhombeus* and from *C. mirianae* presented antimicrobial activity against at least one pathogenic indicator strain.

Table 1 Main preliminary results

SPECIES	ISOLATED	IDENTIFIED	ANTIMICROBIAL ACTIVITY
Serrasalmus rhombeus	71 Regions: A, B and C.	49	45
Cichla mirianae	40 Regions: A and B	22	7

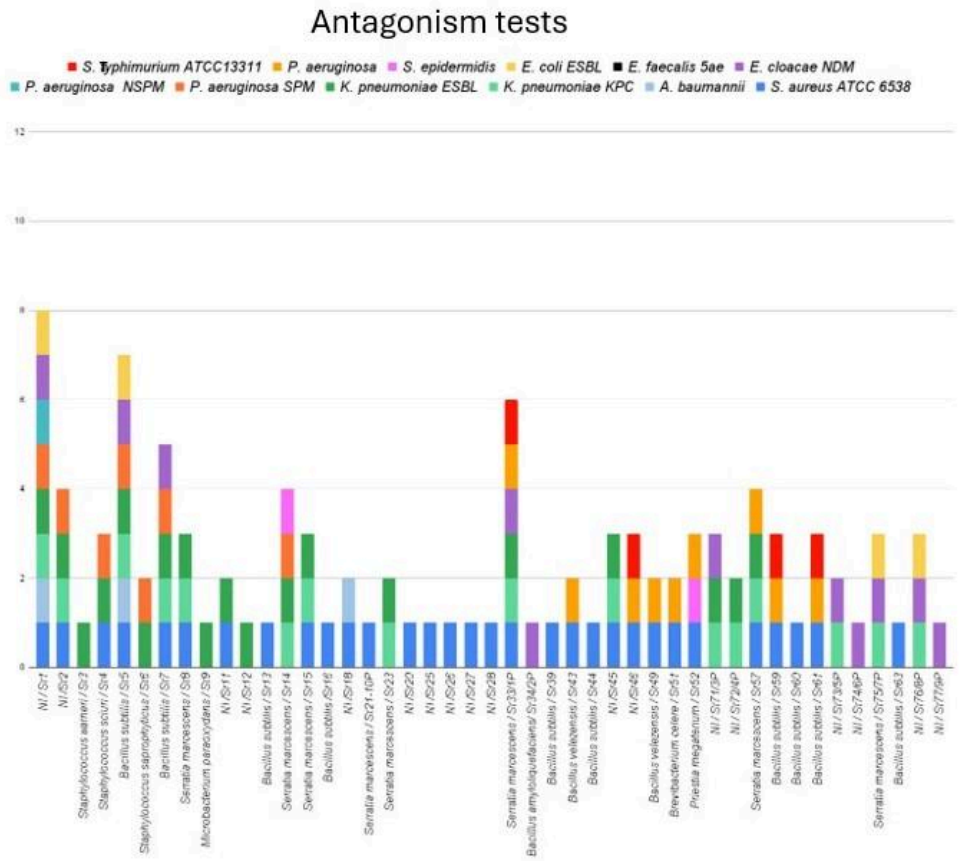
Figure 1 shows one of the strains, isolated from *S. rhombeus*, that presented a good antagonistic activity. *S. aureus* is an important pathogen ,either in clinic and veterinary medicine and its resistance to many antibiotics of routine use, has been a subject of concern[5]. Figure 2 shows the spectrum of activity of strains isolated from *S. rhombeus* and its different origins : A (mouth), B (scales) and C (anus) against 12 indicator strains. From 45 strains that presented antagonistic activity, 27 inhibited at least 2 indicator strains.

Figure 1: Bacterial strain Sr21, isolated from *Serrasalmus rhombeus*, identified as *Serratia marcenses.:* General aspect of bacterial growth on Nutrient Agar (A), Microscopy of Gram staining (B) and its antagonistic activity against *Staphylococcus aureus* GM1 (C).



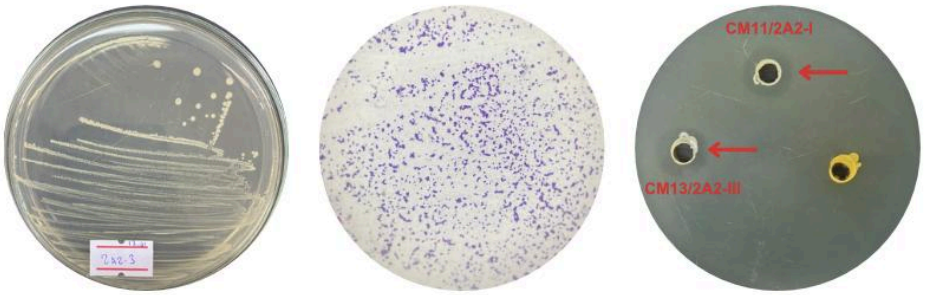
A B C

Figure 2: Antimicrobial activity spectrum of strains isolated from *Serrasalmus rhombeus. The presence of halos in the triplicate tests were represented in the bar graph presenting positive results for antimicrobial activity in antagonism tests. The X axis shows the indicators tested, while the Y axis shows the bacteria that produce antimicrobial substances*



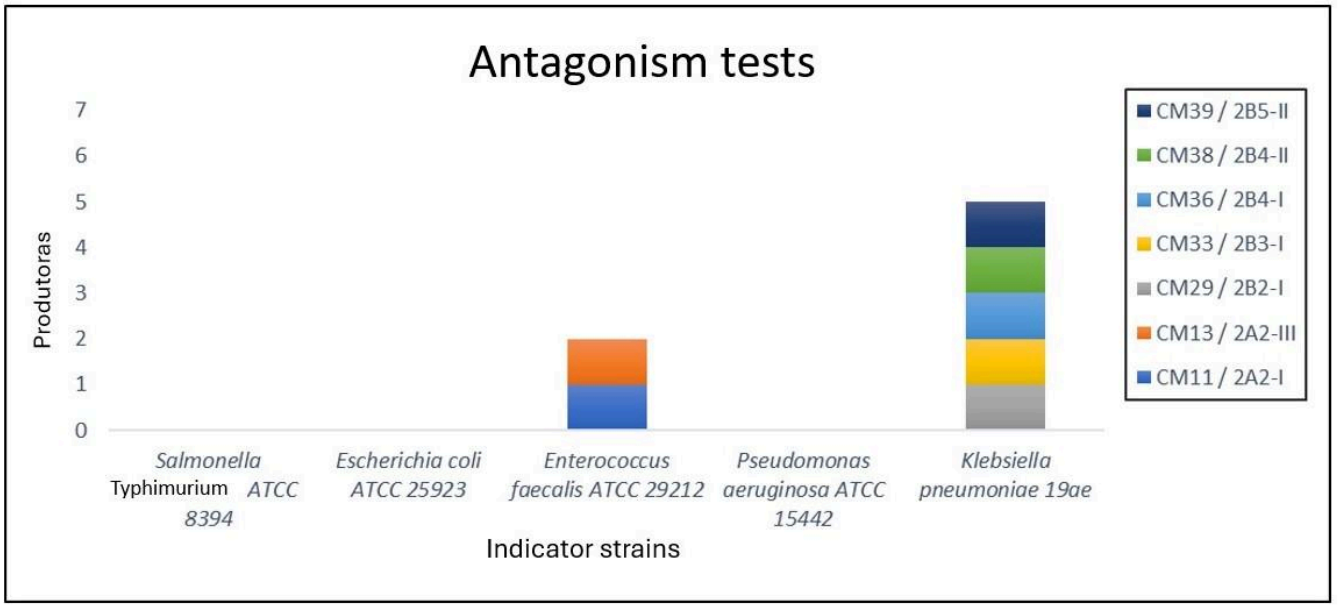
From a total of 40 *C. mirianae* bacterial isolates, 7 strains presented activity against at least one of indicator strains tested, namely *K. pneumoniae* 19ae and *E. faecalis* ATCC 29212. Figure 3 illustrates one of those potential producer strains**.** For the C. mirianae fish samples, bacteria from regions A and B have been isolated this far, from which it was possible to isolate 40 bacteria, among which 22 were identified using Maldi-tof and 7 strains were antagonistic against *Klebsiella pneumoniae* and *Enterococcus faecalis*."(Table1 , Figures 3 and 4).

Figure 3: *Staphylococcus haemolyticus strains ,* isolated from *Cichla mirianae* fish*:* General aspect of bacterial growth on Nutrient Agar (A), Microscopy of Gram staining (B) and its respective antagonistic activity against *E. faecalis* strain (C).



A B C

Figure 4:Antimicrobial activity spectrum of strains isolated from *Cichla mirianae. The presence of halos in the triplicate tests were represented in the bar graph presenting positive results for antimicrobial activity in antagonism tests. The X axis shows the indicators tested, while the Y axis shows the bacteria that produce antimicrobial substances.*



Isolates from *S. rhombeus or C. mirianae* were found that show antagonistic activity against strains of concern that show antimicrobial resistance and are clinical infections . However, data obtained thus far suggests that *S. rhombeus'* bacterial isolates offer a greater diversity of antimicrobial substances.

Antimicrobial bacterial producers isolated from fish have been described and their potential use either as probiotics or as purified substances have been evaluated elsewhere [3] The investigation of new substances in environments where a low human impact is expected to provide new types of antimicrobial substances and thus try to circumvent the resistance matters .

CONCLUSION

The results presented in this work not only contribute to the knowledge of the local microbial biodiversity, but also indicate a promising path in the research and characterization of new antimicrobial substances. The search for bacteria that produce antimicrobial substances in aquatic environments is a potential alternative to combat infections caused by pathogenic bacterial strains. Further studies are essential to explore the full biotechnological potential of these discoveries.

REFERENCES

[1] SILVA, A. F. C., FERREIRA, B. CASTRO, C. T. 2023. Lat. Am. J. Biochem. Process. 27 (1). 429-440.

[2] GREEN, T. PARKER, R. 1999. Interpreting landscapes. *In:* Evolution of Bioinformatics. DAWSON, L. (ed). 2nd ed. Pearson, Los Angeles. 180-205.

[3] Feliatra F, Muchlisin ZA, Teruna HY, Utamy WR, Nursyirwani N, Dahliaty A. Potential of bacteriocins produced by probiotic bacteria isolated from tiger shrimp and prawns as antibacterial to *Vibrio*, *Pseudomonas*, and *Aeromonas* species on fish. F1000Res. 2018 Mar 29;7:415.

[4] Iseppi R, Stefani S, de Niederhausern S, Bondi M, Sabia C, Messi P. Characterization of Anti-*Listeria monocytogenes* Properties of two Bacteriocin-Producing *Enterococcus mundtii* Isolated from Fresh Fish and Seafood. Curr Microbiol. 2019 Sep;76(9):1010-1019.

[5] Vestergaard M, Frees D, Ingmer H. Antibiotic Resistance and the MRSA Problem. Microbiol Spectr. 2019 Mar;7:2.

ACKNOWLEDGEMENTS

CNPq PIBIC UFRJ UFRJ Dimensions Sciences CSIRO THE UNIVERSITY OF WESTERN AUSTRALIA