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EVALUATION OF THE ANTIMICROBIAL POTENTIAL OF ACTINOBACTERIA STRAINS ISOLATED FROM MANGROVES IN THE MUNICIPALITY OF BRAGANÇA, PARÁ, BRAZIL

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ABSTRACT

The actinobacteria are filamentous Gram-positive bacteria with a high content of guanine and cytosine in their genomes. They are highlighted in research on enzyme production and secondary metabolites of scientific and industrial interest in the pharmaceutical sector to produce new drugs. They are free-living organisms, denominated cosmopolitan, because they are widely distributed in terrestrial and aquatic ecosystems, due to their high adaptability, and can even be found in internal plant tissues (endophytic). The growing bacterial multi-resistance and the reduction in the effectiveness of current antibiotics do the search for new antimicrobial sources necessary. The aim of this work was to isolate and evaluate the antimicrobial potential of strains isolated from mangrove soils, leaf and root in the municipality of Bragança-PA. As results, from the 15 isolates, 13 strains, showed a good performance against the tested pathogenic bacteria - *Enterococcus faecalis* and *Staphylococcus aureus.*

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INTRODUCTION

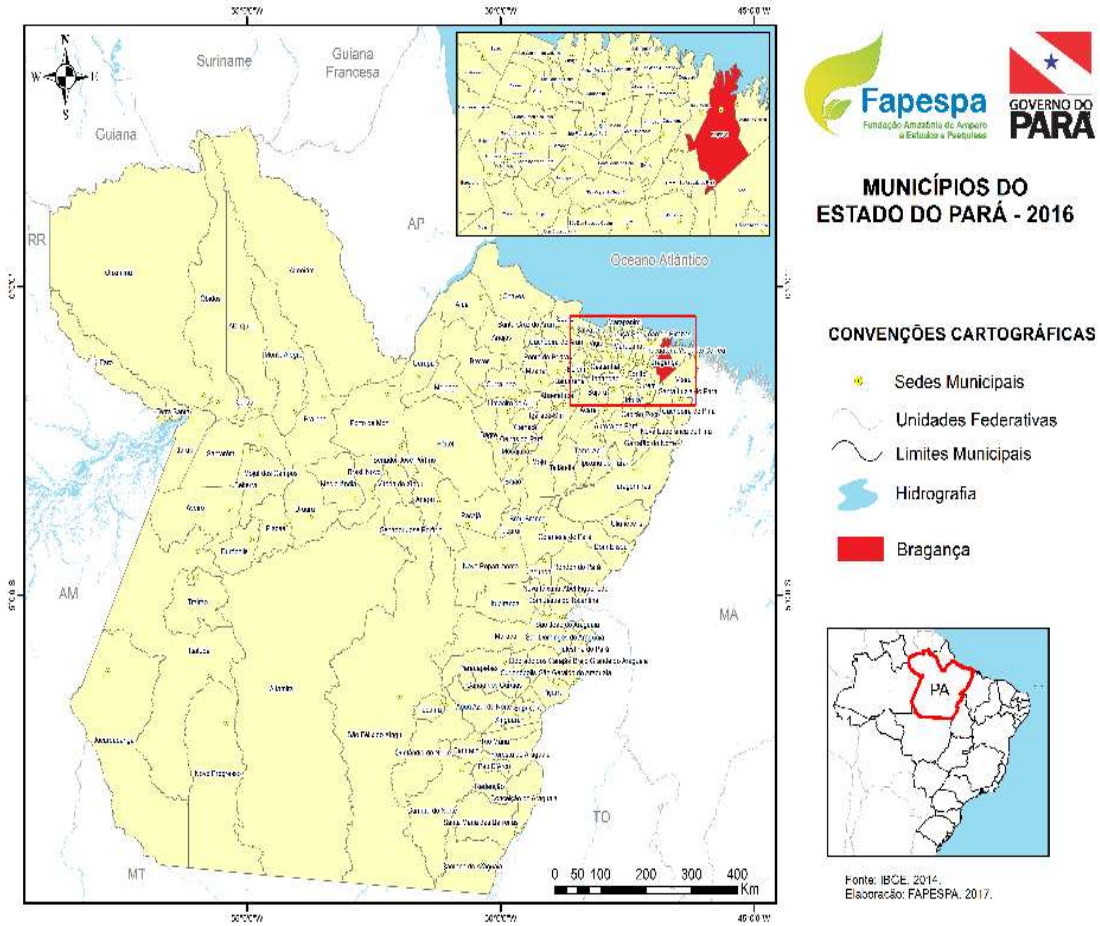
Bacterial resistance is one of the most serious global publichealth problems faced by humanity since the discovery of the first antibiotic, to the most modern antibacterial drug, employed for the treatment of bacterial infections. This phenomenon has been seen, and one of the greatest advances in medicine showed fragility when the high adaptability of these microorganisms when exposed to new environmental conditions was showed (MORAES, ARAÚJO, BRAGA, 2016; OLIVEIRA, 2018). Antibiotics are substances used in the treatment of bacterial infections, and that among their various sources have in the secondary metabolism of bacteria and fungi one of the main ones of the main sources of antimicrobial substance. The bacteria of clinical importance are characterized by being sensitive to these substances, but in some cases, they are indifferent to the presence of these substances, which are called resistant (LIMA, TREVISAN, 2021).

The occurrence of bacteria resistant to multiple drugs is showed particularly in the hospital environment, this situation is determinant in the increase of costs inherent to health care services, as well as in morbidity and mortality rates caused by infections (DIAS, MONTEIRO, MENEZES, 2010; DA SILVA and OMERO-JÚNIOR, 2022). The abusive use of antimicrobials in human, veterinary medicine, and agriculture, used without prescription or by inappropriate prescription, is listed as the major responsible for the emergence of multidrug-resistant strains. Several studies show that these bacteria with characteristics of multiple resistance, without control strategies, will lead to the deathof about ten million people of the world population by 2050. (BRAOIOS *et al*., 2013; BASTOS, 2019). The lack of new antibiotics is a threat to worldwide efforts to hold these infections, and the need to produce new drugs is urgent (DOS SANTOS and COMARELLA, 2021). To circumvent this problem, the World Health Organization (WHO) listed the twelve families of bacteria with a high degree of threat to human health to stimulate the production of new drugs effective to these new strains resistant (WHO, 2020).

The phylum Actinobacteria is one of the largest taxonomic units currently recognized within the domain of Bacteria, they have a wide morphological variety and can present themselves in the format of cocci or bacilli, besides forming structures called hyphae or pseudohyphae, thus, these microorganisms condense forming arrangements characterized as mycelia or pseudo-mycelium the colonies of these bacteria, in vitro, have varied pigmentation and are typical structures in its various aspects. (BARKA *et al*., 2016; LEWIN *et al*., 2016; GOMES, 2018). Actinobacteria, which are filamentous Gram-positive bacteria with high guanine and cytosine content in their genomes, are highlighted in research on enzyme production and secondary metabolites of scientific and industrial interest in the pharmaceutical sector to produce new drugs (BARKA *et al*., 2016; MARTINS *et al*., 2022). These microorganisms manage the production of more than 50% of the antibiotics known today, being remarkable natural sources of antimicrobials (OLIVEIRA, 2020). The genus *Streptomyces*, besides being more prevalent, is the one that stands out as a source of natural products with high impact, responsible for more than two-thirds of all antibiotics of natural origin marketed today. But other genera have also been discovered and shown to be important sources of bioactive compounds with biotechnological potential today (GOMES-JÚNIOR *et al*., 2022).

compounds of biotechnological interest (SOUZA, 2016). Mangroves are areas found in tropical and subtropical climates and coastal zones, with a significant role as shelter and habitat spaces for part of animals, in water filtration, fertilization of the coasts, and protection against coastal erosion. They manage the enrichment of estuaries and coastal waters, being the natural nursery for many species of living beings. (SILVA, SILVA, ARAUJO, 2020). In Brazil, mangrove areas occur along an extensive coastline, from the mouth of the Oiapoque River in the north to Laguna in the state of Santa Catarina in the south. Their Brazilian distribution represents 7% of the total global mangrove land cover. Considering only the northern Brazilian coastline, the country has the largest continuous formation of this type of ecosystem in the world. Despite the clear distribution, only four federative units concentrate about 85% of the national mangrove area, these being: Maranhão (46%), Pará (22%), Amapá (9%), and Bahia (7%) (GOMES-JÚNIOR *et al*., 2022). The fauna and flora of the mangrove ecosystem have specific characteristics, some of which are very typical and determinant for the survival and selection of many, organisms, such as the low concentration of available oxygen and high salinity, makes these microorganisms develop physiological processes and particular metabolites.

Figure 1. Location of the municipality of Bragança, Pará, Brazil



Source: IBGE, 2014.

Actinobacteria are considered free-living bacteria, so-called cosmopolitan because they are widely distributed in terrestrial and aquatic ecosystems, and due to their high adaptability, they are found in extreme environments under varied temperatures and osmotic pressure conditions. One of the most significant findings about these bacteria was their isolation in internal plant tissues, which shows a probable symbiosis between these beings (WOLFF, 2013; OLIVEIRA, 2020). The microorganisms that adapt to the tissues of vegetation and that do not cause damage to the host are called endophytes. The genetic diversity of the microbiota living in plants, with the ability to promote compounds of diverse structures and different bioactive, presentsa high potential to produce new

The mangrove has biota of several organisms such as animals, fungi, and bacteria, among these, the actinobacteria that despite its great diversity and importance, within this ecosystem is still not known, thus it highlights the extreme need for research on the activity of microorganisms present there (COLARES, 2014; OTTONI *et al*., 2021).

MATERIALS AND METHODS

Mangrove location: This study uses mangrove samples from the municipality of Bragança (Figure 1). Belonging to the state of Pará, this Brazilian municipality is found (01º 03' 57"'S and 46º 47' 22"W)

19 meters above sea level, with a territorial area of 2,124.734 Km^2 (IBGE). The samples were collected on 04/09/2022 at 11:12 am in Caeté Bay. The climate in the region is equatorial, hot, and humid, with a rainy season between December and May, and the tides are semi-diurnal and asymmetric. It presents a vegetation characterized by a homogeneous physiognomy with low diversity of species, but notable specializations in morphology and physiology, which include herbaceous, bushy, and woody plants (OTTONI *et al*., 2021, RODRIGUES *et al*., 2021).

Sample Collection: The collection of samples was performed in 5 different random points that were georeferenced with the help of the Google Earth tool. Soil samples were collected from a depth of approximately 5 cm because actinobacteria are mostly aerobic microorganisms that can be found up to 2 meters below the ground, the points had a distance of at least 3 meters from each other (Barka *et al*., 2016). Root and leaf samples of the plant *Laguncularia racemose* (L.) C.F. Gaertn. typical of these sites, found around the collection points were also collected for the research of endophytic bacteria. The total number of samples was 5 soil samples and 2 root and leaf samples. The samples were collected with the help of PVC spatulas and sterile plastic bags, which were identified with the number of the collection point, date, latitude, and local longitude. The samples were stored and transported in an isothermal box to the Microbiology and Genetics of Microorganisms Laboratory of the Pará State University (LABMICRO CCBS/UEPA).

***Sample Processing and Bacterial Isolation*:** The leaf and root samples were submitted to previous processing of washing in running water to remove excess soil, after this cleaning, they were immersed in ethanol 70% for prior disinfection for 15 minutes, washed with a 5% aqueous sodium hypochlorite solution for 15 minutes, and washed in self-classified distilled water for 15 minutes to remove toxic residues of sodium hypochlorite. A second disinfection process was performed in 70% ethanol for 10 minutes, and the samples were again washed in self-classified distilled water to remove alcohol residues (CUNHA, 2015; SOUZA, 2016), with adaptations. After this treatment, 10g of leaves and roots were placed in a crucible and macerated with a pistil aseptically in a biological safety cabinet, and then transferred to a sterile beaker having 90 ml of self-classified distilled water, the sample was slightly agitated in an orbital shaker for homogenization for about 5-10 minutes. After natural sedimentation, the supernatant was used to perform seeding in selected culture media in serial dilutions. For seeding, sterilized 0.001 ml nickel-chromium loops were used, employing the streak depletion technique. The culture media R2A agar, Columbia agar, and Soy Tryptone Agar (TSA), all enriched with 0.5% Nystatin to avoid fungal contamination, were the media used for the primary isolation of bacteria. The choice of media was based mainly on their nutritional composition, taking into account the quantity and quality of nutrients available, thus the bacteria, when growing, presented various morphological characteristics, allowing the selection of strains of interest, and this use of various media is established in the literature (PÉREZ-CORRAL *et al*, 2022; MARQUES *et al,* 2022).

***Antimicrobial sensitivity testing*:** The evaluation of the antibacterial potential of the isolated actinobacteria was performed in two ways: direct sensitivity test and indirect sensitivity test. The bacteria isolated will confront pathogenic bacterial strains resistant to some antibacterial commonly found in clinical practice and that are part of the bacterioteca of LABMICRO CCBS - UEPA, they are:

- *Enterococcus faecalis* - ATCC 29212 is a quality control strain widely used in vancomycin-sensitive clinical and laboratory experiments (KIM *et al*., 2012).
- *Escherichia coli* ATCC - 25922 according to the package insert is a full genome sequenced quality control strain that does not produce verotoxin. This organism is a CLSI control strain for antimicrobial susceptibility testing.
- *Pseudomonas aeruginosa* - ATCC 27853 is a bacterial strain embedded in biofilm, a protective mechanism that allows microorganisms to live adhered to surfaces. Bacteria enveloped by biofilms show more resistance to antimicrobials (BROWN, ALDRICH, GAUTHIER, 1995; CAO *et al*., 2017).
- *Staphylococcus aureus* - ATCC 25923 is a bacterial strain used as a standard laboratory test control strain. It is sensitive to a variety of antibiotics, including methicillin (TREANGEN *et al*., 2014).
- *Klebsiella sp.* Producing carbapenemases (KPC) are enzymes that confer resistance to carbapenems. The bacterial strain was isolated from urine culture and belonged to the LABMICRO bacterial collection.

***Direct sensitivity testing*:** The direct sensitivity test was performed based on the work of Martins *et al*. (2022) with adaptations. The multidrug-resistant bacteria were used to prepare bacterial suspensions with concentrations equivalent to the first tube on the MacFarland scale (10^5 cfu/mL) and seeded on a 140-millimeter (mm) Petri dish having Mueller Hinton Agar (MHA) culture medium with the aid of disposable sterile swabs (MACFARLAND, 1907). After resting for 30 minutes in the MHA medium of the plates seeded with the bacterial suspensions, wells of approximately 4 mm in diameter were made with a spacing of 3 to 4 cm from one to the other. Subsequently, these wells were filled with culture media fragments of equal size and shape, taken from plates having actinobacteria growth previously characterized through macro and micromorphological analysis. The plates with the implants and seeding were incubated at ± 30°C in a humidity chamber for a period of at least 24 to 72 hours, growth inhibition will be evaluated by the formation of inhibition halos around the implants.

***Indirect sensitivity testing*:** The indirect sensitivity test followed the disc-diffusion method proposed by Bauer *et al* (1966), with adaptations. The actinobacteria selected for this study were seeded in Soy Tryptone Agar (TSA), a non-selective culture medium rich in tryptone and peptone, a source of carbohydrates, proteins, and lipids for bacterial growth. Dilutions were prepared with actinobacteria at a concentration equivalent to the second tube of the McFarland scale (10^8 cfu/mL) and seeded on a mat in the culture medium. Filter paper disks were fixed on the plate so that the growth of the actinobacteria could occur on them, which were previously autoclaved to avoid bacterial contamination since it is not a selective culture medium for bacterial growth. After seeding, the plates were placed in an incubator for 48 hours at ± 30 °C in a humid chamber for the growth of the actinobacteria on the disks. After satisfactory growth of the actinobacteria on the disks, 140-mm Petri plates were prepared to havean MHA culture medium seeded with bacteria of clinical importance. After this step, the film paper disks impregnated with actinobacteria were placed on the plates seeded with the selected bacteria. The plates with the filter paper discs and seeding were incubated at ± 30°C in a humid chamber for a period of at least 24 to 72 hours, growth inhibition will be evaluated by the formation of inhibition halos around the discs.

RESULTS AND DISCUSSION

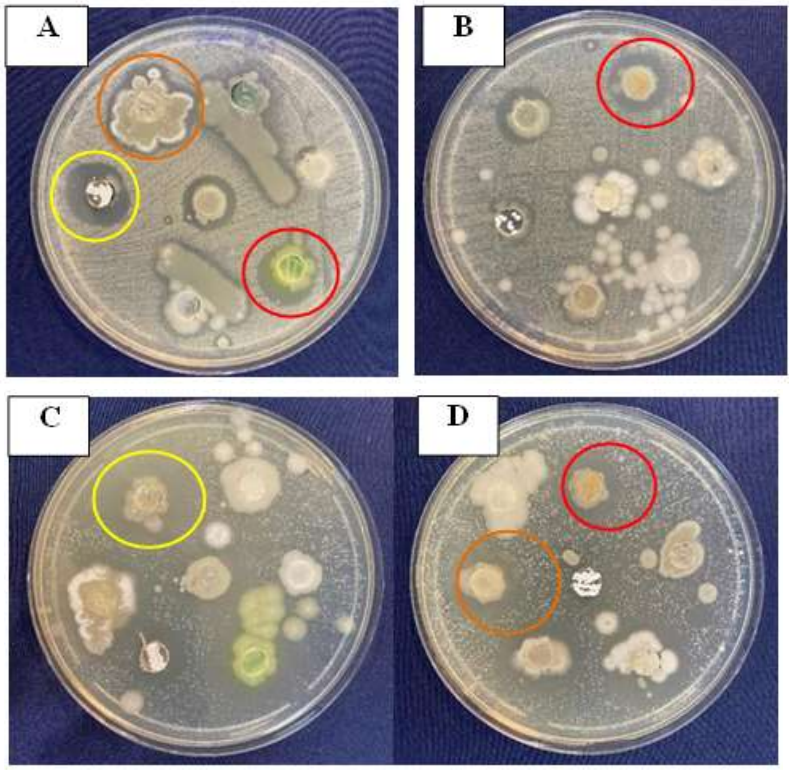
The antimicrobial potential of the 15 isolates found were evaluated, which demonstrated an excellent inhibition capacity against two of the tested pathogenic strains commonly found in clinical practice. It was seen that the actinobacteria produced expressive inhibition halos with sizes varying from 15-30 mm against these pathogenic microorganisms. The dimensions of the halos found are a key factor because it shows the level of sensitivity that the test strains presented to the antibacterial substances produced by the isolates. The complete descriptions of this antimicrobial activity are shown in Table 1. *E. faecalis* and *S. aureus* were the two pathogenic bacteria that showed relevant sensibility to the antimicrobial substances produced by the selected isolates. *E. faecalis* proved to be susceptible to 13 of the isolates (Figure 2/A and B), except for the strains AJ08 (S) and AJ15 (S).

Table 1. Sensitivity test of the isolated actinobacteria against resistant bacteria

Strains	Method	Zone of inhibition (halos)				
		E. faecalis ATCC® 29212	*E. coli* ATCC® 25922	*P. aeruginosa* ATCC® 27853	*S. aureus* ATCC® 25923	*Klebsiella* sp.
AJ01 (S)	Direct	15 mm	-	-	-	-
	Indirect	-	-	-	-	-
AJ02 (S)	Direct	24 mm	-	-	25 mm	-
	Indirect	18 mm	-	-	-	-
AJ03 (S)	Direct	28 mm	-	-	-	-
	Indirect	-	-	-	-	-
AJ04 (E)	Direct	26 mm	-	-	-	-
	Indirect	-	-	-	-	-
AJ05 (E)	Direct	18 mm	-	-	-	-
	Indirect	-	-	-	-	-
AJ06 (S)	Direct	23 mm	-	-	-	-
	Indirect	-	-	-	-	-
AJ07 (E)	Direct	22 mm	-	-	25 mm	-
	Indirect	-	-	-	-	-
AJ08 (S)	Direct	-	-	-	-	-
	Indirect	-	-	-	-	-
AJ09 (E)	Direct	24 mm	-	-	24 mm	-
	Indirect	-	-	-	-	-
AJ10 (S)	Direct	22 mm	-	-	17 mm	-
	Indirect	15 mm	-	-	9 mm	-
AJ11 (E)	Direct	25 mm	-	-	-	-
	Indirect	-	-	-	-	-
AJ12 (E)	Direct	26 mm	-	-	30 mm	-
	Indirect	-	-	-	-	-
AJ13 (S)	Direct	-	-	-	-	-
	Indirect	17 mm	-	-	-	-
AJ14 (S)	Direct	25 mm	-	-	-	-
	Indirect	-	-	-	-	-
AJ15 (S)	Direct	-	-	-	-	-
	Indirect	-	-	-	-	-

LEGEND: (E) Stands for endophytic actinobacteria, (S) Soil actinobacteria, and (-) antimicrobial resistance absent or undetermined.

Figure 2. Direct sensitivity test of *Enterococcus faecalis* ATCC® 29212 and *Staphylococcus aureus* ATCC® 25923

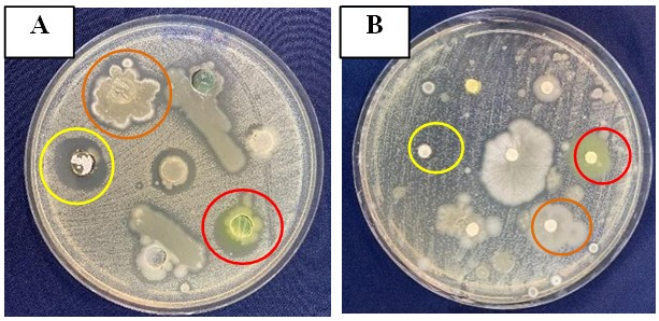


LEGEND: *Enterococcus faecalis* ATCC® 29212: A) red circle: strain AJ11 (E), orange circle: strain AJ03 (S), yellow circle: strain AJ01 (S), and B) red circle: strain AJ02 (S). *Staphylococcus aureus* ATCC® 25923: C) and yellow circle: strain AJ12 (E) and D) red circle: strain AJ02 (S) and orange circle: strain AJ07 (E).
Source: AUTHOR, 2022.

Where as *S. aureus* showed sensitivity to 5 strains of the actinobacteria, these being: AJ02 (S), AJ07 (E), AJ09 (E), AJ10 (S), and AJ12 (E) (Figure 2/C and D). The *E. coli*, *P. aeruginosa* and *Klebsiella sp.* strains evaluated were not sensitive, under the conditions studied, to any of the isolated strains. The low antimicrobial activity found against Gram-negative bacteria (*E. coli*, *P. aeruginosa,*and *Klebsiella* sp.) tested, authors such as Kurnianto, Kusumaningrum, and Lioe (2020) discuss that this finding is related to the complexity of the cell wall of these microorganisms since they have an outer membrane that gives them greater resistance to the action of antimicrobial substances produced by the actinobacteria strains for example in this study, which are unable to pass through the lipid barrier of the pathogenic strains. Martins *et al*. (2022) and Ferreira *et al*. (2016) also when performing the direct sensitivity test, Martins *et al*. (2022) with samples from the mangrove forest soil of São Caetano de Odivelas and Ferreira (2016) from the soil of the Caatinga biome found strains with antimicrobial potential for *S. aureus*, with inhibition halos that presented sizes of 20 mm and 29 mm, respectively. In this study, the largest halo of inhibition found with the Bragança mangrove soil samples against this same bacterial strain was 25 mm. The endophytic actinobacteria also showed an excellent biotechnological potential against this pathogenic strain that is on the list of 12 families of super-bacteria of high lethality or "priority pathogens" of the World Health Organization, where the largest zone of inhibition was 30 mm. Assad *et al* (2021) with samples from the Pantanal also isolated 3 endophytic strains that produced extracts with antimicrobial activity for *S. aureus* evaluated through the MIC - Minimum Inhibitory Concentration and MBC - Minimum Bactericidal Concentration method.

The *E. faecalis* tested against the mangrove soil samples proved to be very susceptible to the isolated actinobacteria, where the largest halo of inhibition was 28 mm. Gomes-Júnior *et al* (2022) with similar mangrove soil samples from São João de Pirabas did not find actinobacteria that could inhibit the growth of this pathogenic strain and Liberal (2018) with soil samples from Santarém found actinobacterial colonies with low antimicrobial activity with inhibition halos as small as 10 mm. In the work of Cunha (2015) who sought to evaluate the antimicrobial activity of endophytic microorganisms isolated *Ipomoea pes-caprae* (L.) R. Br in the city of Recife found good antimicrobial activity for *S. aureus* but found no antimicrobial activity for *E. faecalis* strain. But, in the present study, it was possible to find a good antimicrobial activity for the endophytic bacteria from the mangrove forest against *E. faecalis*, where the inhibition halos ranged from 18 and 26 mm in diameter. Drugs used in clinical practice such as vancomycin, cefazolin, tetracycline, and norfloxacin are drugs in that *S. aureus* and *E. faecalis* present sensitivity to inhibition halos higher than 16-18 mm in laboratory routine. In this context, it is noticeable that the actinobacteria isolated in this study are promising sources of new antimicrobial drugs, since halos higher than 17-30 mm were found like those of antibiotics that are currently offered in the market (NCCLS, 2005).

Figure 3. Direct (A) and indirect (B) sensitivity testing of Enterococcus faecalis



LEGEND: red circle: strain AJ11 (E), orange circle: strain AJ03 (S), and yellow circle: strain AJ01 (S).
Source: AUTHOR, 2022.

The results obtained by the studies cited above demonstrate that the bacteria isolated from the soil and vegetation of the mangrove swamp in the municipality of Bragança present the same potential for antimicrobial activity of the actinobacteria that were isolated in other regions, and by comparing with the results from other mangroves shows that this ecosystem harbors actinobacteria with important and diversified physiological properties, which can be sources of potential products with antimicrobial action. These findings reflect the importance of conducting further research in the mangrove ecosystem. Similar results were found in Gomes-Júnior *et al*. (2022) and Martins *et al*. (2022), where the direct sensitivity test proved to be more efficient, the strains AJ01 (S), AJ03 (S) and AJ11 (E) presented inhibition halos of 15-28 mm in the direct sensitivity test, but when observed in the indirect sensitivity test it was not possible to observe their real antimicrobial potential, these differences can be seen in Figure 3. This finding of the indirect sensitivity test being less efficient may be related to the growth and age of the colony, in which studies have shown that these variables are correlated with the increased production of antimicrobial substances. When the colony is well-developed it can figure out a greater production of antibacterials, with the development of spores, the actinobacteria produce compounds with antagonistic action against pathogenic microorganisms (SINGH; SHARMA; TALUKDAR, 2014; BARKA *et al.,* 2016; DHOLAKIYA *et al*., 2017; KURNIANTO; KUSUMANINGRUM; LIOE, 2020). Moreover, in controlled or natural environments in which actinobacteria are found, these microorganisms take advantage of their secondary metabolism for the production of diversified substances that sustain their survival, this characteristic can be observed in this work in the direct sensitivity test in which strain AJ11-E changed its pigmentation from white to yellow (Figure 4), suggestive that the strain was stimulated to express its secondary metabolism in search of survival (MEDEIROS *et al*., 2018; SHARMA; DANGI; CHOUDHARY, 2014).

Figure 4. Actinobacteria producing yellow carotenoid pigment post sensitivity test

LEGEND: A) Strain AJ11-E pre-sensitivity test with white staining and B) and C) Strain AJ11-E post-sensitivity test with yellow staining.
Source: AUTHOR, 2022

CONCLUSION

Bragança mangrove, located off the coast of Pará State, Brazil, is a rich and prospective source for actinobacteria owning antimicrobial potential against pathogenic bacteria of sanitary contingency there is a lack of characterization works on mangrove-derived actinobacteria in the federative unit pointed, and the present study is a pioneer on isolating actinobacterial owning antimicrobial potential in the region. We hope to supply a piece of baseline information for further local works on mangrove-derived actinobacteria. The isolates from the current study need further characterization towards drugdevelopment, enabling the writing of new chapters in the urgent combat against superbugs.

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REFERENCES

ASSAD *et al*. Endophytic actinobacteria of hymenachne amplexicaulis from the brazilian pantanal wetland produce compounds with antibacterial and antitumor activities. *Microbiological research,* v. 248, e126768, 2021. doi: https://doi.org/10.1016/j.micres.2021.126768.

BAUER, A. W. *et al*. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology,* v. 45, n. 4, p. 493-496, 1966.

BARKA, Essaid Ait, *et al*. Taxonomy, physiology, and natural products of Actinobacteria. *Microbiology and Molecular Biology Reviews*, 2016, 80.1: 1-43.

BASTOS, Luís Felipe Cunha Sardenberg. 2019. "OPAS/OMS Brasil - Novo relatório pede ação urgente para evitar crise de resistência antimicrobiana | OPAS/OMS". Pan American Health Organization / World Health Organization. Disponível em <https://www.paho.org/bra/index.php?option=com_content&view=article&id=5922:novo-relatorio-pede-acao-urgente-para-evitar-crise-de-resistencia-antimicrobiana&Itemid=812> Acesso em: 11 ago. 2022.

BRAOIOS, Alexandre *et al*. Uso de antimicrobianos pela população da cidade de Jataí (GO), Brasil. *Ciência & saúde coletiva*, v. 18, p. 3055-3060, 2013.

BROWN, Matthew L.; ALDRICH, Henry C.; GAUTHIER, Joseph J. Relationship between glycocalyx and povidone-iodine resistance in Pseudomonas aeruginosa (ATCC 27853) biofilms. *Applied and Environmental Microbiology,* v. 61, n. 1, p. 187-193, 1995.

COLARES, G.B. 2014. Ecologia microbiana em sedimentos de manguezais do estado do Ceará. Tese (Doutorado) - Instituto de Ciências do Mar, Universidade Federal do Ceará, 162 p.

CLINICAL AND LABORATORY STANDARDS INSTITUTE/NCCLS. Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. CLSI/NCCLS document M100-S15 [ISBN 1-56238-556-9]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2005.

CAO, H., Lai, Y., BOUGOUFFA, S. *et al.* Comparative genome and transcriptome analysis reveals distinctive surface characteristics and unique physiological potentials of *Pseudomonas aeruginosa* ATCC 27853. *BMC Genomics* 18, 459 (2017). https://doi.org/10.1186/s12864-017-3842-z.

CUNHA, Ivana Gláucia Barroso da. Potencial Biotecnológico de micro-organismos da rizosfera e endofíticos isolados da planta Ipomoea pes-caprae (L.) R. Br., Convolvulaceae. 2015. 167 f. Tese (Doutorado em Ciências Farmacêuticas) – Universidade Federal de Pernambuco, 2015.

DA SILVA, Aridson Erick Ferreira; JUNIOR, Omero Martins Rodrigues. Resistência bacteriana pelo uso indiscriminado dos carbapenêmicos meropenem e imipenem: uma revisão integrativa. *Research, Society and Development*, v. 11, n. 7, p. e44711730195-e44711730195, 2022.

DHOLAKIYA, R. N. *et al*. Antibacterial and antioxidant activities of novel Actinobacteria strain isolated from Gulf of Khambhat, Gujarat. Frontiers in Microbiology, v. 8, p. 2420, 2017.

DIAS, Margarida; MONTEIRO, Micaela S.; MENEZES, M. F. Antibióticos e resistência bacteriana, velhas questões, novos desafios. Cadernos de Otorrinolaringologia: clínica, investigação e inovação. Lisboa, 2010.

DOS SANTOS GARCIA, Josefa Vancleide Alves; COMARELLA, Larissa. O uso indiscriminado de antibióticos e as resistências bacterianas. Saúde e Desenvolvimento, v. 10, n. 18, p. 78-87, 2021.

Escherichia coli (Mingula) Castellani and Chalmers 25922™, 2022. Disponível em: <https://www.atcc.org/products/25922>. Acesso em: 12 set. 2022.

FERREIRA *et al*. Avaliação *in vitro* do potencial antimicrobiano de *Streptomyces* sp G-27 contra microrganismos de interesse clínico. Rev. Bras. Gest. Amb. Sustent. [online]. 2016, vol. 3, n. 6, p. 367-373. ISSN 2359-1412. DOI: 10.21438/rbgas.030610.

GOMES, Thiara Santana. Prospecção de microrganismos obtidos de sedimentos de manguezais do estado de sergipe com potencial para a produção de biossurfactantes/bioemulsificantes. Dissertação (Mestrado em Engenharia de Processo) - Universidade Tiradentes, 2018.

GOMES-JÚNIOR *et al*. "Evaluation of the antimicrobial potential of actinomycetesisolated from mangrove soils in themunicipality of São João de Pirabas, Pará, Brazil", *International Journal of Development Research,* 12, (08), 58305-58313.

IBGE – Instituto Brasileiro de Geografia e Estatística. Cidades. Disponível em: <https://cidades.ibge.gov.br/brasil/pa/braganca/panorama>. Acesso em: 24 aug. 2022.

KIM EB, KOPIT LM, HARRIS LJ, MARCO ML. Draft genome sequence of the quality control strain Enterococcus faecalis ATCC 29212. J Bacteriol. 2012 Nov;194(21):6006-7. doi: 10.1128/JB.01423-12.

KURNIANTO, M. A.; KUSUMANINGRUM, H. D.; LIOE, H. N. Characterization of Streptomyces isolates associated with estuarine fish Chanos chanos and prolifing on their antibacterial metabolites-crude-extract. *International Journal of Microbiology,* v. 2020, 8851947, 2020.

LEWIN, G. R., CARLOS, C., CHEVRETTE, M. G., HORN, H. A., MCDOLNAD, B. R., STANKEY, R. J., FOX, B. G., & CURRIE, C. R. Evolution and Ecology of Actinobacteria and Their Bioenergy Applications. *Annual review of microbiology,70*, 235–254. 2016.

LIMA, T.M.L. & TREVISAN, M. 2021. Processos fermentativos direcionados a produção de antibióticos: uma alternativa a resistência de bactérias. PubSaúde, 6, 159. DOI: https://dx.doi.org/10.31533/pubsaude6.a159.

LIBERAL, Talita Cunha de Faria. Isolamento, caracterização e atividade antimicrobiana de metabólitos secundários produzidos por Streptomyces sp. de solo amazônico. 2018. 81 f. Dissertação (Dissertação em Biociências) – Programa de Pós-Graduação em Biociências, Universidade Federal do Oeste do Pará, Santarém, 2018. Disponível em: https://repositorio.ufopa.edu.br/jspui/handle/123456789/482 Acesso em: 02 nov. 2022.

MCFARLAND, J. Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *J Am Med Assoc,* 1907; 14:1176-8.

MARTINS, Hadassa Hanna Soares *et al*. Evaluation of the antimicrobial potential of actinobacteria strains isolated from mangrove soils in the municipality of São Caetano de Odivelas – Pará, Brazil. *International Journal of Environment, Agriculture and Biotechnology*, Vol-7, Issue-4; Jul-Aug, 2022.

MARQUES, Jéssyca da Silva. Caracterização cultural, morfológica e enzimática de actinobactérias de áreas cultivadas de Ubajara / Jéssyca da Silva Marques. – 2022. 68 f.: il. color. Trabalho de Conclusão de Curso (graduação) – Universidade Federal do Ceará, Centro de Ciências Agrárias, Curso de Agronomia, Fortaleza, 2022.

MARTINS, Hadassa Hanna Soares *et al*. Bioprospecção e avaliação do potencial antimicrobiano de actinobactérias em solos rizoides de Elaeis guineensis e Euterpe oleracea no Município de Igarapé-Açu no Estado do Pará. *Brazilian Journal of Development,* v. 8, n. 4, p. 30812-30822, 2022.

MEDEIROS *et al*. Diversidade cultural de cepas de actinobactérias do semiárido. enciclopedia biosfera, *[S. l.]*, v. 15, n. 27, 2018. Disponível em: https://conhecer.org.br/ojs/index.php/biosfera/article/view/602. Acesso em: 6 nov. 2022.

MORAES, Amanda L.; ARAÚJO, Nayara G. P.; BRAGA, Tatiana de L. Automedicação: revisando a literatura sobre a resistência bacteriana aos antibióticos. *Revista Eletrônica Estácio Saúde*, v. 5, n. 1, p. 122-132, 2016.

OLIVEIRA, Mariane Pereira de. Caracterização cultural e micromorfológica de actinobactérias do semiárido nordestino em diferentes níveis de cobertura vegetal. 2020. 55 f. Monografia (Graduação em Agronomia) - Universidade Federal do Ceará, Fortaleza, 2020.

OLIVEIRA, Raimundo Carmo; BRANCO-AC, R. I. O. Potencial antimicrobiano de actinomicetos de solos amazônicos. 2018. 61 f.

Dissertação (Mestrado) – Universidade Federal do Acre, Programa de Pós-Graduação, Ciência, Inovação e Tecnologia para a Amazônia - CITA. Rio Branco, 2018.

OTTONI, F. P. *et al*. Brazilian mangroves at risk. Biota Neotropica, v. 21, n. 2, p. e20201172, 2021.

PÉREZ-CORRAL *et al*. Caracterização molecular, morfológica e bioquímica de actinomicetos e sua atividade antagonística contra fungos fitopatogênicos. *Revista Fitotecnia Mexicana*, v. 45, n. 1, pág. 103-103, 2022.

RODRIGUES *et al*. Economic vulnerability: resulting from coastal erosion in Ajuruteua - Bragança/PA. Research, Society and Development, *[S. l.]*, v. 10, n. 16, p. e109101623822, 2021. DOI: 10.33448/rsd-v10i16.23822. Disponível em: https://rsdjournal. org/index.php/rsd/article/view/23822. Acesso em: 24 aug. 2022.

SHARMA M, DANGI P, CHOUDHARY M. Actinomycetes: Source, identification, and their applications. *International Journal of Current Microbiology and Applied Science.* v.3, n.2, p. 801-832, 2014.

SINGH, L. S.; SHARMA, H.; TALUKDAR, N. C. Production of potent antimicrobial agent by actinomycete, Streptomyces sannanensis strain SU118 isolated from phoomdi in Loktak Lake of Manipur, India. *BioMed Central Microbiology*, v. 14, n. 1, p. 278, 2014.

SILVA, A.P., SILVA, J.B., ARAÚJO, E.D.S., 2020. Marisma, Manguezal (Mangue E Apicum): Ecossistemas De Transição Terra Mar Do Brasil. *Revista Brasileira de Geografia Física* 13,727-742. DOI: https://doi.org/10.26848/rbgf.v13.2.p727- 742.

SOUZA, Igor Felipe Andrade Costa de. Bioprospecção de actinobactérias endofíticas de folhas de Moiringa oleifera Lam. em três localidades do Estado de Pernambuco e avaliação dos metabólitos secundários produzidos por esses micro-organismos. 2016. 126 f. Tese (Doutorado em Ciências Biológicas) – Universidade Federal de Pernambuco, Recife, 2016.

TREANGEN, Todd J. *et al*. Complete genome sequence of the quality control strain Staphylococcus aureus subsp. aureus ATCC 25923. Genome announcements, v. 2, n. 6, p. e01110-14, 2014. DOI: https://doi.org/10.1128/genomeA.01110-14.

WOLFF, José Luiz Caldas. Isolamento de Actinomicetos endofíticos visando a triagem de compostos bioativos. 2013. Disponível em: <https://dspace.mackenzie.br/bitstream/handle/10899/14519/2408_2010_0_5.pdf?sequence=1&isAllowed=y>. Acesso em: 29 ago. 2022.

WORLD HEALTH ORGANIZATION (WHO). Lack of new antibiotics threatens global efforts to contain drug-resistant infections. World Health Organization, 2020. Disponível em: <https://www.who.int/news/item/17-01-2020-lack-of-new-antibiotics-threatens-global-efforts-to-contain-drug-resistant-infections>. Acesso em: 14 de dez. de 2021.

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