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Evaluation of the antimicrobial potential of actinobacteria strains isolated from mangrove soils in the municipality of São Caetano de Odivelas – Pará, Brazil

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***Abstract*—*The actinobacteria compose a phylum of Gram-positive bacteria that possess a wide morphological and physiological variety. They are cocci or bacilli shaped and their reproduction is characterized by the formation of spores and septate pseudo hyphae similar to fungi. Physiologically, they have a great capacity to produce secondary metabolites, which give this group of bacteria a high pharmacological and commercial value. The aim of this work was to isolate and evaluate the antimicrobial potential of strains isolated from mangrove soils in the municipality of São Caetano de Odivelas-PA. As results, from the 19 isolates, 11 strains, showed a good performance against the tested pathogenic bacteria - S. aureus, E. coli and K. pneumoniae.*

***Keywords*—Actinobacteria; Antimicrobial activity; Bacterial resistance; Mangrove soils.**

I. INTRODUCTION

The bacterial resistance to antibiotics is one of the major global health problems of the 21st century. Studies indicate that superbacteria could lead to about 10 million people dying each year by 2050 (Bastos, 2019). The World Health Organization (WHO) has published a list with 12 families of bacteria that are considered a high degree of threat to human health. The list is divided according to the degree of urgency of the need for new antibiotics, being named in critical, high and medium priority (Who, 2020).

The purpose of the WHO publication is to stimulate the production of new antibiotics, but the development process involved is expensive, requires time and in-depth studies, and requires funding for research and production (Who, 2020). Actinobacteria present themselves as major targets for research of new bioactive compounds due to their great capacity for synthesis of secondary metabolites, presenting high commercial value. Among the genera, *Streptomyces* are the most studied because they are responsible for the production of most of the available antibiotics such as streptomycin, terramycin, aureomycin and others (Anandan; Dharumadurai; Manogaran, 2016).

The phylum actinobacteria comprises one of the largest taxonomic units among the main lineages recognized in the domain Bacteria. They are formed by Gram-positive bacteria and have a high content of guanine and cytosine (G + C) in their genetic material, which, together with the sequencing of the 16S RNA region, are used in studies of the evolutionary relationships between groups of bacteria. As for their morphology, they can be characterized as cocci or rods, and can present highly differentiated mycelia (Barkaet al., 2016; Venturaet al., 2007).

The mangrove is an ecosystem located in coastal regions and considered a transition zone between the terrestrial and marine environments, developing best in tropical and intertropical zones. This ecosystem has peculiar conditions including the high salinity of the interstitial water, low oxygen concentration of the muddy substrate and daily flooding regime, favoring the development of species adapted to the environment (Brasil, 2018; Saleset al.,2009). In this context, microbial activity plays a key role in contributing to the maintenance of the ecosystem, through the cycling of nutrients due to the processing of organic matter, nutritionally supplying plants and animals living in this environment (Mirandaet al., 2020).

A variety of antibacterial and cytotoxic substances are found in mangroves, which makes this ecosystem an attractive option for the discovery of new bioactive compounds, given that the microorganisms present in this environment have particular physiological processes and metabolites(Yuan; Hong; Lin, 2010;Sangkanuet al., 2017). These characteristics are caused by tidal cycles, humidity, temperature, wave action, UV radiation, nutrients and salinity, making mangrove conditions extreme (Azuma, 2011).

In the last years, research on actinobacteria in environments considered unexplored and extreme, such as mangroves, has increased due to the great diversity and biotechnological activity (Corrêa, 2014). Therefore, the objective of this work is to evaluate the antimicrobial potential of actinobacteria strains isolated from mangrove soils in the municipality of São Caetano de Odivelas - Pará, Brazil.

II. MATERIALS AND METHODS

2.1 Isolation

To isolate the samples, 1g of sample was diluted in test tubes to 10 ml of 0.9% saline solution and submitted to vortex agitation for 5 minutes, after which time they were taken to a centrifuge at 3000 rpm for 5 minutes. The supernatant was seeded with disposable loops by the streak method onto Czapek Dox Agar culture medium.

After primary seeding, the remaining dilution samples were submitted to thermal shock, in which the material was placed in a water bath at 90ºC for 10 minutes and then cooled in the refrigerator at -4ºC for the same period of time and seeding was performed again in order to analyze whether the thermal shock could influence the results found. Petri dishes were incubated in a bacteriological incubator for 24 to 72 hours at a temperature of 35°C ± 2°C and bacterial growth was observed daily.

2.2 Antimicrobial Sensitivity Test

From the isolated actinobacteria, 19 strains were selected to perform two tests to evaluate the antibacterial potential, a direct and an indirect confrontation test, against Gram-positive and Gram-negative bacteria resistant to antibiotics commonly used in clinical practice.

The isolated bacteria were confronted with one strain of *Staphylococcus aureus* (ATCC 25923) and two strains of Gram-negative bacteria from the collection of the Laboratory of Applied Microbiology and Microorganism Genetics of the Pará State University (*Escherichia coli* and *Klebsiella pneumoniae*).

2.2.1 Direct confrontation test

From the multidrug-resistant bacteria, suspensions corresponding to the first tube of the MacFarland scale (105 ufc\mL) were prepared and used to perform mat seeding on a 130mm plate containing Mueller Hinton Agar medium. Subsequently, with the help of an adapted platinum loop, wells of approximately 0.4mm were opened in the culture medium, where fragments of the same size (0.4mm) were implanted from the Czapek Dox Agar culture medium with primary bacterial growth.

The technique was performed with all strains previously selected, which were duly identified and incubated in a bacteriological incubator at 35°C ± 2°C for 24-48h. After the incubation period, the antibacterial potential of the tested actinobacteria was evaluated by measuring, in millimeters, the inhibition halos formed around the colonies.

2.2.2 Indirect confrontation test

A bacterial suspension was prepared with the strains of actinobacteria with a concentration similar to the first MacFarland tube (105 ufc\mL).

The disks impregnated with the actinobacteria strains were positioned on the surface of the Mueller Hinton Agar medium, which was previously seeded by means of the spreading technique with the suspension of multi-resistant bacteria used in the direct confrontation. The bacterial strains were previously identified at the base of the petri dishes. They were then incubated at 35 ± 2°C for 24 to 72 hours. The result was read by measuring, in millimeters, the zone of inhibition formed around the discs.

III. RESULTS AND DISCUSSION

According to Sangkanuet al. (2017), the mangrove ecosystem is a habitat rich in microorganisms with high biotechnological potential. In addition, the review study conducted by Azman et al. (2015), reports that there is still limited knowledge about mangrove actinobacteria, making this a location of interest for the development of new studies on strains producing bioactive compounds.

The bacterial diversity of this habitat was evidenced in the present study, in which 57.9% (n=11) of the actinobacterial strains isolated from the mangrove of the municipality of São Caetano de Odivelas showed antimicrobial action on the pathogenic bacteria tested. Such diversity was also reported in the study by Lee et al. (2014), in which 55.2% (n=48) of the 87 isolates were able to inhibit the growth of one or more of the bacteria used in the research.

In relation to the Gram positive pathogenic bacteria (*S. aureus*, ATCC 25923), the results were also significant (Fig. 1).Growth inhibition was verified both in direct confrontation (Table 1)and in indirect confrontation (Table 2). The largest inhibition halos were observed against *S. aureus* in both tests, with halos measuring between 7 and 20 mm. Observing, thus, a predominance of antimicrobial activity for gram-positive pathogens demonstrated in several studies such as Hozzeinet al. (2011), Costa (2012), Silva (2012), Corrêa (2014) and Oliveira (2018).

Table 1. Zone of inhibition in mm of the isolated strains tested in direct confrontation.

Direct confrontationtest			
Strain	Zone of inhibition (diameter in mm)		
	K. pneumoniae	*E. coli*	*S. aureus*
SCO3	-	-	20 mm
SCO4A	-	13 mm	16 mm
SCO12	-	8 mm	16 mm
SCO17B	-	-	17 mm
SCO32	-	18mm	-
SCO34A	-	9 mm	-
SCO35B	-	18 mm	14 mm
SCO39	-	16 mm	-

Source:Authorial.

The spectrum of antibacterial action of actinobacteria on gram-positive bacteria may have great importance for the pharmaceutical industry. In the research by Kemunget al. (2020) carried out with strains from a Malaysian mangrove, actinobacteria were found to produce anti-MRSA (*methicillin-resistant Staphylococcus aureus*) substances, to produce biofilms and to have antioxidant activity, indicating the diversity of bioactive compounds produced by bacteria from this ecosystem.

Table 2. Zone of inhibition in mm of the isolated strains tested in indirect confrontation.

Indirectconfrontationtest			
Strain	Zone of inhibition (diameter in mm)		
	K. pneumoniae	*E. coli*	*S. aureus*
SCO 3	-	-	16 mm
SCO 4 A	7 mm	12 mm	15 mm
SCO 12	11 mm	9 mm	16 mm
SCO 17 A	-	-	10 mm
SCO 19 A	-	-	7 mm
SCO 34 A	-	8 mm	14 mm
SCO 35 A	-	10 mm	-
SCO 35 B	-	8 mm	8 mm

Source:Authorial.

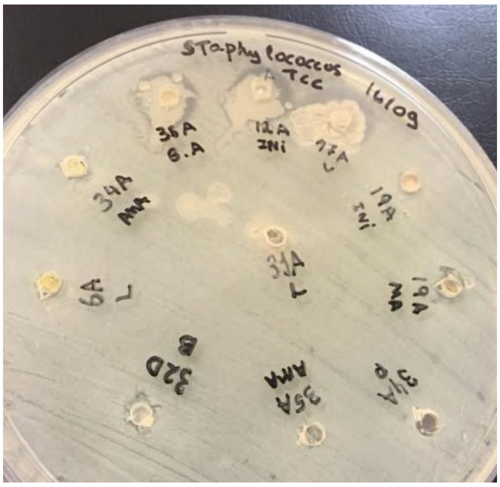

Fig. 1: Direct confrontation against S.aureus

Source: Authorial.

The mangrove isolates showed satisfactory results against *E. coli* (Fig. 2 and 3). This result was similar to that study presented Dhawane and Zodpe (2017), in which there was moderate activity against all strains tested. However, the results were different from those obtained by Oliveira (2018), whose actinobacteria isolates showed no antimicrobial activity against Gram negative bacteria.

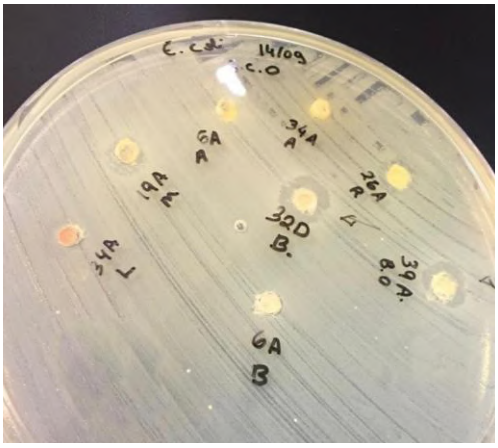

Fig. 2: Direct confrontation test against E.coli

Source: Authorial.

Fig. 3: Indirect confrontation test against E.coli

Source: Authorial.

The reduced activity against Gram-negative bacteria is related to the complexity of the cell wall of these microorganisms, as they have an external membrane that gives them greater resistance to the action of antibiotics, making it impossible to pass the lipid barrier (Silva, 2012; Corrêa, 2014; Nascimento, 2021).

Furthermore, the *K. pneumoniae* strain showed a higher pattern of resistance compared to the other bacteria tested, which can be explained by the presence of a capsule rich in lipopolysaccharides in its structure. This statement can be reaffirmed in the study by Amako, Meno and Takade (1988), in which the authors point out that the capsule may be favorable for protection and resistance against microorganisms in the environment.

The lower spectrum of antibacterial action in direct confrontation compared to indirect confrontation suggests that failures may have occurred during the execution of the technique, such as leveling between the agar block containing the actinobacteria strain and the culture medium, added to that the material used to perform it. Although the technique was adapted from the works of Rincón-enríquez, López-Pérez and Quiñones-aguilar (2014) and Tlemsani et al. (2020), it was noted that there is still a necessity of improvement to obtain satisfactory results in the same way that these authors obtained.

The potential for inhibition of Gram positive bacteria *S. aureus* and Gram negative bacteria *E.coli* corroborates the studies performed by Costa (2012) and Corrêa (2014) with the rhizosphere of Caatinga. Among the microorganisms tested, *K. pneumoniae* was resistant to the metabolites produced by almost all isolates, being sensitive to only two of the isolated strains, in contrast to the results of Costa (2012), in which *K. pneumoniae* was resistant to the metabolites produced by all isolates, which contradicts the study conducted in the Bhitarkanika mangrove in India, in which, of the 15 isolates, 60% inhibited the growth of *K. pneumoniae* (Kishore, 2011).

IV. CONCLUSION

The diversity of actinobacteria present in mangroves is still poorly known due to the difficulties of access and the scarcity of studies focused on these ecosystems. Even so, in the present study, it was possible to identify a great variety of strains of actinobacteria with antimicrobial properties from the mangrove of the municipality of São Caetano de Odivelas-PA, which may have applicability for the research of new antibacterial drugs.The results obtained in the sensibility tests performed were more satisfactory in the indirect confrontation method, highlighting the necessity of improving the direct confrontation technique. Even so, all the results were of great importance for this research and may encourage new studies focused on the bioprospecting of actinobacteria from poorly explored environments.

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