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CHROMOGENIC DIVERSITY OF ACTINOBACTERIA STRAINS ISOLATED FROM MANGROVE SOILS IN THE MUNICIPALITY OF SÃO CAETANO DE ODIVELAS – PARÁ, BRAZIL

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ABSTRACT

Budget The actinobacteria compose a phylum of Gram-positive bacteria that have a wide morphological and physiological variety. They are presented in the form of cocci or bacilli, and their reproduction is characterized by the formation of spores and septate pseudohyphae resembling that of fungi. Among the genera, *Streptomyces sp*. stands out for its complexity and variety in the production of secondary metabolites from which most of the antibiotics known and employed in human and veterinary medicine have been produced. These bacteria are present in the most diverse environments, such as soils, hydric collections, gastrointestinal tract of humans and also in mangroves.

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INTRODUCTION

Mangroves are considered natural nurseries of marine life, several species of economic value spend at least one phase of their life in this environment. Worldwide there are approximately 162,000 km² of this ecosystem and Brazil accounts for about 10,000 to 25,000 km² having the 3rd largest mangrove area in the world (Schaeffer, 1995; Naime, 2019; Brasil, 2018; Hogarth, 1999). It has great importance for exercising protective functions against the natural coastal erosion process, dissipating the sea pressure on the coast, besides acting as a biological filter, digesting organic matter and absorbing nutrients (Salmazo et al., 2018; Miththapala, 2008). The microbiota of the mangrove sediment is diverse and considered unique for being able to survive in an environment considered inhospitable due to high temperature and salinity, tidal cycles, humidity, wave action, UV radiation and scarce nutrients, characteristics that make thesemicroorganisms develop physiological processes and particular metabolites making this ecosystem an attractive option for the discovery of new bioactive compounds. Several species of animals inhabit this ecosystem, among them, thousands of bacteria and fungi being these microorganisms responsible for 91% of the mangrove biomass acting in nitrogen fixation, nutrient transformation, sulfate reduction, metabolization of organic compounds among other functions (Gomes, 2018; Wang et al., 2022). Among these microorganisms, the actinobacteria stand out - they consist in Gram positive bacteria that are present in the most diverse environments, as they are considered cosmopolitan microorganisms. They present in their DNA a high proportion of Guanine and Cytosine and are considered one of the largest taxonomic groups in the domain of bacteria. They have a wide morphological variety and can present themselves in the format of cocci or bacilli, their reproduction is characterized by the formation of spores and septate pseudohyphae resembling fungi (Barka, 2016). Among the groups of dyes produced by actinobacteria, two stand out: melanoids and carotenoids.

Melanoid pigments are dark-colored polymers formed from the oxidative polymerization of phenol and/or indolic compounds, which may be involved in the protection of microorganisms against environmental stress. The carotenoid pigments comprise a family of terpenoids with a range of coloration from pale yellow to reddish orange. The synthesized pigments can vary in coloration depending on the culture medium in which they were seeded, the bacterial species and the incubation time (Gomes, 2018; Oliveira, 2021; Janaki et al., 2014; Lin et al., 2005; Ramos et al., 2015). Actinobacteria have a fundamental role in the production of secondary metabolites. They are the target of research for new bioactive compounds and thus have high commercial value, and among the genera, the 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. The lack of exploitation of a highly found ecosystem in Brazil allows mangroves to become the target of research to elucidate its biotechnological potential (Anandan et al., 2016; Arumugam, et al., 2017). Thus, considering the presence of actinobacteria in these locations, the record of the chromogenic diversity through images will contribute to further related studies. Therefore, the present work had as objective the macro and micromorphological characterization and the creation of a photographic collection of actinobacteria strains isolated in mangrove soils in the municipality of São Caetano de Odivelas in the state of Pará.

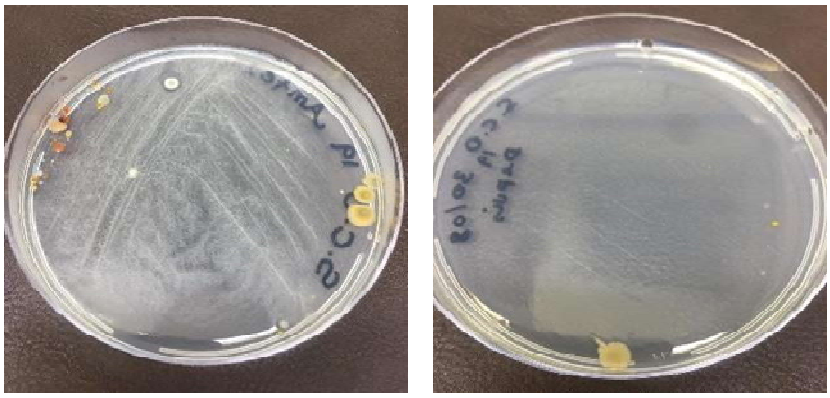
MATERIALS & METHODS

This is an experimental, descriptive, cross-sectional and analytical study, performed from August to November 2021, the collection was carried out in the municipality of São Caetano de Odivelas, located in northeastern Pará and is part of the salt-marsh microregion. For isolation, the samples were diluted in test tubes using 1g of sample to 10 ml of 0.9% saline solution and subjected to agitation for 2-5 minutes in vortex, after this time they were taken to a centrifuge at 3000 rpm for 5 minutes.

The supernatant was sowed with disposable loops by the streak method on Czapek Dox Agar culture medium. After primary sowing, the remaining dilution samples were submitted to thermal shock, in which the material was put in a Bain-Marie at a temperature of 90ºC for 10 minutes and then cooled in the refrigerator at -4ºC for the same period, and then sowed again, with the objective of analyzing whether thermal shock could influence the results found. Petri dishes were placed in a bacteriological incubator for 24 to 72 hours at a temperature of 35°C ± 2°C and daily bacterial growth was observed. Colonies with morphological characteristics of actinobacteria were selected and subjected to the Gram staining method.

RESULTS

The heat shock technique allowed the isolation of some strains that were resistant to the shock, but in the petri dishes sowed before the heat shock there was a more significant growth, presenting colonies with diverse morphologies and pigmentation (Fig. 1). Nineteen bacterial strains with characteristics similar to actinobacteria were isolated from before and after heat shock, and all samples were sowed on Czapek Dox agar. In relation to their cultural or chromogenic diversity, observed from the analysis of the aerial mycelium, samples producing pigments of various shades such as: cream, orange, orange/black, yellow, yellow/pink, light brown, white and whitish gray were obtained (Fig. 3). possible to observe that some of the colonies showed pigmentation changes according to the time they remained in the incubator, as observed in the SCO31B strains. After 24 hours of incubation, SCO31B showed a characteristic carotenoid pigment of orange coloration and, later, it was possible to observe the production of melanoid pigment, which was better visualized after 72 hours of incubation. Of the isolated strains, most presented microscopic characteristics in the form of bacilli, while the others presented characteristics of cocci. It was also possible to observe the presence of pseudo hyphae and spore chains in some isolates (Fig. 4). Actinobacteria forming long, thin, branched filaments and long, short, or verticillate spore chains were found on microscope slides SCO12, SCO16, SCO19A, SCO19B, and SCO31B (Fig. 4)



A B

Figure 1. Macromorphological diversity of strains before and after heat shock; A: plate before heat shock; B: plate after heat shock

SCO31B

Figure 2. SCO31B strain producing pigments in culture medium during different incubation periods

Figure 3. Diversity of actinobacterial strains isolated from mangrove soils

DISCUSSION

The cultural diversity of the actinobacteria can be seen by the production of pigments of various shades. Furthermore, the use of different culture media enables the identification of the great diversity of actinobacteria in different habitats. In a study performed with samples collected from the rhizosphere of Caatinga, the medium used in the isolation was HV-agar (Humic Acid-Vitamin Agar), and the diversity was verified by the production of various shades of pigments (Corrêa, 2014). Similar results were found in this work, which also identified the production of diverse pigments, but using the Czapek Dox agar culture medium. In another study performed with actinobacteria isolated from mangrove sediments in Ariyankuppam, India (Janaki et al., 2014), in which PIYEA (Peptone iron yeast extract agar) was used, cultures with pigment production similar to those presented in this work were observed. Thus, demonstrating the versatility and the ability of this group of bacteria to produce pigments, which can be better evaluated for industrial use. In the present study three bacteria were isolated with yellow pigment coloration, in contrast, in a research involving the collection of samples from the mangrove ecosystem, Bhitarkanika, Odisha (Kishore, 2011) it was observed that only one strain showed this characteristic, having predominance by the presence of colonies with whitish-gray characteristics, whereas in the present study it was observed this pigmentation in only the strain SCO19A (Fig. 3).

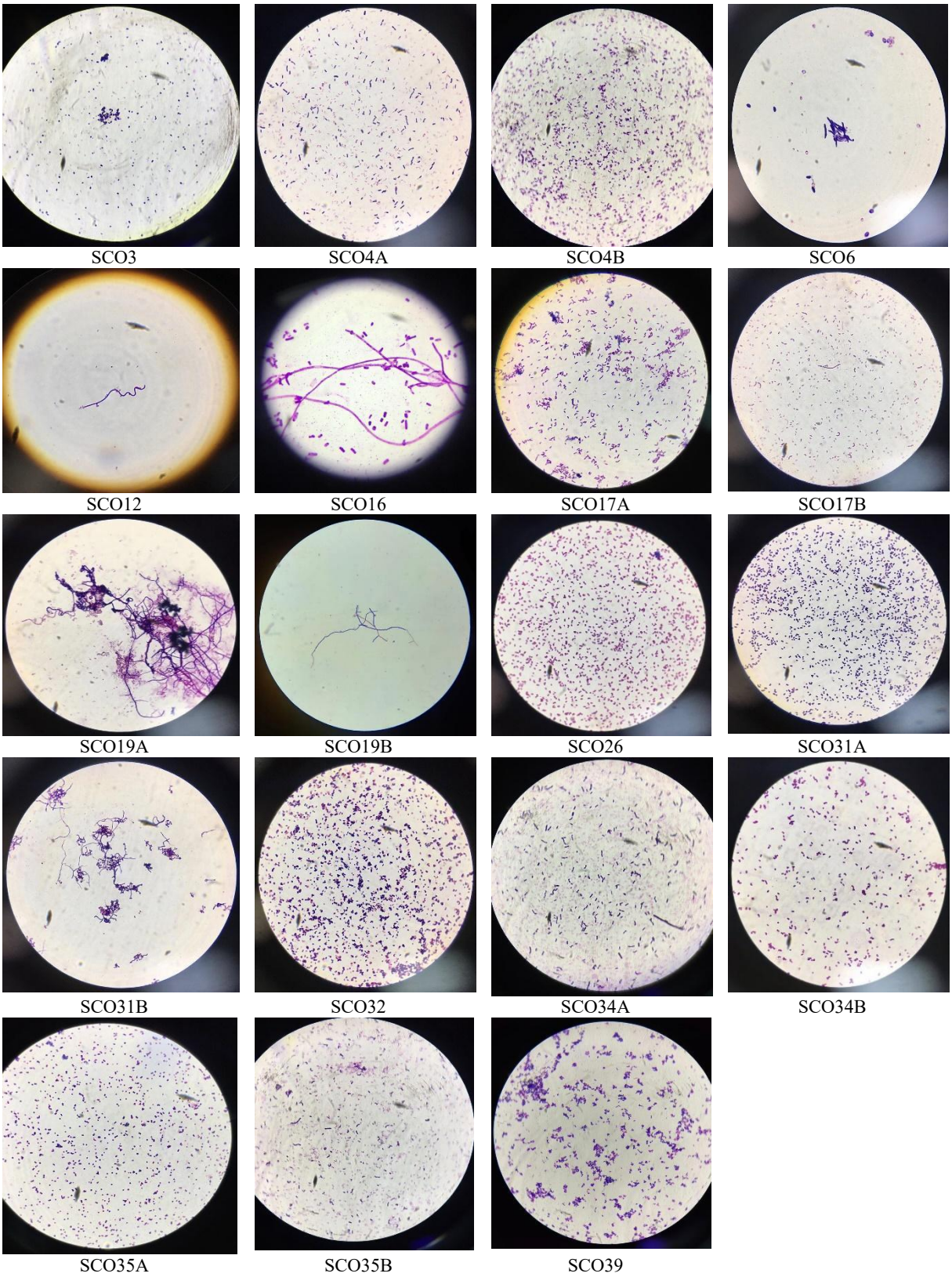


Figure 4. Gram staining of actinobacteria isolated from soil samples in mangroves

This shows that the ecosystem evaluated in this study showed greater diversity in relation to the production of pigments. In relation to thermal shock, it is a process used to reduce the growth of undesirable bacteria or contaminating flora (Uesugi et al., 2021). In this research, the technique allowed the isolation of some strains that were resistant to the shock; however, in the plates sowed before the thermal shock there was a more significant growth, presenting colonies with diversified morphologies and pigmentation. Thus, this technique was not adequate, because it was observed that the procedure inhibited the growth of both the contaminating bacteria and the bacteria of interest in the study. According to Bergey's Manual of Systematic Bacteriology (Goodfellow et al., 2012), actinobacteria are Gram-positive bacteria that form long, thin, branched filaments and may originate chains of spores in the shape of long, short or verticillate spirals - characteristics observed in microscope slides SCO12, SCO16, SCO19A, SCO19B and SCO31B (Fig. 4), of this study, similar to those presented in another scientific work (Nascimento, 2021), confirming the microscopic characterization described by Bergey's Manual of Systematic Bacteriology.

CONCLUSION

Mangrove sediments are a source of actinobacteria that contain great morphological diversity. We isolated 19 strains of actinobacteria from

mangrove soils in the municipality of São Caetano de Odivelas, identified according to their characteristics such as the micro morphology of the isolated bacteria, and the presence or absence of branched hyphae and spores. The importance of controlling the growth parameters of the actinobacteria was observed, in which the alteration of the growth time and temperature influences the production of pigments and metabolites, as was visualized in strains SCO31B and SCO26. The isolates with different pigmentations indicate the cultural diversity of strains in the mangrove, and evidenced the colors: cream, orange, orange/black, yellow, yellow/pink, light brown and whitish gray. With the results obtained it was evident that there is a certain variety of actinobacterial strains in the mangrove soil, demonstrating the necessity of more detailed research for the extraction of the pigments. These characteristics, together with the collection of images, may facilitate ecological studies of actinobacteria from mangroves.

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