

Bioprospection, morphological and biochemical characterization and evaluation of the antimicrobial activity of the bacterial strains of chicken breeding soil (*Gallus gallus domesticus*) in the city of Igarapé-Açu, Pará

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Abstract—The soil is a propitious place for the development of decomposer microorganisms, mainly fungi and bacteria. The soil colonization from those organisms occurs in places with great availability of organic matter, like the rhizosphere and animal breeding soils, like poultry farming. Among the microorganisms present in this type of soil, there are the actinomycetes, which belong to phylum of gram-positive bacteria with filamentous structures that constitute aerial mycelia. The objective of this article was to isolate, bioprospect, characterize morphologically and biochemically and to assess the antimicrobial potential of strains of bacteria from chicken breeding in the city of Igarapé-Açu, Pará. As a result, bacterial colonies with variable features that indicate the genus *Actinobacteria* were found, amongst them one showed potential antibacterial against a strain of *Klebsiella pneumoniae*

Keywords—*Actinobacteria*, *Gallus gallus domesticus*, bacterial resistance.

I. INTRODUCTION

Soil is a promising place for the growth of microorganisms, especially decomposers such as bacteria and fungi (Su et al., 2020). It is estimated that for every 1 gram of soil abundant in organic matter, there are 109 to 1010 prokaryotic organisms (bacteria and archaea) (Srinivasiah, 2008; Tecon, 2017). In places of animal husbandry, such as poultry husbandry, there is a wide availability of this type of material that enriches the soil with primary and secondary macronutrients that promote the proliferation of many organisms (Menegaço, 2017).

Among the microorganisms present in soils intended for poultry farming activities, actinomycetes stand out, which belong to a phylum of gram-positive bacteria that have a filamentous organization, form structures called aerial mycelium and present regions rich in cytosine and guanine

in their DNA (Oliveira, 2021; Ramos, 2015). It is important to emphasize that this phylum has a great morphological variety, which can be bacilli, coccoids, coccobacilli, in short or rudimentary hyphae forms (Barka et al., 2016; Medeiros, 2018).

In addition to actively participating in the degradation of organic matter, this group of bacteria exhibit several physiological and metabolic properties such as the production of extracellular enzymes, antitumor agents, antifungal and antibacterial substances, especially bacteria of the genus *Streptomyces* (Uesugi et al, 2021; Hopwood, 2007; Brito, 2015). Due to the wide variety of metabolites, this phylum has become a great source of compounds of medical importance and of great commercial value for the pharmaceutical industry, especially with regard to the

production of antimicrobial drugs (Espinosa, 2012; Pimentel, 2016).

The actinomycetes are promising sources for the production of some of the most important classes of antibiotics, such as beta-lactams, tetracyclines, aminoglycosides, macrolides, among others (Genilloud, 2017). Its antibiotic action occurs from the synthesis of bactericidal substances from various enzymatic complexes, such as polyketide synthase (PKS's) and non-ribosomal peptide synthases (NRP's) (Quinn, 2020).

Antimicrobial Resistance (AMR) represents a serious public health problem, since medical treatment against bacterial infections has become increasingly limited, resulting in greater clinical complications for hospitalized patients, resulting in a providing number of deaths (Costa, 2017). It is estimated that around 700 thousand deaths annually are related to this problem (Angles, 2018).

One of the biggest barriers in the confrontation against AMR is the development of new technologies, considering that the number of laboratories and pharmaceutical companies that invest in the research of new antibiotics has fallen in the last decades due to low profitability. Allied to this, the production of new drugs with antimicrobial properties cannot keep up with the adaptation of these microorganisms, as it takes years to develop a new drug (Estrela, 2018; Rodrigues et al., 2018).

Although bacterial resistance is a natural event of evolutionary adaptation, the inappropriate use of drugs in hospital, domestic and livestock environments, especially in the production of beef animals such as chickens, significantly contributes to this process occurring in an accelerated way (Furtado et al., 2019; Rodrigues et al., 2018). In view of this scenario, the present work was based on the isolation, identification and evaluation of the antimicrobial potential of microorganisms obtained from the soil of areas intended for the rearing of chickens (*Gallus gallus domesticus*) in the city of Igarapé-Açu, a municipality in the interior of the state of Pará, Brazil.

II. MATERIALS AND METHODS

The material was collected from the soil of an area destined to poultry farming located in Igarapé-açu, inland of the State of Pará. Three samples were taken, one surface, one 5cm deep and one 10cm deep, which were placed in plastic bags and properly identified and transported to the Laboratory of Applied Microbiology of the Center for Biological and Health Sciences of the State University of Pará (LabMicro CCBS/UEPA).

For the preparation of inoculums, a dilution of 1g of soil to 10 ml of saline solution was made, resulting in a concentration of 0.1 g/ml. The mixture was vigorously homogenized for 5 minutes and the supernatant of each sample was inoculated in Petri dishes containing Tryptic Soy Ágar (TSA) and Sabouraud Ágar media and incubated at 37°C for 24 hours.

After that, the samples were subjected to a thermal shock (five minutes at 95°C, followed by 15 minutes at -10°C and another 5 minutes at 95°C) and inoculated again only in Tryptic Soy Ágar (TSA) medium and also, were incubated in an oven at 37°C for 24 hours.

After growth, bacterial strains were isolated by appearance of the colony and placed in test tubes containing Tryptic Soy Agar (TSA). The procedures for characterization of the research samples were carried out using bacterial identification techniques by traditional biochemical series.

The microorganisms were isolated, evaluating colony characteristics and Gram staining and performing biochemical tests such as catalase and oxidase, hemolysis, sugar fermentation and other biochemical tests, including motility, citrate use and sucrose fermentation test and lactose, according to standard microbiological methodology.

The sensitivity test was also carried out, in which the growth inhibition potential of strains such as *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* was evaluated through the method of diffusion in plates containing Ágar Muller Hinton. Bacterial suspensions with concentrations of approximately 0.5 cfu/mL were inoculated, using sterile disposable swabs, in plates containing Mueller Hinton Ágar, which remained at room temperature for approximately 30 minutes before the procedure. Then, the isolated and characterized strains were introduced by pricking, with the use of a properly sterile platinum loop, in specific points of the already seeded plates. It was considered as a potential inhibiting power the formation of inhibition halos of bacterial growth around the bite.

III. RESULTS

In the soil samples from the area destined to the rearing of chickens, a significant growth was observed in all plates. Of the 6 samples collected in this soil, the formation of bacterial colonies observed were: 1 (16.7%) of cotton colony, 1 (16.7%) of mucoid colony, 1 (16.7%) of yellow colony and 3 (50%) of white colony.

From the microscopic analysis, performed from the staining of slides made with the colonies isolated by the Gram technique, considering the morphological aspects of

the colonies found, the bacteria presented the form of isolated gram-positive bacilli or in chains.

Through the Gram stain technique, it was possible to observe the morphology and dye affinity of the isolated bacteria, the morphological characteristics found demonstrate a predominance of bacillary forms, but with a very accentuated pleomorphism.

The biochemical analysis of the samples showed great metabolic variation, the isolated bacteria were characterized according to their metabolism on carbohydrates, motility and enzyme production, the results demonstrate a complex metabolism of this group of bacteria described in table 3.

The bacterial morphologies found showed a predominant biochemical pattern, allowing them to differentiate beyond their morphological characteristics, but also by the production of hemolysins and the enzyme catalase (Table 4).

Bacteria isolated and previously characterized as actinobacteria were evaluated for their potential to inhibit the growth of pathogenic bacteria, only one of the six strains tested showed a positive result inhibiting the in vitro growth of *K. pneumoniae*.

IV. FIGURES AND TABLES

Table 1. Macroscopic characteristics of colonies isolated from the ground in an area destined to the rearing of chickens.

Colony morphology	N	%
White Cotton	4	66.7
Yellow Mucoid	2	33.4
Total	6	100.0

Source: Author's data.

Table 2. Microscopic characterization of isolated bacteria according to colony morphology.

Morphological characteristic	Microscopic
White cotton	Isolated bgp and chain
Yellow mucoid	Isolated bgp and chain

Source: Author's data.

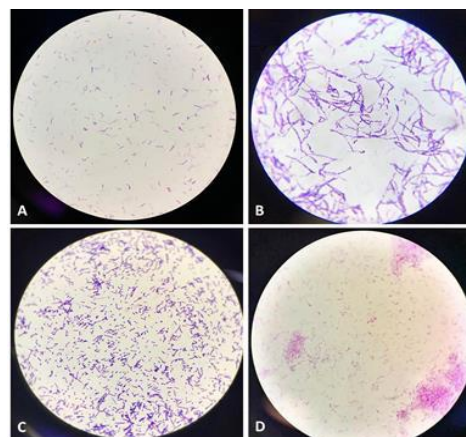


Fig.1: Microscopic characteristics of isolated bacteria from Gram stain.

Source: Author's data.

Caption: (A) Isolated gram-positive bacilli, (B) Gram-positive bacilli in chains, (C) Gram-positive pleomorphic bacilli, (D) Gram-positive coccobacilli.

Table 3. Biochemical characterization of bacteria isolated from agricultural soil samples in Igarapé-Açu – PA.

Biochemical proof	Positive	%	Negatives	%
Lactose	6	100	—	—
Sucrose	3	50	3	50
Motility	1	16.7	5	83.3
Citrate	1	16.7	5	83.3
Hemolysis	4	66.7	two	33.3
Oxidase	4	66.7	two	33.3
Catalase	4	66.7	two	33.3

Source: Author's data.

Table 4. Biochemical characterization according to macroscopic aspect.

	Whiten Cotton	Yellow Mucoid
TSI	AC\AC	AC\AL
lac	+	+
Sac	-	-
Mot	-	-

cit	-	-
hem	-	+
oxy	+	+
cat	+	-

Source: Author's data.

Caption: TSI (Triple Sugar Iron Agar) - AC/AC: Acid base and apex; AC/AL: Acid base and alkaline apex; AL/AC: Alkaline base and acidic apex; Lac: lactose; Sac: sucrose; Mot: motility; Cit: citrate; Hem: hemolysis; Oxy: oxidase; Cat: catalase. (-): negative; (+): positive; (v): variable.

Table 5. Growth inhibition test of pathogenic bacteria involved in human pathologies

	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>
White Cotton	-	+	-
Yellow Muroid	-	-	-

Source: Author's data.

V. CONCLUSION

Bacteria isolated from the soil of areas destined to the rearing of chickens (*Gallus gallus domesticus*) in the city of Igarapé-Açu, in the inland of the state of Pará, presented colonies with variable macroscopic appearance. As for the microscopic characteristics, these were indicative of gram-positive bacteria suggestive of belonging to the phylum Actinobacteria. Among the isolated strains, one showed antimicrobial potential on the growth of *Klebsiella pneumoniae*, demonstrated by the formation of inhibition halos around the colonies. Therefore, the search for actinobacteria in unusual environments can determine the finding of new actinobacterial strains with biotechnological potential.

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