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ISOLATION AND PROBIOTIC POTENTIAL CHARACTERIZATION OF LACTIC ACID BACTERIA (LAB) FROM PERUVIAN AMAZONIAN FRUITS

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ABSTRACT

Lactic acid bacteria (LAB) are an important group of probiotic microorganisms, making them an interesting field of study due to their beneficial health effects. It is estimated that the global market for probiotics will exceed 80 billion USD. Given that these microorganisms are present in various natural sources and have unique characteristics, their search is relevant in understudied Amazonian fruits due to the great biodiversity of their environment and the climatic conditions in which they develop. Three Amazonian fruits (*Genipa americana*, *Pouteria caimito*, and *Solanum mammosum*) were used for the isolation of LAB, yielding a total of 72 strains. The isolates were characterized to evaluate their probiotic potential. However, only 4 strains from *P. caimito* showed probiotic potential, suggesting that they could be used in the future for the development of foods and beverages with beneficial effects for the digestive system

Keywords: Lactic acid bacteria. Probiotics. Amazonian fruits. Isolation.

1 INTRODUCTION

Lactic acid bacteria (LAB) are generally recognized as GRAS (Generally Recognized As Safe) and most are important inhabitants of the intestinal tract in humans and animals at concentrations between 10^5 – 10^8 [1]. LAB are a class of Gram-positive fermentative bacteria characterized by producing lactic acid from carbohydrates [2]. These are a versatile group of microorganisms with numerous uses in the food industry. It is expected that the demand for LAB by the beverage sector will surge during the period from 2023 to 2030 [1]. The global market demand for LAB was estimated at $3.1 billion in 2022, with an annual growth rate of 8.0% [3], and it is projected to exceed $3.6 billion by 2024.

The Amazon is a source of a great diversity of fruits, which in some cases are consumed fresh by the population or serve as raw materials for the preparation of local products [4]. Previous studies have reported the isolation and identification of LAB from different fruits such as blackberries, apples, lulos, cupuaçu, plums, guavas, coconas, and other [1,2]. Fruits from this region grow under specific conditions of temperature, humidity, and rainfall, which could influence their microbiota, making them an interesting source of novel microorganisms with potential use as probiotics or for lactic acid production [5]. In this regard, LAB isolated from natural habitats may exhibit special phenotypic characteristics and high variability compared to traditional strains [6]. The most commonly used LAB as probiotics belong to genera such as *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Lactiplantibacillus, Leuconostoc, Limosilactobacillus, Lacticaseibacillus, Levilactobacillus, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus,* and *Weissella* [6,7].

Various criteria have been used to consider new LAB isolates as probiotics, including surviving gastrointestinal stress (resistance to low pH values, gastric juice, and pancreatic secretions); bile salt hydrolysis; being non-hemolytic; the ability to adhere to gastrointestinal tract walls; antagonistic action against gastrointestinal pathogens; and interaction with host immune cells [8]. However, due to various reasons (ethical, regulatory, technical, and economic), in vitro assays are used as an alternative to in vivo experiments for the characterization of LAB and the evaluation of their probiotic potential [2].

The gastrointestinal lumen contains various substances (essential nutrients, toxins, and antigens) in addition to beneficial and pathogenic microorganisms [9]. The excessive use of antibiotics has detrimental effects on the intestinal microbiota, making the use of probiotics a promising alternative [10]. However, some probiotic microorganisms are limited because they contribute to the transmission of antibiotic resistance and toxin production [2]. Thus, there is a growing need to search for new LAB strains isolated from diverse natural sources with high genetic stability, favorable health effects, and significant industrial potential.

LAB can be found in a wide variety of nutrient-rich environments such as fruits and vegetables, and their beneficial effects are closely related to the strain [6]. The Peruvian Amazon, particularly in the Loreto region and the city of Iquitos, has great biodiversity due to its climatic conditions. In these areas, 162 species of edible fruits have been described, of which around 100 are marketed in Iquitos markets [4]. Therefore, the selection and characterization of new probiotic strains are very important in the research and development of innovative products derived from under-studied Peruvian fruit varieties. In this context, the main objective of the

current work was to report on the isolation of potential probiotic LAB species present in three Amazonian fruits (*Genipa americana, Pouteria caimito,* and *Solanum mammosum*) in order to identify the probiotic potential.

2 MATERIAL & METHODS

Processing of fruits and Isolation of BAL

The samples of *G. americana, P. caimito*, and *S. mammosum* will be superficially swabbed using sterile swabs previously moistened with sterile saline solution (0.85% NaCl). The swabs will be placed in tubes with Man Rogosa Sharpe (MRS) broth. Incubation will be performed under anaerobic conditions at 37°C for 48 hours. The samples will be diluted until to 10^{-6} using PBS, then spread plating will be performed on sterile MRS agar plates. The plates will be incubated at 37°C for 48 hours. Morphologically distinct and isolated colonies will be selected and subjected to Gram staining to verify their purity [1].

Primary Characterization

Preliminary identification of the 72 LAB isolates was based on their phenotypic and biochemical characteristics, which included Gram staining and the catalase test. Additionally, the isolates were streaked onto blood agar plates containing 5% (w/v) sheep blood for evaluation of hemolytic activity [10], deoxyribonuclease (DNase) agar medium to test for production of the DNase enzyme [2] and antibiotic susceptibility on agar plates using the antibiotic disc diffusion method (Amoxicillin 10 µg - AML10, Ciprofloxacin 5 µg - CIP5, amikacin 30 µg - AK30, gentamicin 10 µg - CN10, amoxicillin and clavulanic acid 30 µg - AMC 30, sulfamethoxazole/trimethoprim 25 µg - SXT25, Norfloxacin 10 µg - NOR10, Erythromycin 15 µg - E15, Vancomycin 30 µg - VA30, Nitrofurantoin 300 µg - F300)[2].

Evaluation of Probiotic Properties

After primary characterization, 04 isolates from *P. caimito* were selected, and their characterization was complemented with the evaluation of their ability to auto-aggregate [2], coaggregate with *Staphylococcus aureus* and *Escherichia coli* [8], and their tolerance to both acidic pH 2.0 and bile salts 0.3% [7].

3 RESULTS & DISCUSSION

In this study, the isolated LAB were screened for potential probiotic activities. A total of 72 isolates of LAB with different morphologies were isolated from 3 amazonian fruits. All of them were Gram-positive and 89% were catalase-negative. The results of antibiotic sensitivity test revealed that 95% of the isolates from *G. americana*; 97% from *P. caimito* and 91% from *S. mammosum* were sensitive to AMC30, AML2, AK30, E15, CN10. The sensitivity of the isolates corresponds with many strains of *Lactobacillus spp*. and *Enterococcus spp*., which are sensitive to the majority of antibiotics, especially ampicillin and amoxicillin [8]. However, only 06 isolates from *P. caimito* showed no hemolytic or DNAse activities (safety evaluation), proving the nonpathogenic status of the probiotic isolates [2]. Moreover, bile salt and acidity tolerance are expected for probiotics to have the ability to survive within the varied pH of the intestinal tract [7]. The results revealed that 02 isolates (POU-21 and POU-26) are tolerant to acidic pH and minimum bile salt (0.3%), as a potential probiotic isolate must possess characteristics like survival and colonizing ability under different environmental conditions[2].

Table 1 Nonpathogenic isolates from *P. caimito*, shape and tolerance bilis salt – acidic pH (log UFC/mL) characteristics.

Item	Morphology	Bilis salt (0.3%)	Bilis salt (0.5%)	Tolerance pH 2.0
POU-7	Cocci	6.01	nd	nd
POU-11	Cocci	5.02	3.87	nd
POU-20	Bacilli	nd	nd	1.43
POU-21	Cocci	4.24	1.96	4.74
POU-25	Bacilli	nd	nd	7.34
POU-26	Bacilli	5.58	nd	4.70

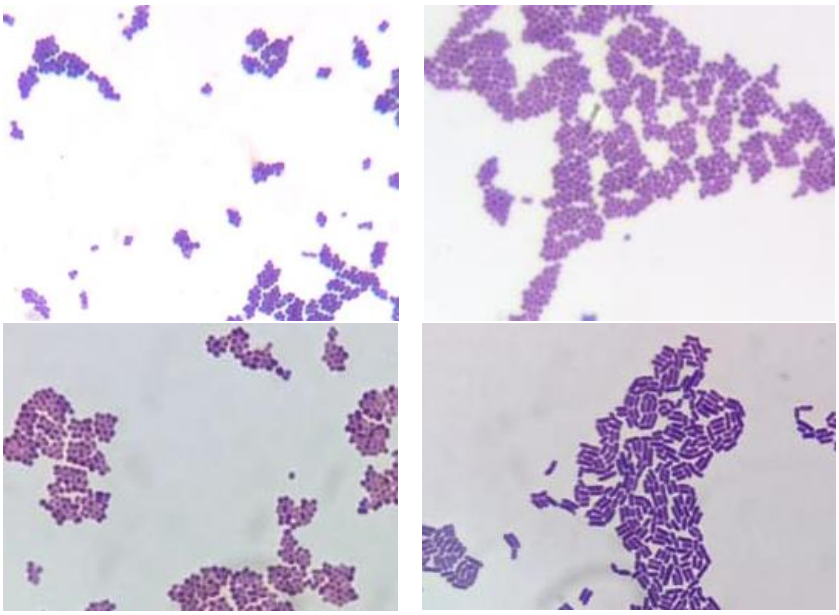

Figure 1 Gram positive BAL from P. caimito. A) POU-7 B) POU-11 C) POU-21 and D) POU-26.

Three isolates exhibited high autoaggregation abilities, which increased for all isolates after 24 hours of incubation (see Table 2). Isolate POU-7 demostrated the highest autoaggregation rate at 69%. All isolates displayed significant coaggregation capability with *E. coli,* and *S. aureus*; POU-26 exhibited the highest coaggregation capability with *E. coli* (64%), and *S. aureus* (56%). In both cases, the aggregations values obtained were greater than those observed for *Lactobacillus rhamnosus* [10].

Table 2 Autoaggregation and coaggregation assays of selected isolates from *P. caimito*.

Item	Coaggregation (%)		Autoaggregation (%)	
	S. aureus	*E. coli*	5 h	24 h
POU-7	56	60	42	69
POU-11	52	56	40	60
POU-21	52	48	17	43
POU-26	56	64	24	64

4 CONCLUSION

Numerous LAB were found in the three Amazonian fruits, although the majority of isolates were discarded due to pathogenic activities such as DNase production and hemolysis. Only 5.5% of the total LAB isolates exhibited probiotic potential. Among them, one isolates (POU-26) displayed particularly interesting characteristics for surviving under gastrointestinal conditions and producing beneficial effects.

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