International Journal of Current Science Research
www.drbgrpublications.in

Unlocking the probiotic potential of *Borassus flabellifer* sap: isolation and biochemical characterization of novel lactic acid bacteria

[1]Rajkumar K, [1]Suba Priya V, [1]Chandika P, [1]Divya S and [2*]Palpperumal S

[1]*PG Student, Department of Microbiology, V. H. N. Senthikumara Nadar College (Autonomous), Virudhunagar, Tamilnadu*

[2]*Head & Assistant Professor, Department of Microbiology, V. H. N. Senthikumara Nadar College (Autonomous), Virudhunagar, Tamilnadu*

**Corresponding Author e-mail id: palpperumal.s@vhnsnc.edu.in*

Abstract

Lactobacillus species are the bacteria that were isolated and identified from the sap of Borassus flabellifer. These species produce lactic acid. In addition to its important role in diabetes control, the probiotic microbiota has other advantageous properties including antibacterial and antibiotic agents and is essential for gastrointestinal health. Various laboratory procedures were used to identify and isolate lactic acid bacteria (LAB).The growth morphology on the selected media, growth at a different temperature, and other biochemical tests were used to identify them. Sugar from Borassus flabellifer was gathered at random from nearby markets for bacteriological analysis. Fresh Palmyra palm sugar samples were collected and cultured on de Man, Rogosa, and Sharpe (MRS) agar to isolate Lactobacillus species. The current study determines Lactobacillus species probiotic capacity. Various laboratory procedures were used to identify and isolate LAB. The growth morphology on the selected media, growth at a different temperature, and other biochemical tests were used to identify them. The purpose of this study was to isolate and characterize LAB with probiotic properties from Palmyra palm sugar.

Keywords: Borassus flabellifer, Biochemical characterization, Lactobacillus

Introduction

Probiotics are beneficial microorganisms found in various parts of the human body, particularly in the gut. The *Lactobacillus* genus consists of lactic acid bacteria (LAB) that are part of the normal microbiota present in the mucosa of humans and animals. According to research, there are 261 species within the *Lactobacillus* genus, which vary at the phenotypic, ecological, and genotypic levels. *Lacticaseibacillus paracasei*, formerly known as *Lactobacillus paracasei*, is included in the microbiota of both human and animal guts. This bacterium contributes significantly by adhering to the gastrointestinal tract, which helps maintain its stability and prevents intestinal infections.

Probiotics also produce antimicrobial peptides that can inhibit various food-borne pathogens such as *Escherichia coli*, *S. aureus*, MRSA, and *Salmonella Typhi*, as well as the biofilms created by these pathogens. Furthermore, the colonization of probiotics in the human gut reduces the areas that can be occupied by pathogens. It has been observed that intestinal probiotics.

Can modify gene expression in the mammalian gut mucosa, ultimately influencing the gastrointestinal tract's function. Palmyra palm sugar is a traditional beverage made from the sap of the Palmyra palm (*Borassus flabellifer*). This juice is popular in tropical Asian regions, particularly in southern Thailand. Derived solely from the sap of the Palmyra palm, Palmyra palm sugar is a natural sweetener comprising several components including reducing sugars, alcohol, volatile compounds, vitamins, essential minerals, and phenolic compounds. Additionally, the juice and products derived from Palmyra palm sugar serve as a vital source of natural bacteria, particularly LAB.

LAB extracted from fermented Palmyra palm sap are regarded as probiotics. Research indicates that probiotic LAB obtained from fermented foods can be utilized in commercially produced fermented foods. Moreover, these probiotics possess functional characteristics that inhibit the growth of significant human pathogens. According to the FAO/WHO guidelines, probiotic bacteria must be safe for both humans and animals. To select and characterize probiotics, the bacteria must endure the gastrointestinal tract's conditions, such as low pH and the presence of pepsin and pancreatin.

Additionally, they should play a crucial role by adhering to the gastrointestinal tract, particularly to epithelial cells, to protect the host from invasive pathogenic bacteria. To date, there have been no studies reported in Asia, including Thailand, regarding the probiotics isolated from Palmyra palm sugar and their effects against MRSA. Therefore, this study aimed to isolate probiotic bacteria from Palmyra palm sugar that can produce antimicrobial compounds targeting MRSA and food-borne pathogens.

Materials and Methods

Collection of samples: Fresh Palmyra palm sugar samples were collected from a local market in Virudhunagar district, Tamil Nadu.

Isolation of LAB from Palmyra palm sugar: A sample of one gram of Palmyra palm sugar was dissolved in 100 ml of sterile water, resulting in a stock solution. Following this, 1 ml from the stock solution was transferred into a test tube that contained 9 ml of sterile distilled water. This mixture was then subjected to serial dilution up to 10^{-5}. Subsequently, 0.1 ml of each diluted sample was taken and spread across appropriate Mann Rogosa Sharpe (MRS) Agar plates.

The plates were incubated at 37°C for 48 hours under aerobic conditions. After 24 hours of incubation, a single colony from the MRS agar was inoculated onto a new MRS agar plate and incubated at 37°C for an additional 24 hours.

The isolated bacterial strains were preserved in MRS broth with 25% (v/v) glycerol at −80°C until they were needed for further experiments.

Identification of LAB from *Borassus flabellifer* Sugar

The isolated colonies were examined for growth and identified based on their morphological and biochemical characteristics. The biochemical tests performed included Gram staining, catalase, oxidase, indole, methyl red, Voges-Proskauer, citrate utilization, triple sugar iron, Starch hydrolysis test.

Result

Isolation and Identification of LAB from Palmyra Palm Sugar: Lactic acid bacteria (LAB) were isolated from Palmyra palm sugar samples. After 24 hours of incubation on MRS agar, the *Lactobacillus* isolates were identified as Gram-positive and non-endospore-forming.

Identification was carried out based on morphological and biochemical characteristics, with reference to Bergey's Manual of Systematic Bacteriology.

Biochemical Test

Table 1 - Biochemical characteristics of *Lactobacillus* sp.

S. No	Biochemical test	Observation
1.	Indole test	Negative
2.	Methylred test	Positive
3.	Voges proskaver test	Negative
4.	Citrate utilization test	Positive
5.	Triple Sugar Iron test	A/A with gas

Discussion

Probiotic LAB and other good bacteria can be found in traditional fermented foods, along with other nutritious nutrients (Sornsenee *et al*., 2020). The lactic acid bacteria that were separated from Palmyra palm sugar were selected for this study and their potential as probiotics was further examined. The three probiotic LAB isolates that were chosen are capable of producing antimicrobial chemicals that combat foodborne diseases, MRSA, and a novel zoonotic pathogen. Ninety-seven percent of the isolates were found to be *Lacticeibacillus* species when compared to the GenBank database. Based on variations at the phenotypic, ecological, and genotypic levels, 261 species of the genus Lactobacillus have been identified (Zheng *et al.*, 2020). *L. paracasei*, referred to as *L. paracasei*, is a component of the gut microbiota in both humans and animals. The findings from this research indicate that the isolated bacteria belong to the *Lacticaseibacillus* genus. The isolate WU 2302 has been characterized A homology of 98.82% was observed through MALDI-TOF analysis (data not presented). It is worth mentioning that the MALDI-TOF analysis instrument's library has not been updated to include the new genus *Lacticaseibacillus spp.* This genus is recognized as a component of the normal gut flora in both humans and animals (Sornsenee *et al.*, 2020). The bacterium is significant for its ability to adhere to the gastrointestinal tract (Li *et al.*, 2020), contributing to the stability of the tract and helping to prevent intestinal infections (Gu *et al.*, 2008): *L.paracasei*, referred to as *L. paracasei*, is a component of the gut microbiota in both humans and animals. The findings from this research indicate that the isolated bacteria belong to the *Lacticaseibacillus* genus. Our findings showed that the isolates could attach to human colon Caco-2 cells. It is essential to highlight that the human Caco-2 cell line is

frequently utilized as an in vitro model. The intestinal epithelial barrier plays an important role in health (Hiebl *et al.,* 2020). Probiotics adhering to the colon can obstruct the establishment of harmful microorganisms. Probiotic lactic acid bacteria (LAB) have shown the ability to combat food-borne pathogens, highlighting their potential as natural alternatives to synthetic medications. Studies have indicated that LAB derived from fermented foods can create biofilms on various surfaces, which can hinder the colonization of numerous bacteria, including *L. monocytogenes, Salmonella Typhimurium,* and *E. coli* O157 H7 (Gómez *et al.,* 2016). The findings indicate the potential advantages of the selected probiotic LAB in managing pathogenic organisms *L. plantarum* K41, derived from traditional Sichuan pickles, demonstrated antibacterial and anti-biofilm effects against Streptococcus mutans. Furthermore, the probiotic decreased the production of exopolysaccharides by the pathogen. Research has indicated that close interactions between pets and humans create an environment conducive to bacterial spread. Moreover, livestock-associated MRSA that leads to human colonization and infection has been documented. Nonetheless, the isolated probiotics' supernatant effectively inhibited these microorganisms. It is widely recognized that antibiotics are prescribed for the treatment of infectious diseases caused by various pathogens. However, the improper use of antibiotics, along with non-compliance, can lead to the emergence of antibiotic-resistant bacteria. Consequently, there has been a shift towards exploring natural products, particularly probiotics, as alternative methods for combating infectious diseases. Probiotics are known to generate antimicrobial substances. In our study, we chose LAB that are known for producing antibacterial substances for further investigation. So far, the antimicrobial agents created by *Lactobacillus* coryniformis BCH-4 have demonstrated the ability to inhibit the growth of *Aspergillusflavus*, a pathogen associated with fungal degradation in maize grains. Additionally, the compounds identified include 2-oxopropanoic acid, 2-hydroxypropane-1,2,3-tricarboxylic acid, 2-hydroxybutanedioic acid, and 2-hydroxypropanoic acid. Propanedioic acid and butanedioic acid have been recognized as bioactive substances among the antimicrobial compounds derived from *L. coryniformis* (Salman *et al.,* 2020). It has been established that the bacteriocin produced by *L. paracasei* WX322 has a significant impact on Pectobacterium carotovorum, a pathogen responsible for soft rot in vegetables (Zhu *et al.,* 2021). Furthermore, the probiotic *L. paracasei* has been identified as a potential protective barrier against bacterium-related damage. In particular, this probiotic has the ability to shield host cells from bacterial enterotoxins (Hassan *et al.,* 2020). The capacity to withstand extremely

harsh conditions, such as those encountered in the gastrointestinal tract characterized by low pH, bile salts, and enzymes is the primary reason for the selection of potential probiotic candidates (Kandylis, P., *et al* 2016). The subject demonstrated considerable resistance to pancreatin. Furthermore, a resistance level between 64% and 75% to bile salts was observed following treatment with 3 g/L of bile salts. A critical factor in the selection of a probiotic candidate is its ability to withstand the low pH found in gastric juice. Our findings indicated 80% resistance to a low pH of 3, yet the isolates did not seem to survive exposure to pepsin or pH 2. Enhancing probiotic viability in gastrointestinal tract conditions can be achieved through encapsulation. The use of calcium alginate and whey protein concentrate for microencapsulating *L. casei* was found to significantly enhance the survival of probiotics both in food carriers and in simulated gastrointestinal environments. Additionally, a symbiotic approach, which combines probiotics and prebiotics, can increase the effectiveness of probiotics and serve as a useful technique in food applications.

Conclusion

The probiotics from Palmyra palm sugar, were isolated and characterized in the current work. It was determined that the three isolates selected from the probiotic LAB were *Lacticaseibacillus spp*., which may produce antimicrobial chemicals against significant pathogenic bacteria.

References

Sornsenee,P., Singkhamanan, K.,Saengsuwan, P.Sangkhathat, S., and Romyasamit, C. (2021) Probiotic Properties of *Lactobacillus species* isolated from fermented palm sap in Thailand. *Probiotics Antimicrob. Proteins*, 13(4): 957-969.

Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.,Watanabe, K., Wuyts, S., Felis, G.E., Gänzle, G.M., Harris, H., Mattarelli, P., O'Toole, P.W., Pot, B., Vandamme, P., Walter, J., and Lebeer, S. (2020) A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int. J. Syst. Evol. Microbiol*., 70(4): 2782-2858.

Li, M., Wang, Y., Cui, H., Li, Y., Sun, Y. and Qiu, H.J. (2020) Characterization of lactic acid bacteria isolated from the gastrointestinal tract of a wild boar as potential probiotics. *Front. Vet. Sci*., 7: 49.

Gu, R.X.,Li, Z.H., Yang, Z.Q., Chen, S.L. and Luo, Z.L. (2008) Probiotic properties of lactic acid bacteria isolated from stool samples of longevous people in regions of Hotan, Xinjiang and Bama, Guangxi, China. *Anaerobe*, 14(6): 313-317.

Hiebl, V.,Heiss, E.H., Schachner, D., Ladurner, A., Stangl, H. and Dirsch, V.M. (2020) Caco-2 cells for measuring intestinal cholesterol transport-possibilities and limitations. *Biol. Proced. Online*, 22(1): 1-1

Gómez, N.C., Quecan, B.X.,Ramiro, J.M. and de Melo Franco, B.D. (2016) Use of potential probiotic lactic acid bacteria (LAB) biofilms for the control of Listeria monocytogenes, Salmonella typhimurium, and *Escherichia coli* O157: H7 biofilms formation. *Front. Microbiol*., 7: 863.

Salman, M., Tariq, A., Ijaz, A., Naheed, S., Hashem, A., Allah, E.F.A., Soliman, M.H. and Javed, M.R. (2020) In vitro antimicrobial and antioxidant activities of *Lactobacillus coryniformis* BCH-4 bioactive compounds and determination of their bioprotective effects on nutritional components of Maize (Zea mays L.). *Molecules*, 25(20): 4685.

Zhu, R., Liu, X., Li, X., Zeng, K. and Yi, L. (2021) Transformation of inferior tomato into preservative: Fermentation by multi-bacteriocin producing *Lactobacillus paracasei* WX322. Foods, 10(6): 1278.

Hassan, M.U., Nayab, H.,Almansouri, T.S., Asim, N., Shafi, N., Shafique, F., Williamson, M.PAttacha, S., Khalid, M. and Ali, N. (2020) Probiotic properties of *Lactobacillus helveticus* and *Lactobacillus plantarum* isolated from traditional Pakistani yogurt. *Biomed. Res. Int*., 2020: 8889198.

Kandylis, P., Bekatorou, A.Pissaridi, K.Kanellaki, M. and Koutinas, A.A. (2016) Dairy and non-dairy probiotic beverages. *Curr. Opin Food. Sci*., 7: 58-63