

# Probiotic Properties of Lactic Acid Bacteria Isolated from Sugar Cane Juice

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## ABSTRACT

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Lactic acid bacteria (LAB) were isolated from different food and food products. There are many such foods that are not been investigated for the presence of these beneficial bacteria. In this study, LAB were isolated from fresh sugar cane juice and their probiotic attributes were investigated. Twenty-five bacterial strains were isolated and, at first screened for morphological and biochemical properties. Twelve Gram-positive and catalase-negative isolates were further tested for acid and bile tolerance. The six isolates which were tolerant to acidic pH and bile were further subjected for their probiotic attributes. These isolates exhibited the best probiotic characteristics. They were finally identified following the 16S rRNA gene sequencing technique.

**Keywords:** 16S rRNA sequencing, lactic acid bacteria, probiotics, sugar cane juice

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## INTRODUCTION

Lactic acid bacteria, especially the genus lactobacillus, lactococcus, and streptococcus are most widely used as probiotics. They are the important members of the intestinal beneficial microflora. They are generally recognized as safe to the consumers. Potential probiotic bacteria should possess special characteristics like colonization and survival in the intestinal tract, pathogenic inhibition, production of anti-microbial compounds, non-toxic to the consumers, increasing nutritional value of the food and food products. Anticancer, antiallergic, reduction in the cholesterol level, enhanced immune response, reduction in intestinal inflammation, irritable bowel syndrome, and antibiotic-induced diarrhea are the other important benefits of probiotics. These potential probiotics can be used as natural preservatives.

## OBJECTIVES

Isolation and characterization of lactic acid bacteria from sugar cane juice

Study of probiotic attributes of the isolated bacteria

## METHODOLOGY

Freshly extracted sugar cane juice samples were procured from the farmers. LAB strains were isolated by serial dilution and spread plate technique on MRS agar plates added with 0.25 % (w/v) L-cystine. The plates were incubated at 37°C for 48 hours in an anaerobic condition. Morphological, physiological, and biochemical characters of the isolates were studied following the standard protocol described by Cappuccino and Sherman (2004). The methods described by Guo et al. (2009) and Ramos et al. (2013) were followed to evaluate the bile salt and acidic pH tolerance of the isolates. Antimicrobial activity is one of the prime tests for the evaluation of probiotics. The cell-free supernatant (CFS) was neutralized and used to test the production of antimicrobial compounds by the isolates. The ability of the strains to auto aggregate was determined as per Zommiti et al. (2017). The hydrophobicity property of the isolates was determined by the assay as described by Kalyaraung

et al. (2008). The survival of LAB isolates under gastric and pancreatic environments were determined in vitro by adopting the standard protocols.

## CONCLUSION/RESULT

Based on morphological, physiological, biochemical, and molecular identification, six potential probiotic strains *Lactobacillus casei* subsp. *casei*, *Enterococcus durans*, *Lactococcus lactis* subsp. *lactis*, *Lactobacillus acidophilus*, *Lactobacillus fermentum* and *Lactobacillus plantarum* were identified. *E. durans* and *L. plantarum* shown survival at 10% NaCl concentration. The isolates *L. casei* subsp. *casei*, *L. acidophilus*, and *L. fermentum* shown tolerance to 50°C. Acid tolerance is a principal measure for the validation of probiotics. *E. durans* was the most acid-tolerant organism. It has shown 26.21% survival at pH 3 after 3 hours of incubation. *L. acidophilus* was very much resistant to 1% bile salt than the other isolates. *L. plantarum* could survive at the rate of 9.67% in a gastric juice at a pH of 2 after 3 hours of incubation. All the isolates showed tolerance to intestinal juice. They were checked for their survival till 6 hours of incubation. These tests were conducted to check their ability to tolerate the harsh environment of the gastrointestinal tract. Auto-aggregation was best exhibited by *L. acidophilus*. All the isolates showed good coaggregation capacity with *S. aureus* (MTCC 9760), and *E. coli* (MTCC 7410). Isolates showed moderate levels of bile salt hydrolase activity. Adhesion to the intestinal epithelium was exhibited well by *L. plantarum* whereas *E. durans* showed the least adherence activity. *L. plantarum* has shown 65% hydrophobicity activity. Antibiotic resistance property of the isolates was carried out with thirteen commonly used antibiotics. *L. fermentum* was the most antibiotic-resistant organism in this study. All the isolates have broad-spectrum antimicrobial activity. *L. acidophilus* produced 5.2 Gm/L of lactic acid whereas *Lc. lactis* subsp. *lactis* could produce only 0.47 Gm/L. *L. fermentum* produced 0.17±0.03 Gm/L of H<sub>2</sub>O<sub>2</sub> whereas *Lc. lactis* subsp. *lactis* produced only 0.0035±0.0012 Gm/L. All the isolated strains exhibited good probiotic attributes, so they can be used as potential probiotics in food and feed formulations. The present investigation aims at increasing the knowledge about the investigation of potential probiotic bacterial strains from non-fermented foods.

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