

ASSESSMENT OF PROBIOTIC EFFICACY OF *LACTOBACILLUS* SPECIES ISOLATED FROM DAIRY PRODUCTS

Debapriya Mohanty* and Pratima Ray

¹Department of Microbiology

Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India

E-mail: debapriyam.h@gmail.com (*Corresponding Author)

Abstract: Probiotics have been used as a treatment modality for over a century. The current work aimed to a screening program for probiotic attributes of *Lactobacillus* species isolated from dairy products.

Keywords: *Lactobacillus* species, probiotic properties, dairy products

Introduction

A series of food and pharmaceutical products have drawn a special attention and interest of consumer community due to their health and medical benefits. Therefore, food and pharmaceutical market has introduced a large number of health beneficial components including probiotics. Probiotics are increasingly seeking special interest of health conscious consumers because of their clinical properties and basic nutritional value (Stanton et al., 2005). Over the years, many potential microbial strains have been used as probiotic cultures mainly including lactic acid producing bacteria, namely, *Lactobacillus* and *Bifidobacterium* (Holzapfel et al., 2001). The FAO/WHO (2002) guidelines propose that the potential probiotic strains should be relevant for surviving in the GI tract at the time of ingestion to confer a health benefits. Therefore, these properties have consequently become important selection criteria that explain the ability of probiotic strains to tolerate physiochemical harsh environment such as highly acidic conditions present in the stomach and concentrations of bile salts found in the small intestine (Tuomola et al., 2001). Probiotics play a crucial role in prevention and alleviation symptoms of intestinal infections, traveler's diarrhea and antibiotic associated diarrhea, inflammatory bowel disease, prevention of colon cancer etc (Wollowski et al., 2001).

Materials and Methods

Preliminary isolation and identification of the *Lactobacillus* species

Representative dairy samples (yogurt prepared from raw cow milk) were collected from rural regions of Odisha for the isolation of potential probiotic *Lactobacillus* species by using selective media de Man Rogosa and Sharpe (MRS) agar. Preliminary identification of *Lactobacillus* species were performed as per Bergey's manual of systematic bacteriology. Gram variability (Gram's reaction), cell morphology, motility, endospore formation, catalase and oxidase reactions of all isolates were tested as per standardized protocols. Carbohydrates (glucose, lactose, xylose, maltose, fructose, dextrose, galactose, riffinose, trehalose, melibiose, sucrose, arabinose, mannose, sorbitol, mannitol and arabitol) utilization capacity of potent probiotic strain was determined by using Hi-carbo KIT A and B (Hi-media).

Screening of *Lactobacilli* isolates for their probiotic attributes

Screening of *Lactobacilli* isolates for their probiotic attributes mainly includes the ability of the organisms to survive in the adverse conditions such as low pH and high bile salt concentration. Acid tolerance capability of all *Lactobacillus* isolates was evaluated in MRS broth by adjusting its pH in different values (2.0, 2.5 and 3.0) with 1N HCl as per standardized protocol. Similarly, bile salt tolerance of *Lactobacillus* cultures were examined by inoculating them in MRS broth containing bile salts at the concentrations of 0.3, 0.6% and 1% (w/v) (Hi-Media, India Pvt. Ltd.). The viability of bacterial cells was assessed by colony counts (CFU/ml) after incubation of 0, 1 and 2 h to estimate the percentage of acid and bile salt tolerance ability of isolates (Kaushik et al., 2009). Haemolytic activity was checked to estimate in vitro safety assessment of isolated probiotic strains. It was performed by spotting 2 µl of a 6 h old culture broth on blood agar plates supplemented with 5% sheep blood. Plates were incubated at 37 °C for 48 h (Maragkoudakis et al., 2006).

Results and Discussion

A number of 23 strains were preliminarily isolated from yogurt (cow milk) by using MRS medium at 37 °C. Out of which, 5 isolates were Gram Positive, non spore forming, non motile, very small rods (cocobacilli) that occurred singly or in pairs. They were facultatively anaerobic, catalase negative and oxidase positive. The preliminary observation was matched with Bergey's manual of systematic bacteriology, Vol. III, 2nd edition that confirmed all 5 strains were belonging to *Lactobacillus* species. In the present study, only one strains, namely, DM60, showed the good viability in form of log CFU/ml at low pH (2, 2.5 and 3) and high bile salt concentration of 0.3%, 0.6% and 1% (w/v) till 2 h that indicated its

probiotic potency in human use. No haemolytic was observed for DM 60. It was confirmed as safe bio-resources. Again, all sugars such as glucose, lactose, xylose, maltose, fructose, dextrose, galactose, riffinose, trehalose, melibiose, sucrose, arabinose, mannose, sorbitol, mannitol and arabitol have been utilized by DM 60. When these biochemical test results are compared with the literature information and Bergey's manual of systematic bacteriology, it seems that DM 60 is like to be *Lactobacillus casei*. In a climate of increasing consumer demand in the way to probiotic products, fermented products prepared from cow milk of Odisha will be very friendly to the public health.

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