

PRELIMINARY ISOLATION OF ANTIMICROBIAL PROBIOTIC *LACTOBACILLUS* SPECIES FROM GOAT MILK OF ODISHA

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Abstract: *Lactobacillus* species were isolated from goat milk collected from different region of Odisha and systematically screened for their probiotic attributes. Probiotic *Lactobacillus* species were again evaluated for their antimicrobial activity.

Keywords: *Lactobacillus* species, probiotic, antimicrobial activity.

Introduction

The term “Probiotic” is relatively a new word which was derived from the Greek word 'pro bios' meaning ‘for life’. Probiotic means “for/in favor of life.” In general, probiotics are defined as “live microbial food supplements or components of bacteria which when taken up in adequate amounts, confer a health benefit on the host” (FAO/WHO 2001). Over the years, many potential microbial strains have been used as probiotic cultures mainly including lactic acid producing bacteria. They are represented by the following genera *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Saccharomyces* (Krishna kumar and Gordon, 2001). Some *Lactobacillus* species with probiotic properties are *L. plantarum*, *L. fermentum*, *L. casei*, *L. paracasei*, *L. lactis*, *L. brevis*, *L. acidophilus*, *L. reuteri*, *L. rhamnosus*, *L. amylovorus*, *L. salivarius*, *L. bulgaricus*, *L. cellobiosus*, *L. gasseri*, *L. johnsonii* and *L. sporogenes* (Gupta and Garg, 2009). Probiotics have been found to promote the GIT homeostasis, stimulate the growth of indigenous beneficial gut microbata and also inhibits the growth of pathogenic or opportunistic pathogenic microbes by producing antimicrobial substances (Sherman et al., 2009). *Lactobacillus* species produce antimicrobial substances, such as organic acids, fatty free acids, ammonia, hydrogen peroxide, bio surfactant and bacteriocins.

Material and method

Samples collection and bacteriological analysis

A total of two goat milk samples were collected aseptically from Khurda and Jagatsingpur (Odisha). One mL of dairy samples was mixed well in nine mL of saline water to make an initial dilution (10^{-1}). The suspensions were used for making suitable serial dilutions up to 10^{-7}

⁸ by transferring 1mL into 9mL of sterile saline water and pour-plated aseptically using MRS agar and plates were incubated at 37 °C for 48 h (Goyal et al., 2013; Yadav et al., 2016). After incubation period, bacterial cultures were randomly picked from master plates on the basis of their colony characteristics.

Preliminary screening of *Lactobacillus* isolates from goat milk

Genus level screening of *Lactobacillus* isolates were performed as per Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). It mainly includes included Gram reaction, catalase, motility test and endospore formation test.

In vitro screening of *Lactobacillus* isolates for their probiotic attributes

As per the FAO/WHO guidelines (FAO/WHO 2002), several aspects, including survival, safety and physiological characteristics were taken into consideration in the selection process of probiotic microorganisms.

In this study, survival criteria was mainly evaluated by acid or low pH tolerance test (pH 2.0, 3.0 and 4.0 for 3h), bile salt tolerance test (0.5 and 1% (w/v) of bile salts for 3h), bile salt hydrolysis (BSH activity) (bile sodium deoxycholate) (Kaushik et al., 2009), gastric and intestinal juice tolerance (Pisano et al., 2014), lysozyme resistance test, adhesion by cell surface hydrophobicity test and aggregation by cell aggregation assay (Kaushik et al., 2009). Similarly, safety criteria was evaluated by hemolytic assay, gelatinase activity, lipases activity, DNase activity, biogenic amine production test, antibiotic resistance test and effect of *Lactobacillus* isolates on other *Lactobacillus* isolates (Lavilla-Lerma et al., 2013).

Antimicrobial activity of probiotic *Lactobacillus* species

In the present study, to evaluate the antimicrobial activity of probiotic *Lactobacillus* isolates, whole cells of all probiotic *Lactobacillus* isolates as well as their cell free supernatant (CFS) and sonicated pellets (P) were taken for consideration. Agar well diffusion method described by Kumar and Kumar, (2015) was followed to determine the antimicrobial activity of probiotic *Lactobacillus* isolates against *E. coli* ATCC 25922, *Bacillus cereus* ATCC 10702, *Salmonella typhi* MTCC3216, *Salmonella enterica* subsp. *enteric* ATCC 35640, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* MTCC 902.

Results

Preliminary isolation of bacterial cultures from dairy products

Preliminarily, 20 bacterial cultures were randomly isolated from these two goat samples by observing their colony characteristics. Furthermore, all bacterial cultures isolated in this study were named as DM116-DM135.

Preliminary screening of *Lactobacillus* isolates

As per *Lactobacilli* comprise a diverse group of Gram positive, rod shaped, non spore forming catalase negative and non motile organisms (Kumar and Kumar, 2015). After this preliminary screening of Gram reaction, catalase, motility test and endospore formation test, a total of 13 *Lactobacillus* isolates (DM116 - DM122 and DM129 - DM134) were obtained from 20 bacterial cultures.

In vitro screening of *Lactobacillus* isolates for their probiotic attributes

Screening for survival criteria of *Lactobacillus* isolates

Out of 13 *Lactobacillus* isolates, only four such as DM 119, DM 120, DM 121, DM 132 were able to tolerate low pH (2.0, 3.0 and 4.0), high bile salt concentration (0.5 and 1% (w/v) for 3h. survival percentages was more than 70%. Again, the same four isolates can survive not only the toxicity of bile salts but also can carry out BSH mediated deconjugation of sodium deoxycholate (DCA). DM 119, DM 120, DM 121, DM 132 also showed resistant to gastric juice more than 70% and to intestinal juice more than 50%. Survival percentage of these 4 *Lactobacillus* isolates were found to be more than 90 % for 2h. The cell surface hydrophobicity and cell aggregation percentage of these *Lactobacillus* isolates were found to be more than 40 %.

Safety assessment of *Lactobacillus* isolates

Above four *Lactobacillus* isolates such as DM 119, DM 120, DM 121, DM 132 were found to be positive for survival criteria. So, they were selected for safety assessment. They were found to be negative for haemolytic, gelatinase, lipases, DNase activity and biogenic amine production and have no inhibitory effect against other *Lactobacillus* strains. They are sensitive to Ampicillin (10µg/mL), Amoxycillin (10 µg/mL), Amphotericin B (10µg/ mL), Chloramphenicol (30µg/mL). These were the required characteristics for safety criteria. DM 119, DM 120, DM 121, DM 132 were found to be safe.

Antimicrobial activity of probiotic *Lactobacillus* species

DM 119, DM 120, DM 121, DM 132 were obtained from goat milk as probiotic *Lactobacillus* isolates. Therefore, they were screened for their antimicrobial activity. Cell free supernatant of DM 120, DM 121 and DM 132 showed activity against *E. coli* ATCC 25922, *Bacillus cereus* ATCC 10702, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* MTCC 902. Most of the isolates showed zone of inhibition more than 10mm, suggesting their antimicrobial activity against the pathogens.

Conclusion

From the above study, it could be concluded that number of probiotic *Lactobacillus* species which have antipathogenic effect are present in goat milk of Odisha. These *Lactobacillus* species are suitable for use in various probiotic products and can be used as an adjuvant or alternative therapy in gastrointestinal disorders over the antibiotics. In a climate of increasing consumer demand in the way to probiotic products, fermented products prepared from goat milk of Odisha will be very friendly to the public health.

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