

## A Study on the Probiotic Aspects of Lactobacillus Isolated From Raw Milk of Vechur



### Biology

**KEYWORDS :** Probiotic, Lactobacillus, Milk, Vechur

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### ABSTRACT

*The objective of this study was to characterize the probiotic attributes of a Lactobacillus species isolated from raw milk of Vechur, a local indigenous cow breed of Kerala. Lactobacillus was isolated by selective enrichment of milk samples in MRS broth and subsequent plating in MRS agar. The results of biochemical characterization were suggestive of the isolate to be L.acidophilus. Considerable growth observed even after 3h of exposure to pH 3.0 and three percentage of bile salt concentration confirmed the ability of isolate to withstand the harsh environment of gastrointestinal tract. Opaque white colonies on Lactobacillus Oxgall agar affirmed the BSH activity of the isolate. CSH values obtained with the different solvents: n-hexadecane, ethyl acetate and chloroform were 36%, 30% and 27% respectively. The aggregation percentage of 52% in 24h indicates the good adhesion potential. Absence of resistance to tetracycline can be taken as an indicator for the absence of acquired antibiotic resistance. Observations made in this trial suggest that the isolate used in this work has ample potential to be exploited as a probiotic starter.*

### Introduction

Lactic acid bacteria are integral components of fermented foods where they carry out primary and secondary fermentation. Their long history of safe use in foods have earned them the status Generally Regarded As Safe (GRAS). Lactic acid bacteria constitute a group of gram positive bacteria united by a constellation of unique morphologic metabolic and physiological characteristics. They are the major starters used in the preparation of fermented milk products. Lactobacillus, one of the most important genus of lactic acid bacteria comprise of a large diverse group of gram positive, non sporing, catalase negative, rods that produce lactic acid as the major end product on fermentation of carbohydrates. Lactobacilli are widely and safely being used as probiotics for medical and veterinary applications. However over the world, search for novel indigenous probiotic strains is continuing to satisfy the ever increasing demand of the market for novel strains to develop new functional products. Foodborne lactobacilli consist of natural, uncharacterized strains, whose biodiversity depends on geographical origin, seasonality, animal feeding/plant growth conditions. The present study is an attempt for the probiotic characterization of a Lactobacillus species isolated from raw milk of indigenous cattle breed Vechur.

### Materials and Methods

Raw milk samples were collected aseptically from the Vechur cows of the University Livestock farm, Kerala Veterinary and Animal Sciences University, Mannuthy. Isolation of Lactobacillus from milk was achieved by selective enrichment in MRS broth and subsequent plating in MRS agar as per the standard procedure. Subsurface colonies of Gram positive rods that were catalase and oxidase negative were selected, and maintained in nutrient agar slants at 4°C. Biochemical characterization of the isolate was carried out (Barrow and Feltham, 1993). For long term storage, isolate was also preserved in 70% glycerol at -20°C.

To ascertain the capacity of the isolate to withstand the hostile environment of acidity and bile in the human GI tract, acid and bile tolerance of the isolate was assessed by judging the nature of growth (slight/ moderate/ heavy) obtained on streaking the culture in MRS agar after 3h exposure to pH 3.0 and 3.0 % bile salt concentration.

Ability of the isolate to produce bile salt hydrolase (BSH) was assessed qualitatively by comparing the colony morphology in MRS agar and bile salt supplemented MRS agar (Dashkevich and Feighner, 1989).

Adhesion potential of the isolate was measured in terms of cell surface hydrophobicity (CSH) by Microbial Adhesion to Solvents (MATS) assay using the apolar solvent n-Hexadecane, acidic solvent chloroform and basic solvent ethyl acetate (Rosenberg *et al.*, 1980). Affinity to hydrocarbons was reported as adhesion percentage as per the formula  $(A_0 - A/A_0) \times 100$  where  $A_0$  and  $A$  were the absorbance before and after extraction with organic solvents respectively. Hydrophobicity was calculated from three replicates as the percentage decrease in the optical density of the original bacterial suspension due to cells partitioning into a hydrocarbon layer.

Auto aggregation ability a property related to adhesion was also determined as procedure of Reniero *et al.* (1992). The standardized broth suspension was incubated in aliquots at 37°C and was monitored for 24h in terms of absorbance (A) at 600nm. Auto aggregation percentage was calculated by using the formula  $(1 - A_{upper} / A_{total}) \times 100$ . Autoaggregation was calculated from three replicates as the percentage decrease in absorbance of the original suspension due to aggregation and sedimentation. Sensitivity of the isolate to tetracycline, the most wide spread antibiotic resistant determinant was determined by disc diffusion assay. (Bauer *et al.* 1966).

### Results and Conclusions

Sub surface colonies obtained in MRS agar was indicative of the microaerophilic nature of the isolate. Microscopic examination of stained smear revealed the presence of Gram positive long bacilli arranged singly and in pairs. Preliminary identification tests and biochemical characterization results were suggestive of the isolate to be *Lactobacillus acidophilus*.

Bacterial adhesion to intestinal epithelial cells is important for colonization of probiotic strains in the gastrointestinal tract as it prevents their immediate elimination by peristalsis. This also provides a competitive advantage for the isolate in this ecosystem. Adhesion potential, a property primarily dependent on physicochemical characteristics of cell surface was determined *in vitro* in terms of CSH and auto aggregation. According to Del Re *et al.* (2000), strains should possess a hydrophobic surface for better adhesion to intestinal cells. The bacterial adhesion to apolar solvent reflects the hydrophobicity of the cells. Maximum CSH value (36%) with the apolar solvent n-hexadecane suggests a hydrophobic cell surface for the isolate. As the difference in CSH values obtained for acidic solvent (27%) and basic solvent (30%) is very less, the results are neither indicative of a strong

acidic nor a strong basic character for cell wall.

Ability to auto aggregate is an advantageous property for probiotic strains as it complements the adhesion potential. The auto aggregation percentage obtained for the *Lactobacillus acidophilus* isolate from Vechur cow milk was 52% within 24h. Similar values have been reported for *Lactobacillus acidophilus* NCFM (Collado *et al.*, 2008). The CSH values and percentage of auto aggregation observed is very much supportive of the good adhesion potential of the isolate obtained in this work.

In recent years, ability of probiotic lactobacilli to produce bile salt hydrolase (BSH) has become the focus of attention, on account of its influence on cholesterol metabolism. To assess the ability of the isolate to hydrolyze bile salt, the colony morphology in MRS agar with 0.3% bile salt was compared to that on simple MRS agar (control). In the control plate, colonies were pale and translucent whereas in MRS agar with 0.3% bile salt, colonies were opaque and white due to the precipitation of bile acids by way of bile salt hydrolase activity of the isolate. Distinctly different colony morphology in control plate and treatment plate was suggestive of BSH activity of the isolate. Probiotics encounter significant amount of bile salts consistently in the mammalian gut. As BSH helps in the detoxification of bile salts, presence of this enzyme definitely improves the survival rate in the competitive environment of gastrointestinal tract (Fuller, 1989). The remarkable bile tolerance shown by the isolate used in this work could be due to BSH activity. Recently there has been an increasing interest in bile salt hydrolytic activity of lactic acid bacteria as they are being identified as biological hypocholesteremic agents (Ramasamy *et al.*, 2010).

The most wide spread antibiotic resistant determinant in Lactobacilli is tet (M) Nawaz *et al.* (2011). Absence of acquired antimicrobial resistance is a prerequisite for the safety of lactobacilli to be used as probiotic starter. As the antibiogram revealed sensitivity to tetracycline, demonstrating the absence of acquired antibiotic resistance, it can be inferred that the isolate obtained in this work is a safe probiotic candidate.

**Table. 1**  
Percentage of cell surface hydrophobicity

| Sl. No | Solvent       | CSH |
|--------|---------------|-----|
| 1      | n- Hexadecane | 36% |
| 2      | Chloroform    | 27% |
| 3      | Ethyl acetate | 30% |

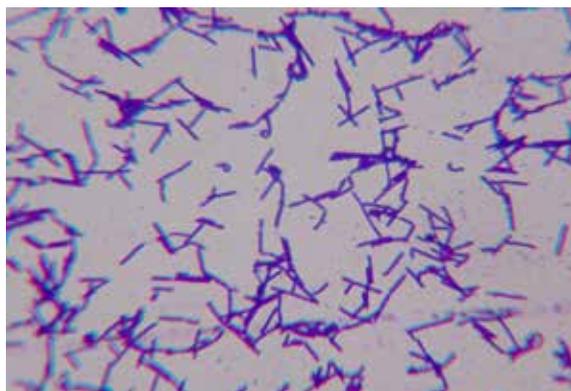
**Table. 2**  
Antibiogram of isolate

| Sl. No | Name of the antibiotic | Result |
|--------|------------------------|--------|
|        | Cefixime               | R      |
|        | Gentamycin             | S      |
|        | Tetracycline           | S      |
|        | Aztreonam              | S      |

|  |                 |   |
|--|-----------------|---|
|  | Sulphadiazine   | R |
|  | Amoxicillin     | R |
|  | Ampicillin      | R |
|  | Oxacillin       | S |
|  | Ciprofloxacin   | S |
|  | Clindamycin     | S |
|  | Chloramphenicol | S |
|  | Ceftazidime     | I |
|  | Chloramphenicol | I |
|  | Rifampicin      | I |

R- Resistant, S- Sensitive, I- Intermediate

**Figure 1**  
Gram's staining of *Lactobacillus acidophilus*



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