

## Characterization of Lactobacilli Isolated From Four Different Sources

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**Abstract:** Lactobacilli, the Gram positive, non spore forming, catalase negative rod shaped lactic acid bacteria are used widely as starter cultures for food fermentations and also as probiotic cultures due to their functional attributes. In this study, four lactobacilli each isolated from different sources namely: carrot, idli batter, dahi and duck feces were assessed for various attributes like antimicrobial, bile salt hydrolysis, proteolytic and lecithinase activities and their ability to grow at different salt concentrations. All the isolates were able to grow in salt concentrations as high as five percent and possessed bile salt hydrolase, an enzyme that imparts bile salt tolerance. All of them were also found to be antagonistic against *S. aureus* with the isolate from carrot giving the largest zone of inhibition of 16mm diameter. Two of the isolates were found to be moderately proteolytic and none of the isolates showed lecithinase activity.

**Key words:** Antimicrobial activity, Bile salt hydrolysis, Lactobacilli, Proteolytic activity, Salt tolerance

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### I. Introduction

Lactic acid bacteria (LAB) are a group of Gram positive bacteria having a long history of safe use in the food industry. They are acid tolerant, microaerophilic, non sporulating rods or cocci that ferment lactose producing lactic acid. The genus of lactic acid bacteria that is most commonly used in food fermentations is *Lactobacillus* [1]. They are widely being used as starter cultures in the preparation of a number of fermented dairy, vegetable and meat products. Members of this genus are also well acclaimed for their probiotic attributes. Considering the functional and technological significance of Lactobacilli there is always a thrust towards isolating new strains of Lactobacilli and functionally characterizing them. Owing to their unique potential for adaptation, lactobacilli are often encountered in a wide range of different environmental niches. As there could be habitat specific variations in the metabolic profile and starter culture potential of lactobacilli, natural habitats are often considered as potential sources to obtain prospective strains for food industry. Considering these facts an attempt was done to characterize lactobacilli isolated from four entirely different niches namely: carrot, idli batter, dahi and duck feces.

### II. Materials and Methods

#### 2.1 Lactobacilli isolates

Four *Lactobacillus* cultures isolated from various sources (carrot, idli batter, dahi and duck feces) and maintained as glycerol stock in the culture collection of Department of Dairy Microbiology were used in the present study. The cultures were activated by growing in sterile deMan Rogosa Sharpe (MRS, Himedia, Mumbai) broth at 37°C for 48 hours. The activated cultures thus obtained were transferred to MRS agar slants and maintained at 4°C with fortnightly subculturing. Active cultures were subjected to Gram staining, catalase test and motility test (hanging drop method) [2]. Gram stained cultures were examined microscopically for the presence of Gram positive rods. The catalase test was performed by the slide method by mixing a small amount of the culture with one drop of 3% hydrogen peroxide and observing for the development of effervescence. Motility of the isolates was tested by the hanging drop method. The ability of the isolates to ferment carbohydrates was determined by observing for color change and gas production upon inoculation and incubation of Andrade peptone water (Himedia, Mumbai) tubes containing the respective carbohydrate discs at 37°C for 7 days. The cultures were also assessed for their ability to grow at various temperatures (37°C, 45°C and 10°C) by incubating the inoculated MRS broth tubes at these temperatures for 48 hours and visually observing for development of turbidity. The ability to grow at different NaCl concentrations (1.5%, 3%, and 5%) was also tested in a same fashion.

#### 2.2 Activity testing of the isolates

Antimicrobial activity of the isolates against *S aureus* was determined by the agar well diffusion method [3]. Ability to produce the enzyme bile salt hydrolase was assessed qualitatively by streak culturing the isolates on MRS and *Lactobacillus* selection Oxgall agar (HiMedia, Mumbai) followed by incubation at 37°C for 48 hours. Development of a white precipitation solely in Oxgall agar was considered as a positive test [4].

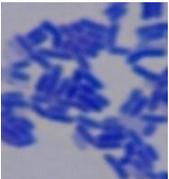
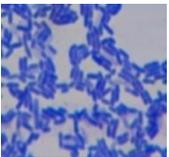
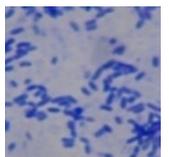
The proteinase and lecithinase activities of the isolates were tested by streaking them on skim milk agar (Nutrient agar with 10% skim milk) and Egg Yolk Agar (Nutrient agar supplemented with 6% egg yolk emulsion) respectively followed by incubation at 37°C for 48 hours [2] with some modifications. Development of zone of clearance around the colonies was considered as positive test for proteolytic activity and development of opaque (precipitation) zone around the colonies indicated the lecithinase production.

### III. Results and Discussion

#### 3.1 Morphological and Biochemical characterization of isolates

All the isolates formed small/ large, dry/smooth creamy white convex or sub- surface spindle shaped colonies on pour plating on MRS agar. Microscopic examination of Gram stained preparations of isolates revealed all the isolates as purple rods arranged singly or in clusters (Table 1). The isolates were also found to be non- motile on performing the hanging drop method. All the isolates failed to form any gas bubbles on treating with hydrogen peroxide indicating their catalase negative nature. Thus all the four isolates were found to be Gram positive, rod shaped non-motile, catalase negative bacteria.

**Table1.** Colony Morphology and Gram reaction of the isolates

Isolates	Source of isolation	Colony morphology	Gram reaction	Microscopic appearance
Isolate 1	Carrot	Small, creamy white, convex, entire margin, sub-surface, dry and spindle shaped colonies	Short, Gram positive rods	
Isolate 2	Idli batter	Large, creamy white, convex, entire margin, sub-surface, smooth and spindle shaped colonies	Short, Gram positive rods	
Isolate 3	Dahi	Small, creamy white, convex, entire margin, sub-surface, smooth and spindle shaped colonies	Short single Gram positive rods	
Isolate 4	Duck feces	Large, creamy white, convex, entire margin, sub-surface, dry and spindle shaped colonies	Short, Gram positive rods	

#### 3.2 Sugar fermentation and growth at different temperatures and salt concentration

Differences were observed among the isolates in their ability to ferment the sugars Arabinose, Galactose, Mannitol, Mellibiose, Raffinose and Xylose (Table 2). None of the isolates were capable of fermenting the sugar Rhamnose. Rest of the sugars namely, Cellobiose, Dextrose, Fructose, Lactose, Maltose, Mannose, Salicin and Sucrose were fermented by all the isolates. None of the isolates produced gas on fermenting Glucose/Dextrose indicating their homofermentative nature. On assessing the ability of the isolates to grow at different temperatures (10°C, 37°C and 45°C), it was observed that all the four isolates could grow at 37°C (Table 2). Only two isolates (1 and 3) could grow at 10°C. *Lactobacillus* isolated from duck feces alone could grow at 45°C exhibiting its thermophilic nature. It is being reported that NaCl concentration of more than 3% hampers the growth of LAB [5, 6, 7, 8]. Contrary to this report it was found that all the isolates used in the current study were able to grow even at 5% NaCl, the highest concentration tested. Isolation of *Lactobacilli* capable of growing at NaCl concentration as high as 18% is reported by other researchers [9, 10]. As salt tolerant lactic acid bacteria are of paramount importance in the ripening and flavor development of pickled cheeses like Domiati and in the pickling of vegetables like cucumber, cabbage and olives [11,12] the isolates could be of use in this direction.

**Table 2.** Biochemical, Physiological and Antimicrobial characteristics of the isolates

SL No.	Parameter	Isolate 1	Isolate 2	Isolate 3	Isolate 4
1.	Catalase	-	-	-	-
2.	Motility	-	-	-	-
3.	Growth at different temperatures				
	10°C	+	-	+	-
	37°C	+	+	+	+
	45°C	-	-	-	+
4.	Growth at different salt concentrations				
	1.5% NaCl	+	+	+	+
	3% NaCl	+	+	+	+
	5% NaCl	+	+	+	+
5	Carbohydrate utilization				
	Arabinose	+	+	-	-
	Cellobiose	+	+	+	+
	Dextrose	+	+	+	+
	Fructose	+	+	+	+
	Galactose	-	+	+	+
	Lactose	+	+	+	+
	Maltose	+	+	+	+
	Mannitol	+	+	+	-
	Mannose	+	+	+	+
	Melibiose	+	+	-	-
	Raffinose	+	+	-	-
	Rhamnose	-	-	-	-
	Salicin	+	+	+	+
	Sucrose	+	+	+	+
	Xylose	-	+	+	+
6	Antimicrobial activity against <i>S aureus</i> –Zone of clearance inclusive of disc diameter of 6mm)				
	16mm	13mm	14mm	13mm	

### 3.3 Antimicrobial activity of the isolates

All the isolates showed antimicrobial activity against *S. aureus* (Table 2). Maximum inhibition was exhibited by isolate 1, obtained from carrot giving a zone of inhibition of 16mm, followed by isolate 3 with a zone of clearance of 14mm. Classification of antimicrobial activity into moderate (6-9 mm), strong (10-14 mm), and very strong (15-18 mm) categories based on zone of inhibition [13] was followed in this study. Based on this criterion, the antimicrobial activity exhibited by isolate 1 could be graded as very strong whereas all other isolates fit into the category of strong inhibition. Antimicrobial activity of lactobacilli strains against different organisms like *E coli*, *S aureus*, *E. aerogenes* has been reported by a number of researchers [13, 14, 15]. Lactic acid bacteria are found to produce a variety of antibacterial agents including organic acids, diacetyl, H<sub>2</sub>O<sub>2</sub> and bacteriocins [16]. So the inhibition zone observed in this study could be due to the production of any of these agents and needs to be further investigated.

### 3.4 Bile Salt Hydrolysis activity of the isolates

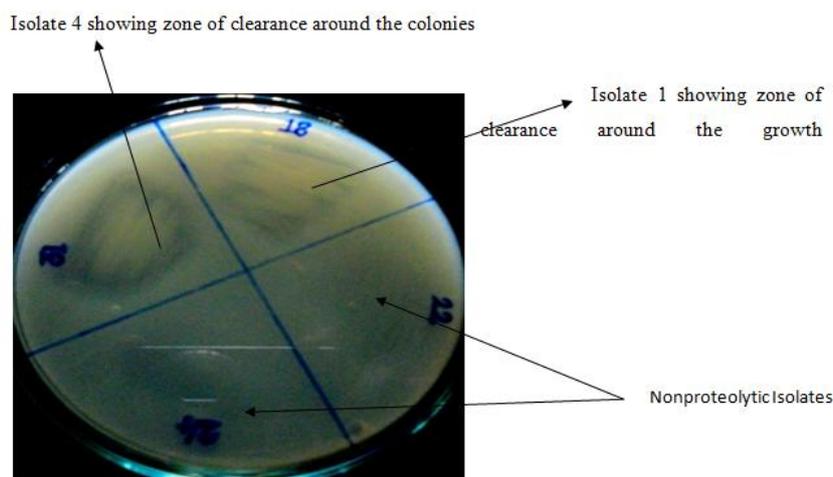
All the isolates formed white colored colonies on oxgall agar indicating their bile salt hydrolase (BSH) activity (Fig. 1). Bile salt hydrolases catalyze deconjugation of conjugated bile salts into amino acid residues and bile acids. This deconjugation process is suggested as an important detoxification mechanism of microorganisms during colonization in the gastrointestinal tract [17, 18]. This process is also hypothesized as one of the indirect means to decrease cholesterol level in humans [19]. Contrary to the reports [20] that the lactic acid bacteria isolated from human intestine or feces are BSH positive and that of food-origin are mostly BSH negative, in the current study all the isolates regardless of their source of isolation exhibited BSH activity. As BSH activity is considered as a property of importance in lowering serum cholesterol levels [21, 22] all the isolates could be considered as potential candidates for their hypocholesteremic effect. Considering that marked differences are reported between the BSH activities of microorganisms derived from various sources [23] it is required to quantify BSH activities of the isolates to derive such a correlation.



**Figure1:** Plate assay for bile salt hydrolase (BSH) activity in MRS agar and Oxgall agar

### 3.5 Proteolytic activity of the isolates

As LAB are fastidious in nature they have developed a complex proteolytic system of proteinases and peptidases to support their growth. Contrary to this general observation, only two isolates 1 and 4, isolated from carrot and duck feces respectively formed zone of clearance around the growth indicating their proteolytic activity. (Fig. 2) Considering the fact that most of the LAB isolated from fermented dairy products have multiple amino acids auxotrophy and their growth in protein rich medium depends on expression of a complex proteolytic system for the degradation of casein [24] it is interesting to note that isolate 3 obtained from dahi, a fermented milk product was unable to degrade the proteins provided by skim milk. The proteolytic activity is of special significance in cheese ripening. Further the range of bioactive peptides released by LAB depend on their proteolytic system. Proteolytic potential of lactobacilli isolated from different sources has been reported by other researchers also [15, 24, 25].



**Figure 2:** Skim milk plate with zone of clearance exhibiting proteolytic activities of isolates

### 3.6 Lecithinase activity of the isolates

In this study, none of the isolates produced lecithinase as there was no opaque (precipitation) zone around the colonies. Lecithinase (phospholipolytic) activity is used as an indicator of food toxicity [26]. Considering the fact that the LAB are widely being used in food fermentations it is essential to ensure that these organisms are safe for human consumption. So assessment of lecithinase activity could be considered as a selection criteria while screening microorganisms for use in food industry.

## IV. Conclusion

Lactobacilli are a group of microorganisms widely used for a number of industrial fermentation processes ranging from the preparation of fermented food products to production of biopolymer. They are also of special significance due to the functional properties attributed to it. In this context there is always a need to isolate them from different sources and screen for various industrial uses. In the present study, isolates from

different sources were found to differ in their activities. Among the isolates, isolate 1, obtained from carrot showed highest antimicrobial activity against *S aureus*. Exhibition of antimicrobial as well as proteolytic and bile salt hydrolysis activities by the same isolate proposes it as a potential candidate for further study on its probiotic and related properties for its efficient utilization as a functional *Lactobacilli*.

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