



Isolation and characterization of bacteria from dairy samples of Solan in Himachal Pradesh for identification of *Lactobacillus* spp.

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ABSTRACT

Lactobacillus is a genus of lactic acid bacteria and described as heterogeneous group of regular, gram-positive, rod shaped, non-motile, non-spore forming bacteria with absence of catalase enzyme. A total of 30 dairy samples were collected from the local areas of Solan in Himachal Pradesh. In total 30 bacteria were isolated after growth on MRS agar medium and pure culture was obtained by sub-culturing on the same medium. Purity of each culture was confirmed by morphological investigation, Gram's staining and further identification by *Lactobacillus* specific biochemical tests. Finally 12 bacterial isolates were identified as *Lactobacillus* spp. after morphological, cultural and biochemical characterization.

Keywords: Culture and biochemical characteristics, Dairy samples, *Lactobacillus* isolation.

INTRODUCTION

Microorganisms play a very important role in food industry. One of the most important groups of acid producing bacteria in food industry is the lactic acid bacteria, which are used as starter culture for dairy products. The preservative effect of lactic acid bacteria during the manufacture and storage of fermented foods is mainly due to acidic conditions that they create, converting carbohydrates to organic acids (lactic acid and acetic acids) in the food during their development. Among all lactic acid bacteria, the genus *Lactobacillus* has some beneficial characteristics which make it useful for the industrial applications. Genus *Lactobacillus* contains over 110 species, which are classified in three major groups: obligate homofermentative, facultative homofermentative and obligate heterofermentative.¹ The *Lactobacillus* genus consists of a genetically and physiologically diverse group of rod-shaped, Gram-positive, non-spore forming, nonpigmented,² catalase negative and microaerophilic to strictly anaerobic³ lactic acid bacteria (LAB) that have widespread use in fermented food production⁴ and are considered as generally recognized as safe (GRAS) organisms and can be safely used for medical and veterinary applications.⁵

The Lactobacilli isolated from dairy products have shown a long history of safe use.⁶ They are used widely as starter cultures in the food industry, e.g. fermented milk or meat products, alcoholic beverages, sourdough and silage.⁷ In raw milk and dairy products such as cheese, yoghurt and fermented milks, Lactobacilli are naturally present or added, for technological reasons or to generate a health benefit for the consumer.⁸ So in the present study we will endeavour to isolate and characterize the *Lactobacillus* from dairy samples on the basis of their phenotypic characteristics.

MATERIALS AND METHODS

Collection of sample and Isolation of bacteria

A total of 30 dairy samples i.e. household milk and curd were collected from local areas of Solan district of Himachal Pradesh. Isolation was done by using serial dilution method.^{9,10} Serial dilutions of dairy samples were prepared and then sample from different dilutions were spread over the solidified MRS (de Man, Rogosa, and Sharpe) medium for isolation. Plates were then incubated at 37°C for 24-48hrs. After incubation isolated colonies were restreaked on MRS agar plate and pure cultures were isolated.

Characterization of Isolates

All the pure cultures isolated from dairy samples were studied for their morphological, cultural and some biochemical characteristics.

Morphological examination

Morphological examination was carried out by examining colony morphology characteristics of all the isolates.

Culture and biochemical characterization

Cultural and biochemical characterization of all isolates were done by Gram (1984) staining, motility test, endospore test, catalase and sugar fermentation test.

For motility test sterilized inoculating needles with culture were stab cultured to 2/3 of the way down to the bottom of test tubes containing motility medium. The tubes were incubated at room temperature for 24-48 hrs and observed for the presence or absence of growth along the line of the stab inoculation. For endospore test bacterial smear was aseptically heat fixed on microscopic slide. The slide was placed over steaming water bath and malachite green stain was added for 5 min. The slide was removed and rinsed with tap water. Counter stain safranin was



applied for 30 seconds and rinsed with water. Blot dried slides were observed under light microscope for the presence of endospores.

For catalase test a drop of hydrogen peroxide was taken on a clean glass slide and culture was picked with sterile inoculation loop and slowly immersed into the drop of hydrogen peroxide. Formation of bubbles was observed for positive test. In sugar fermentation test, to 5 ml of nutrient broth (containing 5µl phenol red), 100 µl of glucose (1%) solution was added. The test tubes were inoculated with bacterial culture and incubated at 37°C for 24-48 hrs. The change in colour of the medium from red to yellow was observed for the positive results of fermentation. In case of homofermentation there will be production of acid with the change in colour of the medium from red to yellow whereas in heterofermentation there will be gas production in durham tube.

RESULTS AND DISCUSSION

Thirty bacteria were isolated from different dairy samples collected from Solan district in Himachal Pradesh. In all 14 isolates were from milk sample and 16 isolates were from curd samples (Table 1).

All isolates were studied for their morphological characteristics viz., type of colony, colour, margin, elevation, opacity and presence of pigment and the results are shown in Table 2. Out of thirty isolates two isolates showed less growth followed by nine with moderate and nineteen with high growth on MRS agar after 24-48h of incubation. All the isolate colonies appeared small and large in their shape (Figure 1). The colour of colonies ranged from off white, shiny white to creamy white. Margins were entire in all the isolates. Elevations of the isolated colonies were flat, raised and convex. The opacity of the isolates was translucent and opaque in nature. Further the colour pigments were absent in all pure colonies of isolates and appeared white to creamish in colour.

Various cultural and biochemical tests were studied for identification of *Lactobacillus* viz., motility and endospore test, catalase and sugar fermentation test and results of which are summarized in Table 3. Grams staining of bacterial isolates were done for distinguishing between Gram +ve and Gram -ve bacteria. The strains exhibited different cell shapes varying from rod to cocci. Out of thirty isolates 18 were Gram +ve rods whereas, 12 isolates were Gram +ve cocci in shape. Motility test showed that the isolates were non-motile, growing in the confined stab line. Endospore test revealed that bacteria were non-endospore forming. Catalase test study showed that in twelve isolates catalase enzyme was absent and other were not able to produce bubbles when mixed with H₂O₂. All the isolates were homofermentative and fermented glucose to acid with change of the colour of medium from red to yellow.

Table 1: Isolation of bacteria from dairy samples collected from Solan in Himachal Pradesh

Place of sample collection	Source of sample	Isolate No.	Growth on MRS Agar
Shilli	Curd	S1	+++
	Milk	S2	+++
	Milk	S3	++
	Curd	S4	+++
	Curd	S5	+
	Milk	S6	+++
	Milk	S7	++
	Curd	S8	++
Jatoli	Milk	J1	++
	Curd	J2	+++
	Curd	J3	+++
	Milk	J4	+
	Curd	J5	+++
	Milk	J6	++
	Curd	J7	+++
Oachghat	Curd	O1	+++
	Curd	O2	+++
	Milk	O3	+++
	Milk	O4	++
	Curd	O5	+++
	Curd	O6	+++
Tatuli	Milk	T1	+++
	Milk	T2	++
	Curd	T3	+++
	Curd	T4	++
Majhgaon	Milk	M1	++
	Milk	M2	+++
	Curd	M3	+++
	Milk	M4	+++
	Curd	M5	+++

+ = Minimum Growth, ++ = Moderate Growth, +++ = High Growth

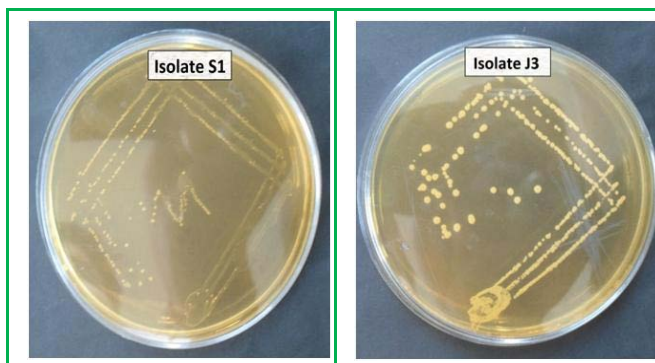


Figure 1: Isolates showing small and large colonies after growth on MRS agar medium

Table 2: Morphological characteristics of cultures isolated on MRS agar from dairy samples collected from Solan in Himachal Pradesh

Isolate No.	Type of colony	Colony colour	Margin	Elevation	Opacity	Pigment
S1	Small	Creamy white, shiny	Entire	Convex	Opaque	-
S2	Small	Off white	Entire	Raised	Translucent	-
S3	Small	Off white	Entire	Flat	Opaque	-
S4	Large	White, shiny	Entire	Convex	Opaque	-
S5	Large	Off white	Entire	Convex	Translucent	-
S6	Large	Creamy white, shiny	Entire	Convex	Opaque	-
S7	Small	White	Entire	Convex	Opaque	-
S8	Small	Creamy white	Entire	Convex	Opaque	-
J1	Large	Off white	Entire	Convex	Translucent	-
J2	Large	Creamy white, shiny	Entire	Convex	Opaque	-
J3	Large	White	Entire	Convex	Opaque	-
J4	Small	White, shiny	Entire	Convex	Opaque	-
J5	Large	Creamy white	Entire	Convex	Opaque	-
J6	Small	White	Entire	Convex	Opaque	-
J7	Large	Off white	Entire	Convex	Opaque	-
O1	Large	Off white	Entire	Convex	Translucent	-
O2	Small	White, shiny	Entire	Convex	Opaque	-
O3	Small	Creamy white	Entire	Convex	Opaque	-
O4	Large	white	Entire	Raised	Opaque	-
O5	Small	Creamy white	Entire	Convex	Opaque	-
O6	Small	White, shiny	Entire	Convex	Opaque	-
T1	Small	Off white	Entire	Convex	Translucent	-
T2	Large	Off white	Entire	Raised	Opaque	-
T3	Small	White	Entire	Convex	Opaque	-
T4	Large	Off white	Entire	Convex	Opaque	-
M1	Small	Creamy white, shiny	Entire	Convex	Opaque	-
M2	Small	Creamy white	Entire	Convex	Opaque	-
M3	Small	White	Entire	Raised	Opaque	-
M4	Large	Off white	Entire	Convex	Translucent	-
M5	Large	Creamy white, shiny	Entire	Convex	Opaque	-

-- Activity absent

In present study, thirty bacteria were isolated from dairy samples. Twelve bacterial isolates were identified as *Lactobacillus* by morphological, cultural and biochemical characteristic study. The results from the present study were compared with the literature. Ahmed and Kanwal (2004)¹¹ isolated different strains of lactic acid bacteria from Camel milk and all the strains were non-motile. All the 30 isolates reported in the present study and isolated from different dairy samples were also non-motile. El-Hadi Sulieman *et al.*(2006)¹² reported that the majority of lactic acid bacteria isolated from Garris (Sudanese traditional fermented camel milk) belongs to the genus *Lactobacillus*. Forouhanden *et al.*(2010)¹³ isolated lactic acid forming bacteria from different traditional and local cheese and yoghurt. Biochemical characterizations of all

the isolates were tested by the utilization of carbon sources. During fermentation of glucose acid was produced with change in colour from red to yellow. In present study, all isolates showed change in colour of sugar from red to yellow during fermentation of glucose. Goyal *et al.*(2012)¹⁴ isolated 28 different lactic acid forming bacterial strains from 14 curd samples. All the strains were Gram positive, non-spore forming and few of them were catalase negative. Similar observations were reported in the present study.

CONCLUSION

In present investigation, thirty bacterial isolates were isolated from dairy samples. All the isolates were characterized on the basis of colony morphology and

biochemical characteristics. Twelve bacterial isolates were identified as *Lactobacillus* by morphological, cultural and biochemical characteristics. Further study of selected

Lactobacillus isolates by 16S rDNA gene sequencing is being undertaken for its species identification.

Table 3: Culture and biochemical characteristics of pure isolates from dairy samples

Isolate No.	Gram Reaction	Morphology	Motility test	Endospore test	Catalase test	Sugar Fermentation
S1	+	Rods	-	-	-	H
S2	+	Rods	-	-	+	H
S3	+	Cocci	-	-	+	H
S4	+	Rods	-	-	-	H
S5	+	Cocci	-	-	+	H
S6	+	Rods	-	-	-	H
S7	+	Cocci	-	-	+	H
S8	+	Cocci	-	-	+	H
J1	+	Rods	-	-	+	H
J2	+	Rods	-	-	-	H
J3	+	Rods	-	-	-	H
J4	+	Cocci	-	-	+	H
J5	+	Cocci	-	-	+	H
J6	+	Rods	-	-	-	H
J7	+	Rods	-	-	+	H
O1	+	Cocci	-	-	+	H
O2	+	Rods	-	-	-	H
O3	+	Rods	-	-	-	H
O4	+	Cocci	-	-	+	H
O5	+	Rods	-	-	-	H
O6	+	Rods	-	-	-	H
T1	+	Rods	-	-	+	H
T2	+	Cocci	-	-	+	H
T3	+	Cocci	-	-	+	H
T4	+	Rods	-	-	+	H
M1	+	Rods	-	-	-	H
M2	+	Rods	-	-	+	H
M3	+	Cocci	-	-	+	H
M4	+	Cocci	-	-	+	H
M5	+	Rods	-	-	-	H

+ = Activity present, - = Negative Test, H = Homofermentation

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