

Isolation of Bile Tolerant Lactobacilli and Their Biochemical Characterization

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A total of 140 strains of lactobacilli were isolated on MRS agar medium supplemented with 0.2% of sodium taurocholate and 0.2% of sodium thioglycolate from 10 samples comprising of 4 samples of cow milk, 4 samples of curd, and 2 samples of camel milk. Among 140 strains 36 were isolated from cow milk, 12 from camel milk and 92 strains were isolated from curd samples. The isolated strains were identified on the basis of their cultural, morphology, and biochemical characteristics. Among the 140 isolates, only 68 isolates showed positive characteristics of genus *Lactobacillus*. A total of 82 of them showed precipitation of deoxycholic acid which was diffused in the surrounding medium. All of them showed gram-positive reaction, growth at 15 °C temperature, white colonies on MRS agar medium and produced yellowish colonies on BCP agar medium. All the 68 isolates showed negative test for catalase, no growth at 45 °C temperature and no gas production from glucose. Among the 68 isolates 35 isolates reduced litmus milk fast whereas 33 isolates were quite slow in this reaction.

Key words: *Lactobacillus*, isolates, biochemical reactions.

The lactic acid bacteria which are abundantly found in intestinal tract are lactobacilli, bifidobacteria, *Enetrococcus faecalis* and *E. faecium*. Among them, lactobacilli are the most common and important member of lactic acid bacteria which comprise a diverse group of gram-positive rod shape food-grade bacteria used as a starter in the preparation of fermented foods (Soomro *et.al.*, 2002). Most of the lactobacilli are represented by non-sporulating catalase negative, non aerobic but aerotolerant, fastidious and acid tolerant rods devoid of cytochromes,

producing organic acids during the fermentation of carbohydrates (Axelsson, 1998). Lactobacilli are also the key member of probiotic group known for exhibiting their health promoting functions in man and also in protecting the gut against foreign invasion (Nowroozi *et.al.*, 2004; Begley *et.al.*, 2006). Probiotics are live microbial food supplements (Fuller, 1989) which, when administered in adequate amounts beneficially affect the host by improving its microbial balance (Gregor, 2005; Farnworth, 2008). The present study was conducted to isolate and characterize the lactobacilli isolates from various sources.

MATERIAL AND METHODS

Isolation of Lactobacilli from various samples

The lactobacilli strains were isolated from various sources such as cow milk, curd and

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camel milk. Samples were collected in pre-sterilized glass vials. All samples were inoculated in MRS broth and incubated at 37°C for 24 hours and then plated out on MRS agar medium (De Man *et.al.* 1960).

Identification and phenotypic characterization of isolates

All the isolates were initially tested for cultural and morphological characteristics. Colony characteristics i.e. size, color, margin,

Table 1. Morphological, cultural and biochemical characteristics of the Selected *Lactobacilli* isolates

Characteristics	Results	Name of Isolates
Agar Colonies	White color (with deoxylic acid precipitation) White color (with out deoxylic acid precipitation)	¹ CM1-CM30,CM34-36, ² CL37-39,CL47-CL48, ³ CD49-CD70,CD76-CD96,CD121
Gram Reaction	Positive	CM31-CM33,CL40-CL46,CD71-CD75,CD97- CD120, CD122-CD140
Cell Shape	Short Rods	CM1-CM7, CM12-CM16,CM18-CM21,CM25, CM26, CM30,CM34-CM36,CL37-CL39 ,CL47, CL48, CD49-CD62,CD64-CD70,CD76,CD78- CD81,CD83-CD96, CD121
	Long Rods	CM27-CM30,CM34-CM36,CL37-CL39,CL47, CD48, CD49 ,CD62, CD64, CD80 ,CD83, CD89, CD93, CD95
Catalase Test	Negative	CM1-CM7, CM12-CM16,CM18-CM21,CM25, CM26, CM30,CM34-CM36,CL37-CL39,CL47, CL48,CD49-CD62,CD64-CD70,CD76,CD78- CD81,CD83-CD96, CD121
Growth at 15°C	Positive	CM1-CM7, CM12-CM16,CM18-CM21,CM25, CM26, CM30,CM34-CM36,CL37-CL39,CL47, CL48,CD49-CD62,CD64-CD70,CD76,CD78- CD81,CD83-CD96, CD121
Growth at 45°C	Negative	CM1-CM7, CM12-CM16,CM18-CM21,CM25, CM26, CM30, CM34-CM36,CL37-CL39, CL47, CL48, CD49-CD62,CD64-CD70,CD76,CD78- CD81,CD83-CD96, CD121
Litmus Milk Coagulation	Slow	CM26-CM30,CL37-CL48,CD51-CD60,CD61- CD70,CD76,CD78,CD79,CD80,CD82-CD88,CD90, CD91,CD92,CD94,CD96
	Fast	CM1-CD25,CM34-CM36,CD49,CD49,CD50,CD81, CD89,CD93,CD95,CD121
Gas from glucose	No gas production	CM1-CM7, CM12-CM16,CM18 CM21, CM25, CM26, CM30,CM34-CM36,CL37-CL39, CL47, CL48,CD49-CD62,CD64-CD70,CD76,CD78- CD81,CD83-CD96, CD121
Growth on BCP	Typical Yellowish Colonies	CM1-CM7, CM12-CM16,CM18-CM21, CM25, CM26, CM30,CM34-CM36,CL37-CL39, CL47, CL48,CD49-CD62,CD64-CD70,CD76,CD78- CD81,CD83-CD96, CD121

¹CM Source of isolation i.e. Cow Milk sample;

²CL Source of isolation i.e. Camel Milk sample.

³CD Source of isolation i.e. Curd sample.

elevation etc. were studied. The isolates were stained by Gram staining procedure and the microscopic examinations were taken under the Olumpus microscope (Model CH20 B1MF 200) on 100 X under oil immersion.

Biochemical characterization of isolates

The biochemical characterization of the isolates was done using different biochemical tests (Rogosa and Sharpe, 1959; Reque *et.al.* 2000). The biochemical tests included catalase test using 3%

hydrogen peroxide, growth at different temperature like 15°C and 45°C for 24 hours, growth in litmus milk, gas production from glucose, streaking on BCP-MRS agar medium.

Catalase test

This test was preformed by adding 2-3 drops of 3% hydrogen peroxide in fresh MRS broth cultures of isolates.

Growth at Different Temperature: Overnight grown cultures of isolates were streaked



Fig. 1. Isolated colonies of lactobacilli CM25 on MRS- agar medium supplemented with 0.2% sodium taurocholate and 0.2% of sodium glycocholate



Fig. 2. Growth of selected isolates on MRS agar medium (A) at 45°C no growth and (B) at 15°C thick growth

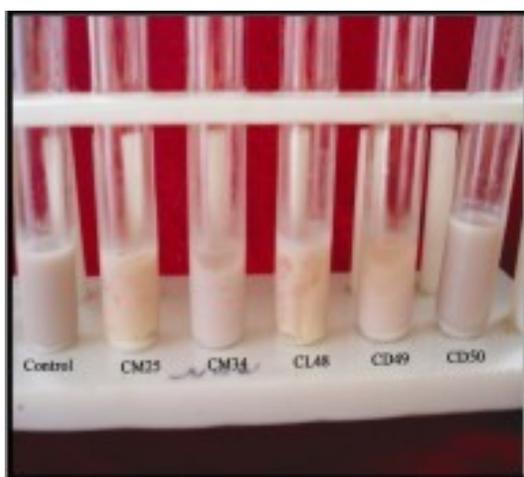


Fig. 3. The litmus milk reaction of selected isolates



Fig. 4. Growth of selected lactobacilli isolates on BCP agar medium

on MRS agar and incubated at 15°C and 45°C (Buchaman & Gibbons, 1974). The growth was checked after an incubation period of 24 hours.

Gas production from glucose: Overnight grown cultures of isolates were inoculated in MRS broth containing Durham tubes and incubated at 37°C. The gas production from glucose was checked after 24 hours (Davis, 1955).

Growth in litmus milk: One loop of overnight grown cultures of isolates were transferred into litmus milk and then incubated at 37°C for 24 to 48 hours (Gerhardt, 1981).

Growth on BCP-MRS agar medium

Overnight grown cultures of isolates were streaked on BCP-MRS agar medium. The plates were incubated at 37°C for 24 hours.

RESULTS AND DISCUSSION

A total 140 strains were isolated from 4 cow milk samples, 2 from camel milk samples and 4 from curd samples on MRS agar medium supplemented with 0.2% sodium taurocholate and 0.2% sodium glycocholate. Among the 140 isolates, 36 (CM1 - CM36) were isolated from cow milk, 12 (CL37 - CL48) from camel milk and 92 strains (CD49 - CD140) were isolated from curd samples. All the 140 isolates were tested for cultural, morphological and biochemical characteristics and the results for some selected isolates are presented in table-1. Colonies of the isolates were appeared off-white in color, smooth, shiny with entire margin and convex elevation. The isolated colonies of lactobacilli CM25 on MRS agar medium supplemented with 0.2% sodium taurocholate and 0.2% sodium glycocholate is shown in Fig. 1. Similar type of colony morphology was reported for the lactobacilli isolated by Brenner, 1986. In the colonies of 82 isolates deoxycholic acid precipitated and diffused into the surrounding medium. Similar kind of precipitation around lactobacilli colonies was also reported by Dashkevicz & Feighner 1989. The precipitation observed in the study, may be due to the deconjugation activity of *Lactobacillus* colonies that results in the production of deoxycholic acid. No such precipitation was observed in the remaining

58 isolates. Among 140 isolates 68 isolates showed gram- positive reaction and rest of them showed gram- negative reaction. All of them were appeared as rods but differed in size (Table 1). Among 140 isolates 68 showed catalase negative test and rest of the 72 isolates showed catalase positive test. On the basis of Gram reaction and catalase test 68 isolates were selected for further biochemical reactions. All the 68 isolates showed growth at 15°C and didn't showed growth at 45°C. The result of few selected isolates for the same is shown in Fig. 2. It is evident from table-1 that 35 were reduced litmus milk fast whereas 33 isolates were quite slow in the reaction. The litmus milk reaction for few isolates is shown in Fig.3. These results are in conformity with Batish *et.al.* (1990). All the 68 isolates didn't produce gas from glucose but produced typical yellowish colonies on BCP - MRS agar medium. Typical yellowish colonies produced by few lactobacilli isolates are shown Fig. 4.

CONCLUSION

In the present study a total of 68 isolates were tentatively identified as *Lactobacillus* on the basis of cultural, morphology, and biochemical characteristics. All the 68 isolates have the capability to tolerate the bile salt. These isolates also bear the deconjugation activity. Further research related to the detection of bile salt hydrolase activity can be done to explore the probiotic potential of these isolates.

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