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Isolation and identification of genus *Lactobacillus* from different curd samples

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ABSTRACT

Lactobacillus is a genus of lactic acid bacteria. Lactic acid bacteria mainly found in fermented dairy products. This study was carried out for the identification and characterization of *Lactobacillus* isolated from curd. A total of 14 curd samples were collected from different places of Gurgaon (Haryana) and Lakshmangarh (Rajasthan). Their identification was carried out based on their morphological and biochemical characteristics. A total of 28 isolates were screened on the basis of their phenotypic characteristics. *Lactobacillus* showed cream-white colonies, rod-shaped, gram-positive, non-spore forming, non-motile, and catalase negative bacteria. Some of the *Lactobacillus* species are also showing the characteristics of homofermentative or heterofermentative categories.

Key words: *Lactobacillus*, curd samples, Lactose.

INTRODUCTION

Lactobacillus is a gram-positive, facultative anaerobic bacteria. They are the major part of lactic acid bacteria group because most of its members convert lactose and other sugars to lactic acid. Some of the species of *Lactobacillus* are homofermentative and some are heterofermentative, a few of them are homo and heterofermentative. *Lactobacillus* species can be selected on solid culture media having high acidic pH.

Among all lactic acid bacteria, the genus *Lactobacillus* has some beneficial characteristics which make it useful for the industrial applications. They can resist weak acids pH 3.5-4.5 and the yield of lactic acid is 90%.

Lactic acid bacteria play an important role in food fermentation, as the products obtained with their aid characterized by hygienic safety, storage stability and attractive sensory properties. The ability to produce lactic acid from lactose is probably the most important property of dairy lactic acid bacteria, known as lactic acid fermentation.

Lactobacillus is highly used in controlled fermentation. Lactic acid bacteria widely used in traditional fermented milk, in industrial, fermentation process and starter culture in dairy industry. Lactic acid bacterial starter cultures are used to produce lactic acid at a suitable rate for the fermentation process. The primary function of bacterial culture is to convert the lactose into glucose or galactose, and then these sugars are converted to the end product of lactic acid. Now a day's lactic acid is being used by the food industry as acidulent and preservative for the production of cheese and yoghurt.

Genus *Lactobacillus* isolated from curd shows absence of catalase activity. The genus produces lactic acid during fermentation of glucose which can be detected by Hugh and Leifson's test.

The objective of the present study was aimed to identify and characterised the strains of lactic acid bacteria isolated from the curd samples on the basis of their phenotypic characterization.

MATERIAL AND METHODS

Sample collection

A total of 14 samples of curd were collected from different places of Gurgaon (Haryana) and Lakshmangarh (Rajasthan) and stored at 4ºC. Then all the samples were taken to the Biotechnology and Microbiology Laboratory of MITS (Deemed University), Lakshmangarh for further microbiological analysis.

Isolation of the Bacteria

Selective media for lactic acid bacteria is MRS (de Man, Rogosa and Sharpe) agar media. A loopful of the samples was streaked on the sterile MRS agar petri plate by quadrant streaking method, under aseptic conditions. After streaking all the petri plates were incubated at 37ºC for 24-48 hours. Then from these plates isolated colonies were restreaked on the MRS agar slants and then stored at 4ºC.

Phenotypic Characterization

Identification of the isolates was performed on the basis of their morphological and biochemical characteristics as followed:

Gram Staining

Initially identification of the isolates was done by gram staining method (Hans Christian Gram, 1884).

Microscopic Morphology

Gram staining slides were observed under microscope oil immersion.

Endospore Test

Bacterial smear was made on microscopic slide under aseptic conditions and heat fixed. Then slide was placed over the steaming water bath and malachite green (primary stain) was applied for 5 minutes. Slide was removed from the water bath and rinsed with water until water run clear. Then the slide was flooded with the counter stain safranin for 20 seconds and rinsed with water. Then slides were blot dried and observed under the light microscope.

Hugh and Leifson's test

The purpose of this test was to determine whether an organism is an oxidizer or a fermenter on the basis of production of acid in aerobic and anaerobic conditions. Hugh and Leifson's medium was prepared into culture tubes. All these test tubes were autoclaved at 121ºC for 15 minutes. A syringe filter sterilized solution of 10% carbohydrate (glucose) was aseptically added to the medium to a final concentration of 1%. Medium was cooled and inoculated by stabbing with the test organism. After stabbing, all the culture tubes were kept in incubator under aerobic and anaerobic conditions at 37ºC for 24-48 hours. After incubation, all the test tubes were observed for fermentation.

Motility test

Hugh and Leifson's medium was also used for the testing that the bacteria were motile or non-motile through stab inoculation.

Catalase test

This test was used to check the production of enzyme catalase. For this test a clean microscopic slide was taken. A drop of 3% H_2O_2 was taken on the slide aseptically. A loopful of bacterial culture was taken and mixed with 3% H_2O_2 solution on the slide and the presence of the bubble production was observed.

Sugar Fermentation test

Approximate 100 ml of the nutrient broth solution was prepared in conical flask and 1 ml phenol red was added to it. This medium was autoclaved at 121ºC for 15 minutes and cooled at room temperature. A syringe filter sterilized solution of 1% glucose was prepared under aseptic conditions.

In all sterilized test tube 5ml of the broth and 100µl of the glucose solution was taken and labelled. All the test tubes were kept at room temperature for 24 hours to check the contamination. After 24 hours, all these test tubes were inoculated with freshly grown bacterial culture and incubated at 37ºC for 48 hrs.

In case of homofermentation colour changes from red to yellow. And in heterofermentation there is gas production in Durham tube along with the change in the colour.

RESULTS

A total of 28 different isolates were isolated from 14 different curd samples after streaking a loopful of curd sample on the MRS agar plate by quadrant streaking method. Two different types of small and large creamy to white coloured colonies were obtained.

Gram staining of the bacterial culture showed they were gram positive and their cell morphology was rod shaped and some of them were coccoid shaped.

Endospore test showed that the bacteria were non-spore forming, showing negative result (red colour) instead of forming positive result (green colour). Hugh and Leifson's test showed that all the bacterial cultures were capable of producing fermentation. Fermentative organisms developed acidic conditions in anaerobic conditions, while oxidative organisms produced acid in aerobic conditions (Fig. 1).

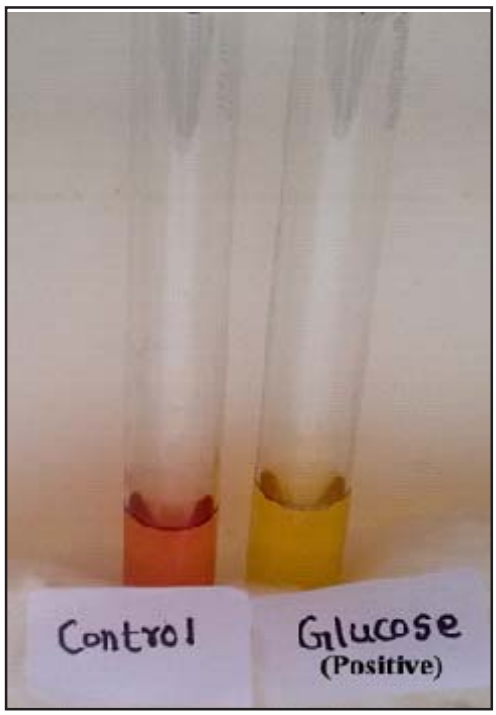

Fig. 2: Sugar Fermentation test

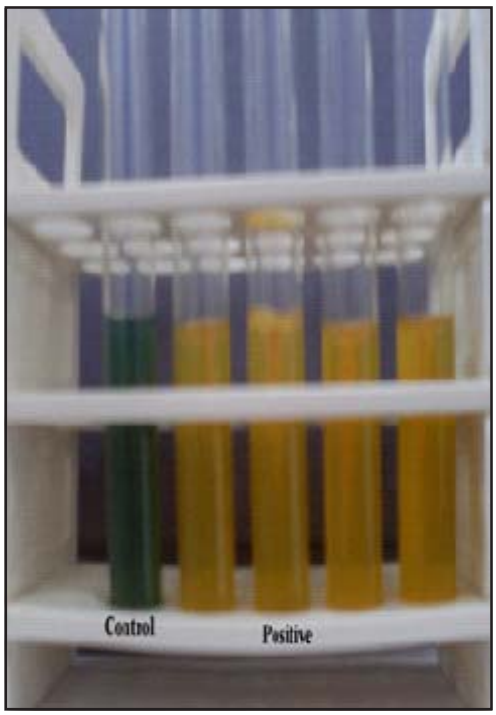

Fig. 1: Hugh and Leifson's test

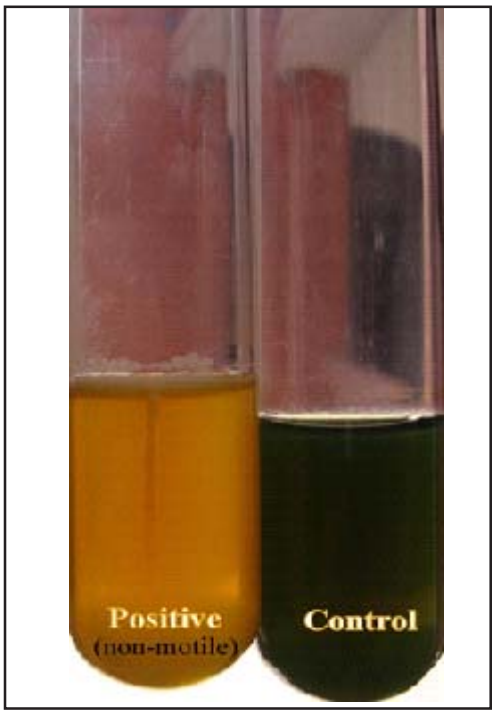

Fig. 3: Motility test

Table 1: Identification of bacteria

S. No.	Isolates	Types of colonies	Gram staining	Microscopic morphology	Endo-spore test	Hugh and leifson's test	Motility test	Catalase test	Sugar fermen-tation test
1	S1	Small	+	Rod shaped	-	F	-	+	H
	L1	Large	+	Rod shaped	-	F	-	+	H
2	S2	Small	+	Rod shaped	-	F	-	+	H
	L2	Large	+	Rod shaped	-	F	-	+	H
3	S3	Small	+	Rod shaped	-	F	-	+	H
	L3	Large	+	Rod shaped	-	F	-	+	H
4	S4	Small	+	Rod shaped	-	F	-	+	H
	L4	Large	+	Rod shaped	-	F	-	-	H
5	S5	Small	+	Rod shaped	-	F	-	-	H
	L5	Large	+	Rod shaped	-	F	-	+	H
6	S6	Small	+	Rod shaped	-	F	-	+	H
	L6	Large	+	Cocci	-	F	-	-	H
7	S7	Small	+	Cocci	-	F	-	+	H
	L7	Large	+	Cocci	-	F	-	+	H
8	S8	Small	+	Rod shaped	-	F	-	+	H
	L8	Large	+	Rod shaped	-	F	-	+	H
9	S9	Small	+	Rod shaped	-	F	-	+	H
	L9	Large	+	Rod shaped	-	F	-	-	H
10	S10	Small	+	Cocci	-	F	-	+	H
	L10	Large	+	Rod shaped	-	F	-	+	H
11	S11	Small	+	Rod shaped	-	F	-	+	H
	L11	Large	+	Rod shaped	-	F	-	+	H
12	S12	Small	+	Rod shaped	-	F	-	-	H
	L12	Large	+	Rod shaped	-	F	-	+	H
13	S13	Small	+	Rod shaped	-	F	-	-	H
	L13	Large	+	Rod shaped	-	F	-	-	H
14	S14	Small	+	Cocci	-	F	-	+	H
	L14	Large	+	Rod shaped	-	F	-	+	H

Key, F-Fermentation, H-Homofermentation (production of lactic acid but not gas)

And when this medium culture tubes were used to determine the motility of bacteria, then it was found that the bacteria were non-motile, growing in a confined stab line (Fig. 3) instead of making the whole medium turbid.

Catalase test showed that the only 7 strains out of 28 strains did not produce bubbling when mixed with3% H_2O_2 this showed that there was absence of catalase enzyme. The absence of catalyse enzyme showed that identified bacteria was from *Lactobacillus* species.

Further, if these cultures were used for sugar fermentation showed that the bacteria was of homofermentative nature by changing colour of the medium (fig: 2) from red to yellow because of acid production.

DISCUSSION

Mehmood *et al* (2009) isolated *Lactobacillus* from dairy samples. All the isolates were gram positive, rod shaped, non- spore forming and showing fermentation and the absence of

catalase enzyme. In our present investigation a total of 28 isolates from curd were screened for various characteristics and it was found that the all isolates were gram positive , rod shaped, non-spore forming, and a few of them were catalase negative.

Catalase test was used for the detection of the enzyme catalase which has been involved in conversion of H_2O_2 into H_2O and O_2. A positive result was screened by the presence of the bubble (O_2), indicated that bacteria survive under aerobic conditions. During aerobic respiration bacteria produce hydrogen peroxides, a natural by product of metabolism. In our present investigation few of strains were showing the absence of catalase enzyme.

Forouhandeh *et al* (2010) and Mehmood *et al.,* (2009) determined that during sugar fermentation test, *Lactobacillus* produce acid from glucose while gas was not produced. In our investigation acid production was evidence by change in colour of sugar from red to yellow. However there was no gas production in Durham tube.

Ahmed and Kanwal (2004) isolated different strains of *Lactobacillus* from camel milk and reported that all the strains were non-motile. In our present study it was found that the strains which we isolated from curd samples were also showing the characteristics of non-motile nature. Coeuret *et al* (2003) isolated, characterised, and identified the *Lactobacilli* mainly from cheese and other dairy products and reported that *Lactobacilli* species were non-spore forming. In our results all the strains were non-spore forming.

Nair and Surendran (2005) isolated lactic acid bacteria from various samples of fresh and frozen fish and prawn. All the cultures were identified on the basis of their morphological, cultural, physiological and biochemical characteristics. For Hugh and Leifson's test oxidation-fermentation medium used for evaluating the production of acid and gas from 1% glucose. And reported that some of the strains were showing positive result and some were negative result. In our isolates all of the strains were showing the positive result with the production of acid.

CONCLUSION

A total of 28 different strains were isolated from 14 different curd samples were performed. All the strains were identified on the basis of their colonies morphology and other biochemical characteristics. And it was found that all the strains were non-motile, non-spore forming, Gram-positive, rod shaped bacteria that produce lactic acid homofermentatively from glucose. Few strains were showed the absence of catalase enzyme because bacteria use peroxidase to detoxify H_2O_2, an enzyme that does not evolve O_2.

It was concluded that we successfully isolated *Lactobacillus* from curd samples by using a range of *Lactobacillus*- specific morphological and biochemical test to determine the presence of *Lactobacillus* in the isolates. In our investigation we could not isolates *Lactobacillus* from all the curd samples. Only out of 28 isolates, seven isolates will show positive test for *Lactobacillus*.

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