African Journal of Biotechnology Vol. 11(79), pp. 14448-14452, 2 October, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.310
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Characterization of the *Lactobacillus* isolated from different curd samples

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Accepted 16 February, 2012

Lactic acid bacteria are commonly found in the fermented dairy products. *Lactobacillus* is a genus of lactic acid bacteria and described as heterogeneous group of regular, non-spore forming, gram-positive, rod shaped, non-motile bacteria and absence of catalase enzyme. The aim of this study was to isolate *Lactobacillus* from different curd samples. A total of 14 curd samples were collected from the local areas of Gurgaon (Haryana) and Lakshmangarh (Rajasthan). From these, 28 isolates were obtained by growing on de Man, Rogosa and Sharpe (MRS) agar medium and characterised by their phenotypic characteristics. The *Lactobacillus* isolates also possess homofermentative and heterofermentative characteristics.

Key words: Lactobacillus, curd, microorganism

INTRODUCTION

Microorganisms are important in dairy products. One of the most important groups of acid producing bacteria in the food industry is the lactic acid bacteria, which are used in making starter culture for dairy products.

Genus *Lactobacillus* contains over 110 species, which are classified in three major groups: obligate homofermentative *Lactobacillus* which ferment hexoses to lactic acid; facultative homofermentative *Lactobacillus* which ferment hexoses to lactic acid only or together with acetic acid, ethanol, formic acid under glucose limitation and obligate heterofermentative *Lactobacillus* fermenting hexoses to lactic acid, acetic acid, ethanol, CO_2, and ferment pentoses to lactic acid and acetic acid (Amin et al., 2009).

Lactic acid bacteria play an important role in dairy and meat fermentation process and have a great influence on the quality and preservation of the end products. Lactic acid bacteria are characterised as gram positive, usually non-motile, non-sporulating that produce lactic acid as a major product of fermentative metabolism. The preservative effect of lactic acid bacteria during the manufacture and subsequent 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. They can resist weak acids of pH 3.5 to 4.5 resulting to a yield of 90% lactic acid. *Lactobacillus* is highly used in controlled fermentation. Lactic acid bacteria are widely used in traditional fermented milk, in industrial fermentation process and starter culture in the dairy industry. The major function of the starter culture was to produce lactic acid at a suitable rate for the fermentation process. Lactic acid is used today by the food industry as acidulent and preservative for the production of cheese and yoghurt.

Conformation of the *Lactobacillus* isolated from curd by testing for the absence of catalase enzyme and the presence of acid produced fermentation of glucose. This present study was to characterize the *Lactobacillus* isolated from different curd samples on the basis of their phenotypic characteristics.

MATERIALS AND METHODS

Sample collection

A total of 14 samples of curd were collected from local areas of Gurgaon (Haryana) and Lakshmangarh (Rajasthan). Then all these

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samples were stored at 4°C. Afterwards all these samples were taken to the Biotechnology and Microbiology laboratory of MITS, Lakshmangarh (Deemed University) for further microbiological analysis.

Isolation of the *Lactobacillus*

The medium which was selected for the lactic acid bacteria was de Man, Rogosa, and Sharpe (MRS) agar medium. A loopful of the curd samples was streaked on the sterile MRS agar Petri plate by quadrant streaking method, under aseptic conditions. After streaking all the Petri plates, they were incubated at 37°C for 24 to 48 h. After the incubation, colonies were restreaked on the MRS agar Petri plate for the formation of isolated colonies. Then from these plates isolated colonies were restreaked on MRS agar slants and stored at 4°C.

Phenotypic characterization

Characterization of all the isolates was performed on the basis of their morphological and biochemical characteristics as described.

Morphological examination of culture

Morphological and cultural examination was carried out by using Gram's staining method described by Hans Christian Gram (1884).

Identification of the pure culture

Pure culture isolated on MRS agar slant was identified with the help of biochemical tests like endospore test, Hugh and Leifson's test, motility test, catalase test and sugar fermentation test (Table 1).

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 min. 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 s and rinsed with water. After these slides were blot dried, they were 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. Then these test tubes were autoclaved at 121°C for 15 min. A 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 37°C for 24 to 48 h. After incubation, all the test tubes were observed for fermentation.

Motility test

Hugh and Leifson's medium was also used for the testing if 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 microscopic 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 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 121°C for 15 min and cooled at room temperature. A syringe filter sterilized solution of 1% glucose was prepared under aseptic conditions.

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

In case of homofermentation, there will be production of acid along with the change in colour of the medium from red to yellow, and in heterofermentation there will be gas production in Durham tube alongside the change in the colour.

RESULTS

From the tested samples, 28 presumptive lactic acid producing strains were isolated from 14 different curd samples. Colonies were observed on the surface of MRS agar Petri plate. More than one type of colony was observed on surface of MRS agar Petri plate. Small and large two types of colonies were observed. Most of colonies were creamy to white. The cultural and morphological characteristics were further resolved on the basis of microscopic examination. Majority of the microorganisms were Gram positive rods and cocci shaped bacteria. After characterization, some of them were determined as representative of lactic acid cocci and the rest of isolates were referred to genus *Lactobacillus*. The strains were phenotypically characterized on the basis of their morphological, cultural, and biochemical characteristics. Gram's staining of the bacterial culture showed they were gram positive and their cell morphology was rod shaped and some of them were coccid shaped. Endospore test showed that the bacteria were non-endospore 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. And when these 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 instead of making the whole medium turbid (Figure 1).

Catalase test showed that the only seven isolates out of 28 isolates were not able to produce bubbling when mixed with 3% H_2O_2. This showed that there was absence

Table 1. Identification of bacteria.

S/N	Isolate	Types of colony	Gram staining	Microscopic morphology	Endospore test	Hugh and Leifson's test	Motility test	Catalase test	Sugar fermentation test
1	S1	Small	+	Rods	-	F	-	+	H
	L1	Large	+	Rods	-	F	-	+	H
2	S2	Small	+	Rods	-	F	-	+	H
	L2	Large	+	Rods	-	F	-	+	H
3	S3	Small	+	Rods	-	F	-	+	H
	L3	Large	+	Rods	-	F	-	+	H
4	S4	Small	+	Rods	-	F	-	+	H
	L4	Large	+	Rods	-	F	-	-	H
5	S5	Small	+	Rods	-	F	-	-	H
	L5	Large	+	Rods	-	F	-	+	H
6	S6	Small	+	Rods	-	F	-	+	H
	L6	Large	+	Cocci	-	F	-	-	H
7	S7	Small	+	Cocci	-	F	-	+	H
	L7	Large	+	Cocci	-	F	-	+	H
8	S8	Small	+	Rods	-	F	-	+	H
	L8	Large	+	Rods	-	F	-	+	H
9	S9	Small	+	Rods	-	F	-	+	H
	L9	Large	+	Rods	-	F	-	-	H
10	S10	Small	+	Cocci	-	F	-	+	H
	L10	Large	+	Rods	-	F	-	+	H
11	S11	Small	+	Rods	-	F	-	+	H
	L11	Large	+	Rods	-	F	-	+	H
12	S12	Small	+	Rods	-	F	-	-	H
	L12	Large	+	Rods	-	F	-	+	H
13	S13	Small	+	Rods	-	F	-	-	H
	L13	Large	+	Rods	-	F	-	-	H
14	S14	Small	+	Cocci	-	F	-	+	H
	L14	Large	+	Rods	-	F	-	+	H

F, Fermentation; H, homofermentation (acid production not gas).

of catalase enzyme. The absence of catalase enzyme showed that identified bacteria were from *Lactobacillus* species. The bacteria use peroxidase to detoxify H_2O_2, an enzyme that does not evolve O_2. Further, if these bacterial cultures were used for sugar fermentation, it showed that the bacteria were homofermentative. The microorganism fermented glucose to acid which was evident by changing colour of medium from red to yellow

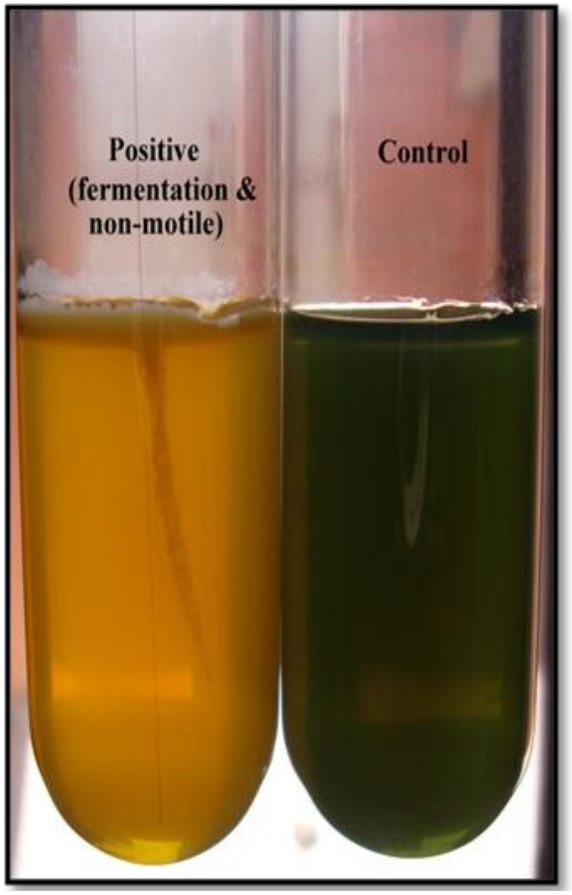

Figure 1. Hugh and Leifson's test and motility test.

(Figure 2). There was no gas production in the Durham's tube.

DISCUSSION

Guessas and Kihal (2004) isolated lactic acid bacteria from goats milk in Algerian arid zone and reported that all the isolates were gram positive, catalase negative and non- spore forming. In our present study, we isolated lactic acid forming bacteria from different curd samples. We 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 showing catalase negative.

Zourari et al. (1992) reported that lactic acid bacteria are facultative anaerobes with a preference of anaerobic conditions. They cannot synthesise porphyrins and consequently they do not synthesise cytochromes or catalase. Oxygen is sometimes used for formation of hydrogen peroxide, which is toxic for lactic acid bacteria and do not contain catalase to break it down. Aerobic organisms that have the enzyme catalase break down hydrogen peroxide in following reaction:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

In our investigation, some of the isolates showed the absence of catalase enzyme and these were from the genus *Lactobacillus*.

Ahmed and Kanwal (2004) isolated different strains of lactic acid bacteria from camel milk. They reported that all the strains were non-motile. All the 28 isolates that we isolated from different curd were also non-motile growing in a confined stab line.

Forouhanden et al. (2010) 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 it was reported that acid was produced not gas in Durham's tube. In our present investigation, all the 28 isolates during fermentation of glucose acid was evidence by change in colour of sugar from red to yellow without production of any gas in the Durham's tube. And the bacterial cultures were found to be homofermentative after glucose fermentation test.

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

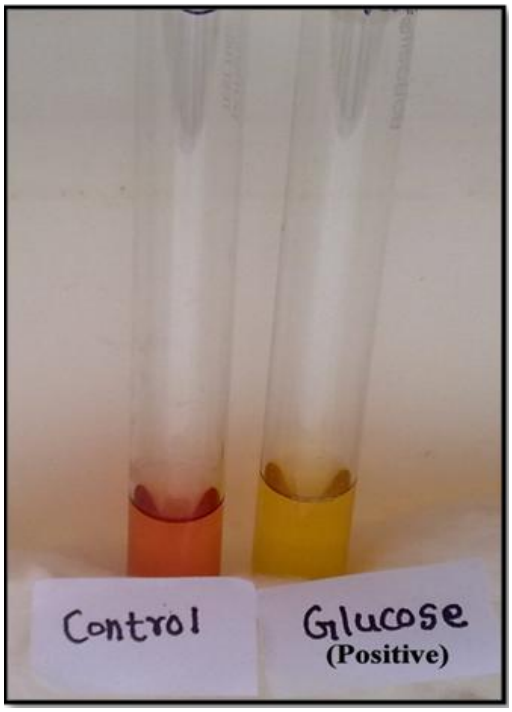

Figure 2. Sugar fermentation test.

characteristics. For Hugh and Leifson's test, oxidation-fermentation medium was used for evaluating the production of acid and gas from 1% glucose and reported that some of the strains showed positive result and some were negative result. In our isolates, all of the strains showed positive result with the production of acid.

Conclusion

From a number of different 14 curd samples, 28 different strains were isolated. All the strains were characterized on the basis of their colonies morphology and other biochemical characteristics. Colonies were circular, small and large and cream-white after incubation on MRS agar plate. All the strains studied were non-motile, non-spore forming, Gram-positive, rod-shaped bacteria that produce lactic acid homofrementatively from glucose. In most of the strains, catalase was not produced.

It was concluded that all the comparative studies including cultural, morphological and identification of the pure culture by biochemical tests showed that the strains that does not produce catalase enzyme were *Lactobacillus*. Only seven strains showed the positive results for *Lactobacillus*.

ACKNOWLEDGEMENT

We thank Dr. R. K. Gaur, Head of the Department of Biotechnology and Microbiology and Dr. S. Jhakoria, Dean of FASC, MITS Lakshmangarh (Deemed University) for their support in completing the work.

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