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Isolation and Screening of Lactic Acid Bacteria from Dairy and Fermented products

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ABSTRACT: *A survey was conducted on isolation and characterization of lactic acid bacteria from dairy products from Rahuri taluq Ahmednagar district of Maharashtra. Around 60 isolates of lactic acid bacteria from different sources such as curd, idli batter, paneer, milk and khawa. Among the total 60 isolates were isolated from different sources, 10 isolates from Curd, 18 isolates from Idli batter, 10 isolates from Paneer, 8 isolates from Khova and 14 isolates from Milk. Further these isolates were screened on MRS media, among 60 isolates 32 isolates were able to grow on MRS media. In which 6 isolates from Curd, 8 isolates from Idli batter, 4 isolates from Paneer, 5 isolates from Khova and 9 isolates from Milk. Further these isolates subjected to different biochemical characteristic.*

Keywords: *Lactic acid bacteria, MRS.*

INTRODUCTION

Lactobacillales or lactic acid bacteria Gram-positive, low-GC, acid-tolerant, generally non-sporulating, no respiring, either rod- or cocci-shaped bacteria that share common metabolic and physiological characteristics Lactic Acid Bacteria (LAB) are Gram-positive, non-sporeforming cocci, coccobacilli or rods with a DNA base composition of less than 53 mol% G + C. They generally are non respiratory and lack catalase. They ferment glucose primarily to lactic acid, or to lactic acid, CO_2 and ethanol. All LAB grow an aerobically, but unlike most anaerobes, they grow in the presence of O_2 as "aero tolerant anaerobes". Although they lack catalase, they possess superoxide dismutase and have alternative means to detoxify peroxide radicals, generally through peroxidase enzymes.

In contrast to vegetables, fruits have good record from public health standpoint. Many fruits possess a natural defense mechanism. Fruits contain organic acids in quantities adequate enough to contribute a pH value of 4.6 or lower. The pH and the type of the acid itself are the major influence that select for predominant micro flora in fruits. Acid tolerant microbes like fungi Splittstoesser (1) and lactic acid bacteria are demonstrated as a part of autochthonous micro flora of tomatoes owing to its low pH and organic acids Brackett, (2); Sajur (3). Food borne bacteria capable of causing human illness cannot grow at a pH less than 4.0, so edible portion of most fruits precludes the involvement as substrate for proliferation of human pathogen.

Lactic acid bacteria (LAB) form a taxonomically diverse group of microorganisms that can convert fermentable carbohydrates into lactic acids. A large number of LAB species are involved in the production and consumption of fermented foods and beverages. Most LAB are omnipresent members of the intestinal flora. Bacteria in the human intestine play an important role in human physiology, most of which are beneficial or neutral for the host.

Although many genera of bacteria produce lactic acid as a primary or secondary end-product of fermentation, the term Lactic Acid Bacteria is conventionally reserved for genera in the order *Lactobacillales*, which includes *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus* and *Streptococcus,* in addition to *Carnobacterium, Enterococcus, Oenococcus,*

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Tetragenococcus, Vagococcus, and *Weisella.* Stiles (5), Lactic acid bacteria (LAB) are widely distributed in nature and occur naturally as indigenous microflora in raw milk, drinking yoghurt, etc. They are gram positive bacteria that play an important role in many food fermentation processes. Some species of the genus Lactobacillus (Lb.), Lactococcus (Lc.) and Leuconostoc (Ln.) are included in this group. The lactic acid fermentation has long been known and applied by humans for making different food stuffs. For many centuries, LAB has been an effective form of natural preservation. In addition, they strongly determine the flavour, texture and frequently, the nutritional value of food and feed products. However the application of well-studied starter cultures has been established for decades (Lee, 4; Tserovska 5).

Lactic acid bacteria are among the most important groups of microorganisms used in food fermentations. They contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid. As agents of fermentation LAB are involved in making yogurt, cheese, cultured butter, sour cream, sausage, cucumber pickles, olives and sauerkraut, but some species may spoil beer, wine and processed meats. Hence due to its outstanding functional properties the investigation was carried out at M.P.K.V., Rahuri on Isolation and Screening of Lactic Acid Bacteria from Dairy and fermented products.

MATERIAL METHODS

Isolation and Identification of LAB Strains

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. (Vijai Pal 6)

Phenotypic Characterization

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

Table 1
Isolation of lactic acid bacteria from different sources

Sr. No.	*Tentative isolate code*	*Location*	*Source of sample*
1.	LAB-1	Rahuri	Curd
2.	LAB-2	Rahuri	
3.	LAB-3	Rahuri	
4.	LAB-4	Rahuri	
5.	LAB-5	Rahuri	
6.	LAB-6	Rahuri	
7.	LAB-7	Rahuri	
8.	LAB-8	Rahuri	
9.	LAB-9	Rahuri	
10.	LAB-10	Rahuri	
11.	LAB-11	Rahuri	Idli better
12.	LAB-12	Rahuri	
13.	LAB-13	Rahuri	
14.	LAB-14	Rahuri	
15.	LAB-15	Rahuri	
16.	LAB-16	Rahuri	
17.	LAB-17	Rahuri	
18.	LAB-18	Rahuri	
19.	LAB-19	Rahuri	
20.	LAB-20	Rahuri	
21.	LAB-21	Rahuri	
22.	LAB-22	Rahuri	
23.	LAB-23	Rahuri	
24.	LAB-24	Rahuri	
25.	LAB-25	Rahuri	
26.	LAB-26	Rahuri	
27.	LAB-27	Rahuri	
28.	LAB-28	Rahuri	
29.	LAB-29	Rahuri	Paneer
30.	LAB-30	Rahuri	
31.	LAB-31	Rahuri	
32.	LAB-32	Rahuri	
33.	LAB-33	Rahuri	
34.	LAB-34	Rahuri	
35.	LAB-35	Rahuri	
36.	LAB-36	Rahuri	
37.	LAB-37	Rahuri	
38.	LAB-38	Rahuri	
39.	LAB-39	Rahuri	
40.	LAB-40	Rahuri	Khova
41.	LAB-41	Rahuri	
42.	LAB-42	Rahuri	
43.	LAB-43	Rahuri	
44.	LAB-44	Rahuri	
45.	LAB-45	Rahuri	
46.	LAB-46	Rahuri	
47.	LAB-47	Rahuri	
48.	LAB-48	Rahuri	Milk
49.	LAB-49	Rahuri	
50.	LAB-50	Rahuri	
51.	LAB-51	Rahuri	
52.	LAB-52	Rahuri	
53.	LAB-53	Rahuri	
54.	LAB-54	Rahuri	
55.	LAB-55	Rahuri	
56.	LAB-56	Rahuri	
57.	LAB-57	Rahuri	
58.	LAB-58	Rahuri	
59.	LAB-59	Rahuri	
60.	LAB-60	Rahuri	

Table 2
Characterization of LAB isolates

Sr. No.	*Isolate code*	*Source of sample*
1.	LBD-1	Curd
2.	LBD2	Curd
3.	LBD-3	Curd
4.	LBD-4	Curd
5.	LBD-5	Curd
6.	LBD-6	Curd
7.	LBI-1	Idli batter
8.	LBI-2	Idli batter
9.	LBI-3	Idli batter
10.	LBI-4	Idli batter
11.	LBI-5	Idli batter
12.	LBI-6	Idli batter
13.	LBI-7	Idli batter
14.	LBI-8	Idli batter
15.	LBP-1	Paneer
16.	LBP-2	Paneer
17.	LBP-3	Paneer
18.	LBP-4	Paneer
19.	LBK-1	Khova
20.	LBK-2	Khova
21.	LBK-3	Khova
22.	LBK-4	Khova
23.	LBK-5	Khova
24.	LBM-1	Milk
25.	LBM-2	Milk
26.	LBM-3	Milk
27.	LBM-4	Milk
28.	LBM-5	Milk
29.	LBM-6	Milk
30.	LBM-7	Milk
31.	LBM-8	Milk
32.	LBM-9	Milk

Morphological Examination of Culture

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

BIOCHEMICAL CHARACTERIZATION

Lactose Utilization

In lactose utilization test, the selected colonies were then streaked onto nutrient agar NA with the addition of lactose containing 0.005 g/L bromo-cresol purple, as a pH indicator dye. The plates were incubated at 30°C for 24 h. Isolates that were able to utilize lactose and produce acid were differentiated by the change of media color from violet to yellow. Ahmed (7).

Nitrate Reduction Test

The basic principal of nitrate reduction test was, if a bacterium producing nitrate reductase is grown in a medium containing nitrate, the enzyme converts the nitrate to *nitrite*. *Nitrite* reacts with certain chemicals to yield a red-colored product. If the bacterium also produces *nitrite reductase*, nitrogen gas will be liberated. Bubbles collecting in an inverted Durham tube indicate that nitrogen has been produced.

An inoculums from a pure culture was transferred aseptically to a sterile tube of nitrate broth containing an inverted Durham tube. The inoculated tube was incubated at 35-37 C for 24 hours and the results were determined. A positive test for both enzymes consists of a turbid (cloudy) broth with pronounced gas bubbles trapped in the Durham tube. If results like this are not observed, testing for the individual enzymes can be done through addition of reagents, with a positive test indicated by the broth turning red.

Catalase Activity

Catalase activity was tested by adding a drop of 30% hydrogen peroxide solution onto the cell smears. Positive reaction would be seen as bubbles or froths generated from the colonies, indicating a rapid production of oxygen gas. Only isolates that showed negative reaction were subjected to further identification test.

Indole Test Methyl Red Test VP Citrate Utilization Test

ImVIC test were carried according to standard protocol in methyl red medium.

Oxidase Tests

Fresh cultures (18 to 24 hours) of LAB were grown on nutrient agar using the streak plate method so that well-isolated colonies were present. One or two drops of 1% Kovács oxidase reagent were placed on the organisms. Do not invert or flood plate.

Further plates were observed for color changes. Microorganisms were oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms were oxidase negative if the color does not change or it takes longer than 2 minutes. (Jurtshuk 8)

RESULT AND DISCUSSION

The lactic acid bacteria were isolated from indigenous dahi samples. The isolation was performed by the routine microbiological procedure and inoculation on a solid medium. Selective media were used for LAB were MRS plates. In most of the cases more than one colony was observed on the surface of MRS

Table 3
Morphological characterizations of LAB isolates

Sl. No.	*Isolate code*	*Colony colour*	*Colony Shape*	*Cell shape*	*Gram reaction*	*Colony on Nutrient media*
1.	LBD-1	Creamy white growth	Small	Rod	Gram + ve	White
2.	LBD2	Creamy dull colonies	Small	Rod	Gram + ve	White
3.	LBD-3	Rough, creamy white colonies	Small	Rod	Gram + ve	White
4.	LBD-4	White to light colonies	Small	Rod	Gram + ve	White
5.	LBD-5	Dull white colonies	Small	Rod	Gram + ve	White
6.	LBD-6	Dull white colonies	Small	Rod	Gram + ve	White
7.	LBI-1	Creamy white growth	Small	cocci	Gram + ve	White
8.	LBI-2	Creamy dull colonies	Small	Rod	Gram + ve	White
9.	LBI-3	Rough, creamy white colonies	Small	cocci	Gram + ve	White
10.	LBI-4	White to light colonies	Small	cocci	Gram + ve	White
11.	LBI-5	Dull white colonies	Small	Rod	Gram + ve	White
12.	LBI-6	Dull white colonies	Small	Rod	Gram + ve	White
13.	LBI-7	creamy, white smooth	Small	cocci	Gram + ve	White
14.	LBI-8	Dull white colonies	Small	Rod	Gram + ve	White
15.	LBP-1	Dull white colonies	Small	Rod	Gram + ve	White
16.	LBP-2	Whitish, creamy slimy	Small	Rod	Gram + ve	White
17.	LBP-3	Creamy white growth	Small	Rod	Gram + ve	White
18.	LBP-4	Creamy dull colonies	Small	Rod	Gram + ve	White
19.	LBK-1	Rough, creamy white colonies	Small	Rod	Gram + ve	White
20.	LBK-2	White to light colonies	Small	Rod	Gram + ve	White
21.	LBK-3	Dull white colonies	Small	Rod	Gram + ve	White
22.	LBK-4	Dull white colonies	Small	cocci	Gram + ve	White
23.	LBK-5	Dull white colonies	Small	cocci	Gram + ve	White
24.	LBM-1	Dull white colonies	Small	Rod	Gram + ve	White
25.	LBM-2	Dull white colonies	Small	Rod	Gram + ve	White
26.	LBM-3	Dull white colonies	Small	cocci	Gram + ve	White
27.	LBM-4	Creamy white smooth	Small	cocci	Gram + ve	White
28.	LBM-5	Dull white colonies	Small	Rod	Gram + ve	White
29.	LBM-6	Dull white colonies	Small	Rod	Gram + ve	White
30.	LBM-7	Whitish, creamy slimy	Small	Rod	Gram + ve	White
31.	LBM-8	Rough, creamy white colonies	Small	Rod	Gram + ve	White
32.	LBM-9	Creamy dull colonies	Small	Rod	Gram + ve	White

plates. In most of the cases more than one colony was observed. The cultural and morphological characteristics were examined within the help of microscope. Different types of microorganisms were observed, majority of them belonged to Gram positive rods and cocci shaped bacteria. The Gram positive rods and cocci shaped bacteria were specifically transferred to the plates of selective media MRS respectively to purify the isolated. Subculturing of the isolate was done until pure isolates were obtained. Once pure colonies were obtained they were cultured in MRS and stored at 4°C in refrigerator until it was used.

Total 60 isolates were isolated from different sources in which, 10 isolates from Curd, 18 isolates from Idli batter, 10 isolates from Paneer, 8 isolates from Khova and 14 isolates from Milk. Further these isolates were screened on MRS media, among 60 isolates 32 isolates were able to grow on MRS media. In which 6 isolates from Curd, 8 isolates from Idli batter, 4 isolates from Paneer, 5 isolates from Khova and 9 isolates from Milk. Table 1, 2.

The colonies of LAB isolates were creamy white to light dull, in colour and rough to smooth in appearance. All colonies were small shaped. The morphological characteristics showed that, 24 LAB isolates were rod in shape where as 8 isolates were cooci shaped and all were gram positive. The organisms identified in this study are listed in Table 3 (Deibel 9, Rogosa 10).

All the biochemical test were carried out according to standard procedure and result showed that 8 isolates were able to give positive Nitrate reduction test, 23 negative and 3 default isolates and Citrate utilization test showed 10 positive and 22 negative isolates. Also they were tested for ImVIC test and oxidase test and result were showed in table 4.

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Table 4
Biochemical characterizations of LAB isolates

Sr. no.	Isolate code	Lactose utilization	Nitrate reduction test	Catalase activity	Indole test	Methyl red test	VP test	Citrate utilization test	Oxidase tests
1.	LBD-1	+	–	–	±	+	–	+	–
2.	LBD-2	+	–	–	–	+	–	–	–
3.	LBD-3	+	–	–	–	+	–	–	–
4.	LBD-4	+	+	–	–	+	–	–	–
5.	LBD-5	+	–	–	–	+	–	+	–
6.	LBD-6	+	–	–	–	+	–	–	–
7.	LBI-1	+	+	–	–	+	–	–	–
8.	LBI-2	+	±	–	–	+	–	–	–
9.	LBI-3	+	–	–	–	+	–	–	–
10.	LBI-4	+	+	–	–	+	–	–	–
11.	LBI-5	+	–	–	–	+	–	+	–
12.	LBI-6	+	–	–	–	+	–	–	–
13.	LBI-7	+	+	–	–	+	–	+	–
14.	LBI-8	+	+	–	–	+	–	+	–
15.	LBP-1	+	+	–	–	+	–	–	–
16.	LBP-2	+	–	–	–	+	–	–	–
17.	LBP-3	+	–	–	–	+	–	–	–
18.	LBP-4	+	+	–	–	+	–	–	–
19.	LBK-1	+	+	–	–	+	–	–	–
20.	LBK-2	+	+	–	–	+	–	+	–
21.	LBK-3	+	±	–	±	+	–	–	–
22.	LBK-4	+	–	–	–	+	–	–	–
23.	LBK-5	+	–	–	–	+	–	–	–
24.	LBM-1	+	–	–	–	+	–	–	–
25.	LBM-2	+	–	–	–	+	–	+	–
26.	LBM-3	+	+	–	–	+	–	+	–
27.	LBM-4	+	–	–	–	+	–	–	–
28.	LBM-5	+	–	–	–	+	–	–	–
29.	LBM-6	+	–	–	–	+	–	+	–
30.	LBM-7	+	–	–	–	+	–	–	–
31.	LBM-8	+	–	–	–	+	–	–	–
32.	LBM-9	+	+	–	–	+	–	+	–

+ = positive and – = negative

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