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ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM DIFFERENT FOOD SAMPLES

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Abstract:

Lactic acid bacteria occur naturally as indigenous micro flora. Lactic acid bacteria isolated from different food samples. Lactic acid bacteria is a heterogeneous group of regular, gram-positive, rod shaped, non motile, non-spore forming bacteria with absence of catalase enzyme. Totally 80 LAB colonies were isolated after growth on MRS and M 17 agar medium and the pure culture obtained from the same medium. Among the 80 LAB isolates, 10 LAB isolates viz., Lactobacillus spp., Lactococcus sp., and pediococcus spp., were identified by morphological, physiological and Biochemical characterization.

Key words: *Lactic acid bacteria, Lactobacillus spp., Lactococcus sp., and pediococcus spp.,*

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INTRODUCTION:

Lactic acid bcteria is a gram-positive, non sporing, catalase negative, devoid of cytochromes, non aerobic habit but aerotolerant, fastidious, acid tolerant and strictly fermentative [13]. They have two different metabolic pathways for hexose fermentation. In homofermentative pathway, lactic acid (more than 85%) is major end product whereas in heterofermentative pathway lactic acid, ethanol/acetone and CO_2 are the terminal products [18, 5]. Lactobacilli are considered especially as beneficial bacteria because they have their ability to break down proteins, carbohydrates and fats in food and help in absorption of necessary elements and nutrients such as minerals, amino acids and vitamins required for the survival of humans and other animals. The antimicrobial activity of LAB may be due to the production of a number of antimicrobial substances such as lactic acid, hydrogen peroxide, diacetyl and bacteriocins [1]. Most Lactic acid bacteria (LAB) are considered generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) [3, 14]. Orla-jenson classified LAB according to morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, ability to grow at high salt concentration, acid or alkaline tolerance. These characteristics are a basic and still very important to identify lactic acid bacteria [5, 12]. LAB are used in the production of foods prepared by lactic fermentation such as dairy products, fermented vegetables, fermented meats, and sourdough bread (*15*). The most important genera are *Lactobacillus*, *Lactococcus*, *Enterococococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Bifidobacterium*. *Bifidobacterium* shares certain physiological and biochemical properties with LAB and some common ecological niches such as the gastrointestinal tract.

Lactic acid bacteria are an important role in the preservation of foods and fermented products. They can be used as natural competitive microbiota or as specific starter cultures under controlled conditions [8].

MATERIALS AND METHODS

Collection of samples

Samples of fresh meat, minced meat and fresh vegetables, were collected in aseptic plastic bags from various butcher shop and local market of Chidambaram, Tamil Nadu, India. After the collection of samples were transported to the laboratory of Department of Microbiology, Annamalai University, stored at 4° C for a maximum of 24 hrs before analysis. (11).

Isolation of Lactic acid bacteria

Lactic acid bacteria were isolated from fresh vegetables and meat products. Approximately 10 gram of sample was added to 90 ml of sterile peptone water (0.1% w/v) and homogenized in the stomacher [4].The sample was diluted up to $10^{-1} – 10^{-7}$ by using sterilized phosphate buffer. Then, the appropriately diluted samples were placed on MRS agar [10]. The plates were incubated at 37° C for 24 hrs. After incubation period, different morphological colonies were picked up randomly, and maintained on MRS agar slants at 4° C.

Characterization of LAB

The LAB isolates were subcultured on MRS and M-17 agar at 37° C. After incubation the pure culture were characterized based on colony morphology, cell morphology and biochemical tests [7, 17].

Morphological characterization of LAB

Morphological characters of the LAB isolates like colony morphology (color, shape, margin, elevation and surface) and cell morphology (shape, arrangement, and Gram reaction) were studied.

Physiological and Biochemical characterization of LAB

The physiological characters such as catalase test, oxidase test, motility test, growth of different temperature, pH and Nacl concentration and the biochemical characteristics of carbohydrate fermentation, and IMVIC tests were studied.

Gram's staining

A loop full of culture was smeared on a clean glass slide and then heat fixed. The cells were stained with crystal violet for one minute and then washed with a gentle stream of water. On a bacterial smear gram's iodine was applied as mordant, washed after one minute and immersed in a jar containing 95% alcohol for 10 seconds. The smear was then washed and counter stained with saffranin for one minute. Then the slide was washed, air dried and observed under oil immersion objectives of the microscope. The observation of gram's staining reaction was recorded [9].

Catalase test

Catalase is an enzyme produced by many microorganisms that breaks down the hydrogen peroxide into water and oxygen and causes gas bubbles. The formation of gas bubbles indicates the presence of catalase enzyme.

$$2H_2O_2 \rightarrow 2\ H_2O + O_2$$

Catalase test was performed to isolates in order to see their catalase reactions. Overnight cultures of isolates were grown on MRS agar at suitable conditions. Also fresh liquid cultures were used for catalase test by dropping 3% hydrogen peroxide solution onto 1 ml of overnight cultures and observed for gas bubble production.

Oxidase test

This oxidase test was done with the help of a commercially available disc coated with a dye N-tetramethyl paraphenylenediamine dihydrochloride (HI media), to detect the presence of cytochrome 'C' oxidase which is responsible for the oxidation of the dye. Rubbing a little quantity of the bacterial culture by means of a sterile toothpick on the disc causes formation of purple colour within 10-30 sec indicating positive reaction whereas no colour change indicates negative reaction.

Motility test

Tubes of semisolid medium were inoculated with a pure culture of suspected isolates by stabbing to a depth of approximately 2 cm with a bacteriological needle. After overnight incubation at suitable temperature, motility was evident as a haze of growth extending into the agar from the stabbing line. Growth of non-motile organism is restricted only to the stabbing line.

Gas Production from Glucose

In order to determine the homofermentative and heterofermentative characterization of isolates, CO2 production from glucose test was applied. Citrate lacking MRS broth and inverted Durham tubes were prepared and inoculated with 1%overnight fresh cultures. Then the test tubes were incubated at 37 °C for 5 days. Gas occurrence in Durham tubes was observed during 5 days which is the evidence for CO_2 production from glucose.

Nitrate Reduction Test:

Nitrate reduction is an important criterion for differentiating and characterizing different types of bacteria. Therefore, the isolates were incubated at 37 °C for 24 hrs in trypticase nitrate broth. After incubation, each of 0.5 mL sulphanilic acid (0.8%, in 5N Acetic acid) and α-naphthylamine (0.5%, in 5N Acetic acid) were added into the tubes. The appearance of red or pink color indicated the positive test for nitrate reduction and was recorded accordingly for the isolates tested in the present study.

Carbohydrate fermentation test

The fermentation of carbohydrates (fructose, glucose, lactose, maltose and sucrose) were performed in MRS broth (prepared without sugars) containing 1% solution of carbohydrate and added to 0.025% bromocresol purple as pH indicator. Results were recorded after 48 hrs of incubation at 30°C.

Determination of different temperatures

The growth of LAB isolates were tested at different temperatures *viz.*, 15ºC, 20ºC, and 37º C by inoculating 0.1 ml of the inoculums of LAB in MRS broth and incubated at above three temperatures for 48 hrs and examined for the intensity of growth.

Determination of different pH

The growth of LAB isolates were tested at two different pH levels *viz.*, 4.5 and 6.5 by inoculating 0.1 ml of the inoculums of LAB in MRS broth adjusted to the pH 4.5 and 6.5 and incubated at 37◦ C for 48 hrs and examined for the intensity of growth.

Determination of different NaCl concentrations

The growth of LAB isolates were tested at different NaCl concentration such as 4.5 % and 6.5 %. The MRS broth was prepared and NaCl was added to NaCl concentrations of 4.5% and 6.5% and inoculated with 0.1 ml of the inoculum of LAB and incubated at37◦ C for 48 hrs and examined for the intensity of growth.

RESULTS:

The lactic acid bacteria were isolated from different food samples collected from Local market. The isolation of LAB was performed the selective media MRS and M 17 agar plates. In most cases the typical colonies were observed and characterized on the surface of MRS and M 17 agar plates. The cultural and morphological characters were identified under the microscope.

Among eighty isolates only ten different colonies of LAB were selected from various food samples. The isolated colonies were examined for morphological characteristics. Size, shape, and colour of colonies were recorded. The surface of colonies smooth, rough, cottony soft, and glistening. Margins were entire, spherical, circular and irregular in all the isolates. Elevations of the isolated colonies were flat, raised and convex. Further the colour pigments of isolates white, dull white, off white and grayish white were observed. (Table 1).

Table 1: Morphological characteristics of LAB isolated from different food samples

S.No	Isolates	Colony surface	Colony size	Colony margin	Colony colour	Cell morphology
1	Lb-1	Rough	0.1-0.5mm	Entire	Dull white	Rods in pairs/ short chain
2	Lb-2	Smooth	1.0 mm	Spherical	Off white	Filamentous colony
3	Lb-3	Glistening	1 mm	Circular	White	Rods in pairs/ short chain
4	Lb-4	Smooth point	0.5×0.9 mm	Circular	White	Short rods in single/pairs
5	Lb-5	Cottony rough	1.0 mm	Irregular	White	Long rods in chains
6	Lb-6	smooth	0.7µm×2.0µm	Irregular	White	Tendency to chain
7	Pc-1	Smooth	1-2 mm	Entire	Grayish white	Cocci in pairs or tetrads
8	Pc-2	Smooth	1-2 mm	Entire	Grayish white	Cocci in pairs or tetrads
9	Pc-3	Smooth	1-2 mm	Entire	Grayish white	Cocci in pairs or tetrads
10	Lc-1	Smooth	0.1-0.5 mm	Entire	White	Cocci in chains

Table: 2 physiological characteristics of LAB isolated from different food samples

s.no	Isolates	Gram reaction	Catalase test	Oxidase test	Motility test	Growth at 15◦C	Growth at 20◦C	Growth at 37◦C	Growth at pH 4.5	Growth at pH 6.5	Growth at 4.5%NaCl	Growth at 6.5%NaCl
1	Lb-1	G +	-	-	-	-	+	+	+	-	+	-
2	Lb-2	G +	-	-	-	-	-	+	+	-	+	-
3	Lb-3	G +	-	-	-	+	+	-	+	ND	+	-
4	Lb- 4	G +	-	-	-	-	+	+	+	ND	+	-
5	Lb-5	G +	-	-	-	+	-	+	-	+	+	+
6	Lb-6	G +	-	-	-	-	+	-	+	-	-	=
7	Pc-1	G +	-	-	-	+	+	+	ND	-	+	+
8	Pc-2	G +	-	-	-	+	-	+	-	ND	-	-
9	Pc-3	G +	-	-	-	-	-	+	+	-	+	-
10	Lc-1	G +	-	-	-	+	+	-	+	-	+	-

(+) – POSITIVE REACTION, (-) – NEGATIVE REACTION, ND- NOT DETERMINED

Table: 3 Biochemical characteristics of LAB isolated from different food samples

S.No	Isolates	Glucose	Fructose	Lactose	Sucrose	Maltose	Indole	Methyl red	Voges proskauer	Citrate utilization
1	Lb-1	+	+	+	+	+	-	+	-	-
2	Lb-2	+	+	+	-	-	-	+	-	-
3	Lb-3	+	+	+	+	+	-	+	-	-
4	Lb-4	+	+	+	+	+	-	+	-	-
5	Lb-5	+	+	+	+	-	-	+	-	-
6	Lb-6	+	+	+	+	+	-	+	-	-
7	Pc-1	+	+	+	-	-	-	+	-	-
8	Pc-2	+	+	+	+	-	-	+	-	-
9	Pc-3	+	+	+	-	+	-	+	-	-
10	Lc-1	+	+	+	-	-	-	+	-	-

(+) – POSITIVE REACTION, (-) – NEGATIVE REACTION,

The physiological characteristics were studied such as gram reaction, catalase test, oxidase test, growth at different temperatures, pH and NaCl concentration and the result which summarized in Table 2. The isolated LAB strains were Gram positive. Motility test showed that the isolates were non-motile, growing in the confined stab line. Catalase test study showed that in catalase enzyme was absent and other were not able to produce bubbles when mixed with H_2O_2.

Various biochemical tests were studied for identification of isolated LAB colonies viz., carbohydrate fermentation test and IMVIC test and results of which are summarized in Table 3.

DISCUSSION:

In the present study, 80 Lactic acid bacteria were isolated from different food samples. Ten LAB isolates were identified as *Lactobacillus spp., Lactococcus sp., and pediococcus spp.,* by morphological, physiological and biochemical characteristic study [2]. There are several methods of identifying different probiotic LAB. In this study, the following tests were used: carbohydrate fermentation, gas production from glucose, growth at different temperatures, pH and NaCl.

In the case of carbohydrate fermentation, different carbohydrates were used. For example some authors used to API kits [6]. Others tested without using kit [19]. In the present study, 5 types of carbohydrates were used: fructose, lactose, maltose, sucrose, and glucose. All the LAB strains were Gram positive, non-spore forming, catalase and oxidase negative. Similar observations were reported in the present study. [20].

CONCLUSION:

In the present investigation, eighty lactic acid bacterial isolates were isolated from different food samples. All the isolates were characterized on the basis of colony morphology and biochemical characteristics. Among the 80 LAB isolates ten isolates were identified as *Lactobacillus spp.,* (6), *Lactococcus sp.,*(1), *and pediococcus spp.,*(3) by morphological, physiological and biochemical characteristics.

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