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# ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA (LAB) AND SCREENING IT FOR PROBIOTIC PROPERTIES.

**Aishwarya Rajendra Mhaske ***
**Mayuri Jagannath Pawar ***
**K.A.A.N.M.Sonawane college Satana**

**Abstract:** The present study was carried out to isolate and identify *lactic acid bacteria(LAB)* lactobacillus in curd. *Lactobacillus* species play a major role in fermented dairy products and also contribute to the therapeutic aspects of human health.LAB were isolated using MRS media. Total five lactic acid bacteria isolated from different curd sample. Probiotic *Lactobacillus* strain was isolated from yogurt and the characterization of the bacteria was performed using gram stain, motility, catalase, biochemical tests and morphological features were confirmed using microscope. Finally the identification was confirmed by carbohydrate fermentation. Effect of inoculation methods and cultivation conditions on the growth of the bacteria was studied.
**Key words;** LAB, Probiotic, Characterization, Identification.

**Introduction**: Probiotics are, "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [(16)]Yogurt is the most common source of probiotics. Yogurt consists of milk fermented by bacteria that modify lactose into lactic acid[(10)]. Lactic acid bacteria (LAB) produce lactic acid either through homofermentive or heterofermentive pathway and are wide spread in nature[(1)]. Lab bacteria have a probiotic effect on human health[(12,13)]. Also found in human digestive system. *Lactobacillus* species play a major role in fermented dairy products and food industry and also contribute to the therapeutic aspects of human health[(9)]. *Lactobacilli* are considered as beneficial bacteria because they have their ability to break down proteins, carbohydrates & 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[(1)]. Lactobacilli represent a significant part of our intestinal microflora, and their friendship with the general state of human health is under serious investigation[(3)]. The genus *Lactobacillus* is one of the major groups of lactic acid bacteria used in food fermentation and is thus of great economical importance. Lactic acid bacteria (LAB) are a group of Gram-positive, non-spore forming, cocci or rods, catalase-negative. For an organism to be a probiotic, it must essentially be non-pathogenic, be generally regarded as safe (GRAS), tolerate low pH, tolerate high concentrations of bile salts[(14).] showing a DNA having G+C content less than 50 mol% and organisms of interest in food processing industries because of their typical roles in inhibiting the growth of food spoilage bacteria. The lactic acid bacteria(LAB) are comprised of at least ten genera: *Aerococcus, Carnobacterium,Enterococcus, Lactobacillus, Leuconostoc,Pediococcus, Streptococcus,tetragenococcus and Vagococcus.*Most representatives of these group have been consumed for thousands of years, do not pose any health risk to humans and are designated as GRAS organisms (generally recognized as safe)[(2)].The search for new bacteriocin producing lactic acid bacteria is of great significance because of their potential use in fermented food and feed industries[(1)].LAB exerts a strong antagonistic activity against many food spoilage organisms and food borne pathogens. The aim of the study was to isolate lactic acid bacteria from fermented food curd and screening it for

probiotic properties. Yogurt consists of milk fermented by bacteria that modify lactose into lactic acid [10]. LAB are the most important group of microorganisms commercially used as starter cultures for the manufacture of dairy based probiotic foods and have been established as a natural consumer"[16].

## II. MATERIALS AND METHODS

The research work was conducted in Department of Microbiology at Sanjivani Arts Commerce and Science College, Kopergaon, Maharashtra.

**Isolation of Lactobacillus:**

The different samples of fresh curd were collected in sterilized screw capped bottles.1 loopful of curd samples were inoculated in 50 ml of MRS broth separately in an aseptic condition and incubate flask at 37◦ c for 24hrs.After sufficient growth was observed in MRS broth were streak on sterile MRS agar plate. Incubation was carried out at 37 ˚C for 24 – 48 hours.

**Identification of lactic acid bacteria:**

The identification was done mainly on the basis of morphological, biochemical & cultural characteristics. Morphology was examined by Gram staining, motility was observed by hanging drop method. Biochemical characterization was done by sugar fermentation test, Indole, MR-VP & citrate utilization test and catalase test. Cultural characterization based on colony characteristics.

**Screening of LAB for probiotic characteristics:**

1. Acid tolerance:

From the curd sample isolate 10 LAB were screened for the acid tolerance activity of different pH. MRS broth were prepared such as pH 2, 4, 7, 11.

2.Bile tolerance:

From the curd sample isolate 10 LAB were screened for the bile tolerance activity of different range. MRS broths were prepared with bile salt concentrations such as 0.5%, 1%, 1.5%, 2%.

3 .Salt tolerance:

From the curd sample isolate 10 LAB were screened for the salt tolerance activity of different range. MRS broths were prepared such as 1%, 2%, 3%, and 4%.

4. Temperature tolerance:

From the curd sample isolate 10 LAB were screened for the temperature tolerance activity of different range. . MRS broth were prepared and screened for temperature such as $27^0$ C, $37^0$C, $40^0$C, $60^0$C, $80^0$C.

**Results and Discussion:**

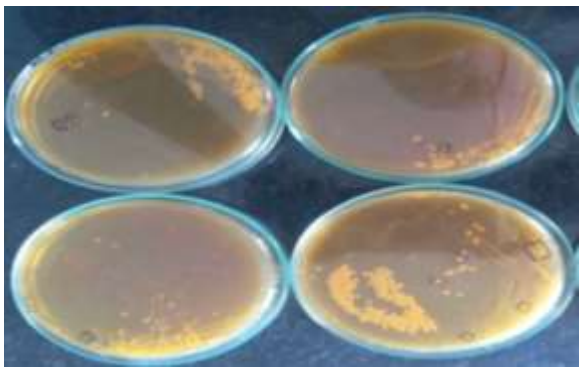
**Isolation Identification and Characterization of LAB:**

The Characterization of LAB isolates was carried out by study of cultural and morphological characters. The morphological characters observed are shown in table. The isolate L1, L2, L3, L4, L5, L6, L7, L8, L9 and L10 produced, white colored, entire, flat, opaque, smooth, colonies. All isolates were detected Gram positive (Photo Plate 1), non- motile and circular shaped bacteria. Spore staining of all LAB was performed by Schaeffer's and Fulton's method and all isolates were found to be non spore former.

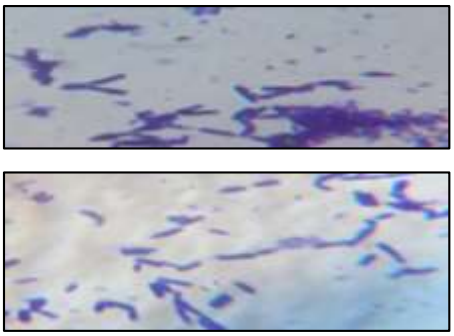
**Table 1: Isolation Identification and Characterisation of LAB**

| Sr No. | Isolate code | Morphological characters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Colony characteristics | | | | | | | | |
| | | Shape | Color | Margin | Elevation | Opacity | Consistency | Grams nature | Motility | Endospore staining |
| 1 | L1 | Circular | White | Entire | Flat | Opaque | Smooth | Gram Positive rods | Non-motile | Non-spore former |
| 2 | L2 | Circular | White | Entire | Flat | Opaque | Smooth | Gram Positive rods | Non-motile | Non-spore former |
| 3 | L3 | Circular | White | Entire | Flat | Opaque | Smooth | Gram Positive rods | Non-motile | Non-spore former |
| 4 | L4 | Circular | White | Entire | Flat | Opaque | Smooth | Gram Positive rods | Non-motile | Non-spore former |
| 5 | L5 | Circular | White | Entire | Flat | Opaque | Smooth | Gram Positive rods | Non-motile | Non-spore former |
| 6 | L6 | Circular | White | Entire | Flat | Opaque | Smooth | Gram Positive rods | Non-motile | Non-spore former |
| 7 | L7 | Circular | White | Entire | Flat | Opaque | Smooth | Gram Positive rods | Non-motile | Non-spore former |
| 8 | L8 | Circular | White | Entire | Flat | Opaque | Smooth | Gram Positive rods | Non-motile | Non-spore former |
| 9 | L9 | Circular | White | Entire | Flat | Opaque | Smooth | Gram Positive rods | Non-motile | Non-spore former |
| 10 | L10 | Circular | White | Entire | Flat | Opaque | Smooth | Gram Positive rods | Non-motile | Non-spore former |

**Photo Plate 1**



**Isolation of Lactic Acid Bacteria on MRS Agar Plate**



**Gram Staining of Isolated Lactic Acid Bacteria**

## Biochemical Characterization of LAB:

After the cultural and morphological study of LAB, all LAB isolates such as L1, L2, L3 L4 and L5 were examined for sugar fermentation of Glucose, Mannitol, Maltose and Sucrose. From the results (Table 2), it was observed that all these LAB were ferment sugars with acid production in Glucose, Dextrose, Maltose except Mannitol and Sucrose while no gas formation was observed.

**Table 2: Biochemical Characterization of LAB**

| Sr.no | Isolate code | Biochemical character | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sugar fermentation | | | | | | | | | |
| | | Glucose | | Sucrose | | Maltose | | Mannitol | | Dextrose | |
| | | Acid | Gas | Acid | Gas | Acid | Gas | Acid | Gas | Acid | Gas |
| 1 | L1 | + | - | - | - | + | - | - | - | + | - |
| 2 | L2 | + | - | - | - | + | - | - | - | + | - |
| 3 | L3 | + | - | - | - | + | - | - | - | + | - |
| 4 | L4 | + | - | - | - | + | - | - | - | + | - |
| 5 | L5 | + | - | - | - | + | - | - | - | + | - |

**Photo Plate: 2 Sugar fermentation tubes**


Maltose


Dextrose


Sucrose


Mannitol


Glucose

**Screening of *Lactobacilli* for probiotics characteristics:**

In present study, a total of five LAB isolate were analyzed for their probiotic potential on the basis of their acid, bile salt, salt tolerance, and high temperature tolerance.

**Table 3: Screening of *Lactobacilli* for probiotics characteristics**

| Isolate code | Growth in NaCl | | | | Growth in Bile salt | | | | Growth at pH | | | | Growth at temperature | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1% | 2% | 3% | 4% | 0.5% | 1% | 1.5% | 2% | 2 | 4 | 7 | 11 | 37°C | 40°C | 60°C | 80°C |
| L1 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| L2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| L3 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| L4 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| L5 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |

(+ : Growth , - : No growth)

Here all the isolate of Lactic acid bacteria L1, L2, L3, L4 and L5 tolerated the extreme condition of various parameter such as NaCl 4%, bile salt 2%, pH 2 and temperature of 60°C and showed their growth. From the results (Table no 3), it was observed that these entire LAB showed probiotic potential.

**Confirmation of isolate by sequencing 16S rRNA:**

The potential strain of Probiotic L5 was selected for sequencing and identified as Lactobacillus plantarum. Lactobacillus plantarum subsp. plantarum strain LB1-2 chromosome, complete genome Sequence ID: CP025991.1Length: 3359427Number of Matches: 1

**Sequence:**

GTGCTGGATCGTAATGACTTATGGAATCTCATTAAGTTCTCTATGGAACAA
GATCTGTCCAAGATCACGTTTCAAACGTTTGTCGAACCGGCAAAACCACT
CCAACTCGATAAAGCCCAAATGACGATCGAAGTCCCAACACAACTTCATC
GTGACTATTGGGAGAAAAACTTGGCCGCAAAGTTCACGGACATTGCGATG
CAAGCGACTAACGAGCAGATTCGGCCGGTCATGATAACGGAAGAAGAAC
GTCAGCAACTTACGAGAGACAAGGACTCGCAAGTGACTACCGGTAACGTT
GCGGGACAACAACCAACAACCGCAACTACCCCCACATTTATGCGGGAAAC
GAAACTCAACCCGAAATACACTTTTGATACTTTCGTGATCGGTAAAGGCA
ATCAAATGGCCCATGCCGCTGCGTTAGTTGTGTCGGAAGAACCCGGCACC
ATGTATAATCCGTTGTTTTTCTACGGGGGCGTTGGTCTGGGAAAAACCCAC
CTAATGCACGCTATCGGTAACAAATTGTTAGAAACCGATCCGACTAGTAA
CATTAAATATGTGACTAGCGAATCTTTTACGAATGAATTAATTAATGCCAT
TCAAACTAAAAAACAGGAGGCGTTCCGCGAAGAATATCGGAACGTTGACC
TGTTATTAGTCGACGACATTCAATTTTTTGCCAATAAGGAAGCAACCCAAG
AAGAGTTCTTCCATACATTTAATGCTTTATATGAAGATGATAAGCAAATCG
TGCTTACATCCGATCGCTTACCGAACGAAATTCCGCAACTCCAAGATCGCC
TAGTTTCTAGGTTTAAGTGGGGATTATCCGTTGATATTACCCCACCTGATC
TCGAGACGCGGATTGCTATTTTGCGCAATAAAGCCGATCTTGAAGGGATC
GAAATCCCTGACGATACGTTAAGTTATATCGCCGGTCAGATCGACTCAAA
CGTGCGAGAACTCGAAGGTGCCTTAGCACGAGTGCAGGCATACTCACGAC
TGAATAATTCACCGATTA

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