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Isolation and Identification of *Lactococcus spp*. from Buttermilk

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A B S T R A C T

Lactic acid bacteria have been used for the fermentation of food products since ancient days. It plays an essential role in the production of all dairy products and is involved in the production of many other fermented foods. Isolation and identification of *Lactococcus spp.* in various milk products reveals the indigenous micro flora of that region. Isolation of such regional strains helps in identification the best isolates which can be utilized for further study. In the present study, the lactic acid bacteria identified from buttermilk sample was *Lactococcus spp*. The study was successful in isolating and identifying the naturally occurring lactic acid bacteria from buttermilk. From this we can conclude that buttermilk is a good source of lactic acid bacteria.

Introduction

Small, single-celled creatures are known as bacteria. Nearly every place on Earth has bacteria, which are essential to the health of the planet's ecosystems. Certain species can endure situations of high pressure and temperature. In fact, it's believed that bacterial cells make up a larger portion of the human body than do human cells. The majority of microorganisms in the body are beneficial and even harmless. Disease is caused by a very limited number of species. Bacteria can be classified into various categories based on their features and characteristics. The classification of bacteria is mainly based on the following: Shape, Composition of the cell wall, Mode of respiration, Mode of nutrition.

Lactococcus is a family of lactic acid-producing bacteria that was previously classified as *Streptococcus* Group N1. They are non-motile, gram-positive, catalase-negative cocci that can be encountered by themselves in pairs, or in chains. The genus comprises strains that are reported to grow at or below 37°.

Materials and Methods

Collection of Buttermilk sample

The sample of buttermilk was collected from local dairy of Mohali city to isolate and identified the naturally occurring Lactococcus spp from raw buttermilk. A total of 1raw buttermilk sample was collected. Buttermilk sample was collected in sterile

bottle and brought to laboratory with ice box and then transported to be analysed.

Isolation of *Lactococcus* species

In 25ml of distilled water, we have prepared a solution of MRS agar with a variable composition. Media have next undergone a 15-minute autoclave at 121°C. After that, the medium has been put into a laminar air flow for solidification. Once the medium has solidified in 10 to 15 minutes, we have taken a sample of butter milk, such as curd or buttermilk.

With a cotton swab, we have evenly spreaded a buttermilk sample over the MRS agar media plate at a 90-degree angle on all four sides. The MRS plate have been incubated at 37°C an aerobically for 24-48 hours. At the end of 48 hour when the colonies became predominate, morphological and well isolated colonies were picked and transferred to new MRS media plate by streaking.

MRS Broth: Colonies showing tropical characteristic of *Lactococcus species* on MRS medium surface were picked up randomly and transferred into MRS broth for further enrichment.

Identification of *Lactococcus* Species

The pure isolate subjected to identification according to their morphological, cultural, phycological and biochemical characteristic. The isolate were strained by gram's method and examined under microscope at 40x. The biochemical identification was performed according to Bergey's Mannul of determinate of bacteriology.

Gram Straining

Positive and negative are two-way which are present in gram straining. One clean glass slide has been used to prepare the smear. A drop of water has placed on the slide, and using an inoculating loop, microorganisms has added to the drop of water. Inoculate a loop to stir it in a circular motion. After 20 minutes of air drying the smear in the laminar air flow, add the reagents. After adding one drop of crystal violet, wait one minute, and then rinse with water. One to two drops of iodine should be added. After waiting for a minute, wash it with water. Then add decolorizing ethanol (95%) and quickly wash it. Safranine should be added for 45 seconds, then reins it out. After 20 minutes of LAF drying, observe them under a microscope.

Biochemical Test

By separating various bacterial species based on their metabolic activity, biochemical tests are performed to identify specific bacterial species.

MR/VP Test

In this MR/VP Test made the composition of NAM solution in 20ml distilled water. After the composition is prepared, transferred the media into four test tubes and label them Control MR, Control VP, MR Test, and VP Test. After that, put all four test tubes in an autoclave for 15 minutes at 121°C.

By using laminar air flow to cool the test tube when the autoclave is done. In the meantime, we have sterilized a 100 µl tip box, a 100 µl micropipette, a lighter, and fresh broth under UV for three minutes. After that, we have to inoculated 100µl isolated *Lactococcus* colonies in a fresh MRS Broth. The test tube will be incubated for 24 hours at 37°C. After 24 hours incubation, Methyl Red Indicator is added from the side wall in the MR test, while 1% alpha-naphthol in ethanol and 1% KOH in distilled water is added in the VP test (600 µl KOH, 400 µl alpha naphthalol).

Oxidase Test

To perform this test a drop of MRS Broth using micropipette is placed on a filter paper again with a micropipette 100µl of oxidase reagent is placed at the centre of a MRS Broth; if the colour changes to purple, the result is positive; otherwise, the result is negative.

Catalase Test

A drop of hydrogen peroxide is placed on a clean microscopic slide. With a inoculating loop pick up cells from a test culture and transfer them into a drop of hydrogen peroxide. Both are mixed together and observed for exam bubble production.

Citrate Agar Test

Prepared the media composition in 20ml of distilled water and transferred it into two test tubes, each holding 10 ml of media. Test tube should be in tilted position for solidification. Using MRS Broth Media performed a citrate test (Test Tube). The outcome can be noted after 24 hours of incubation.

Ureases Test

With 15ml of distilled water, we have prepared the mixture. After preparing the mixture, divide it into two test tubes, each holding 7.5 ml, and autoclave them for 15 minutes at 121°C. We have kept the media in slant position in LAF and let it solidify. As soon as the medium has set, we have streak it onto the urease test tube with an inoculation loop that is already dipped in MRS Broth. 24-hour incubation of the medium at 37 °C.

Indole Test

Tryptone broth composition is prepared. Transferred the media into two test tubes, each holding 5 ml media. The test tube is autoclaved at 121 °C for 15 minutes. Kovac's Reagent is added in test tubes after 24 hours of incubation; if a red ring forms, the test is positive; otherwise, it is negative.

Nitrate Test

Prepared the nitrate media composition in 15 ml of distilled water and transferred it into two test tubes with 7.5 ml each, and label one as a control and the second as a test. Added the nitrate reagent after 24 hours. If the colour changes to pink, the result is positive; otherwise, the result is negative.

Carbohydrate Utilization Test

Phenol red broth base medium was used as a medium for this test. Different sugar substrate namely glucose, sucrose, lactose and D-mannitol were used 1% sugar substrate was add to 50ml of medium. Each mixture was transferred into each test tube. All the tube were sterilized for 15 min at 121°. The tubes were inoculated with a single colonies of the bacteria under study. The positive reaction of bacteria was indicated by the changes in the colour of the media.

Results and Discussion

The goal of this work was to isolate and charterers potential probiotic bacteria from raw buttermilk sample of Mohali. Based on the morphology character was identified as probable *Lactococcus lactis* from raw buttermilk as shown in figure 1

Under the microscope, the isolated bacteria were examined. The gram-positive, coccus-shaped, and chain-forming nature of the bacteria was observed as shown in figure 2. According to the findings of the gram staining, *Lactococcus lactis* is recognised as the probable bacteria. The catalase test is one of the most effective confirmatory diagnostic procedures for identifying *Lactococcus lactis* bacteria because of its ease of use. The isolated bacteria are catalase negative and cannot mediate the breakdown of H_2O_2 to create O_2, according to the results of the catalase test, which revealed no bubble. It is commonly known that *Lactococcus lactis* does not catalyse.

Table 1 lists the findings of the several biochemical tests used to identify the bacterial isolate were shown Catalase negative, Oxidase negative, Indole negative, Methyl Red negative as shown in figure 3, Voges-Proskauer negative as shown in figure 4, Citrate Utilization negative, Urease test negative.

Table.1 Biochemical test for *Lactococcus spp*

Biochemical test	Result
MR Test	Negative
VP Test	Negative
Oxidase Test	Negative
Catalyse Test	Negative
Indole Test	Negative
Nitrate Test	Positive
Ureases Test	Negative
Citrate Test	Negative

Table.2 Carbohydrate Utilization Test

Carbohydrate utilization	Result
Glucose	Positive
Lactose	Negative
Sucrose	Positive
D-mannitol	Positive

Fig.1 *Lactococcus spp*. on MRS agar media

Fig.2 Gram -positive *Lactococcus spp*. Under light microscope at 10x magnification

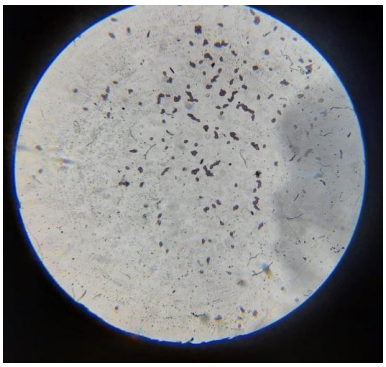


Fig.3 MR Test **Fig.4** VP Test

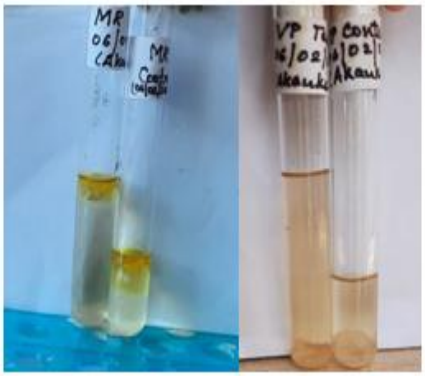
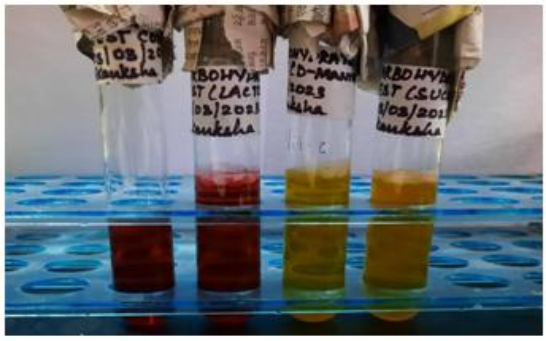


Fig.5 Carbohydrate Utilization Test



Carbohydrate Utilization Test

The colour of the medium changes from red to yellow as shown in Table 2 and figure 5.

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