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ISOLATION AND IDENTIFICATION OF PROBIOTIC LACTOBACILLUS SPP. FROM LOCAL YOGURT SHOPS IN THE NARC REGION, ISLAMABAD

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ABSTRACT

Lactobacillus is a genus of Gram-positive, non-spore-forming lactic acid bacteria with significant probiotic potential. These bacteria contribute to human health by producing bioactive compounds such as bacteriocins and hydrogen peroxide, which enhance digestion, support gut microbiota, and reduce the risk of gastrointestinal and other diseases. Yogurt is a rich source of probiotic bacteria, particularly *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which are widely used as starter cultures in dairy fermentation. This study aimed to isolate and identify probiotic *Lactobacillus* strains from yogurt samples collected from ten different local shops in the NARC region of Islamabad. The samples were cultured on de Man, Rogosa, and Sharpe (MRS) agar, and the isolates were subjected to

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morphological and biochemical characterization, including Gram staining, catalase, oxidase, and methyl red tests. The results revealed the consistent presence of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in all yogurt samples, with characteristic Gram-positive morphology and negative results for catalase, oxidase, and indole tests. These findings confirm the probiotic potential of locally available yogurt and support its beneficial role in promoting human gut health. The isolated strains may have future applications in the development of probiotic supplements and functional foods.

INTRODUCTION

Long before the scientific discovery of probiotics, fermented foods were traditionally used for their health benefits. Ancient Egyptians, for example, are believed to have consumed fermented milk, such as yak milk, to preserve it during long travels (Suvarna & Boby, 2005). In the 1800s, scientists began to observe the positive health effects of fermented milk, although the underlying microbial mechanisms were not yet understood (Barnett, 2000). A major breakthrough occurred in the early 20th century when Russian scientist Elie Metchnikoff, a collaborator of Louis Pasteur, proposed that the consumption of fermented milk containing lactic acid bacteria could promote health and longevity. He identified Lactobacillus species in yogurt and the colon and introduced the concept of probiotics—microorganisms that provide health benefits to the host.Further progress was made by Bulgarian physician Stamen Grigorov, who, at the age of 27, discovered Lactobacillus bulgaricus (often referred to as the "Bulgarian bacillus") in fermented yogurt. His findings supported Metchnikoff's hypothesis that regular consumption of fermented dairy products contributed to the robust health and longevity of people in rural Bulgaria. This laid the foundation for modern probiotic research and the widespread use of probiotic cultures in food and health applications(McFarland, 2015). He continues on claiming that the Lactobacilli counteract the gastrointestinal system's putrefactive metabolism, which can lead to disease and aging. Elie Metchnikoff connects this to his recommendations for probiotics (Hill et al., 2014). Not all bacteria, their components, or their metabolites qualify as probiotics. To be considered a probiotic, a microorganism must be a live, beneficial organism that provides therapeutic effects when consumed in adequate amounts. Among the well-known probiotic strains are Lactobacillus bulgaricus and Streptococcus thermophilus, both of which are widely used in the fermentation of yogurt and have demonstrated beneficial effects on human health (Estifanos Hawaz, 2014). Yogurt lowers the symptoms of moderate lactose digesting, which is one of its most significant and scientifically supported health advantages (Guarner et al., 2005).

The majority of clinical studies have shown that the effectiveness and health benefits of probiotic bacteria depend on their ability to survive, multiply, and persist in the host's gastrointestinal tract. Their ability to colonize the gut is essential for exerting beneficial effects, including improved digestion, immune modulation, and pathogen inhibition. Additionally, yogurt starter cultures, particularly Lactobacillus bulgaricus and Streptococcus thermophilus, play a significant role in enhancing the vitamin B complex profile, especially in young and adult women, by contributing to the synthesis and bioavailability of B vitamins during fermentation(Fabian et al., 2008). Lactobacillus, a genus of lactic acid bacteria, has garnered significant attention for its role in preventing gastrointestinal disorders. It promotes the growth of beneficial gut microbiota while inhibiting harmful microorganisms through various mechanisms, including the production of organic acids, bacteriocins, and competitive exclusion (Hawaz, 2014). In addition to their role in promoting growth, Lactobacillus species are now widely recognized for enhancing human defense mechanisms against infections and contributing to overall health protection. In the present study, several strains of Lactobacillus were isolated from yogurt samples collected from the NARC region of Islamabad and examined using standard biochemical assays, including Gram staining, catalase, and oxidase tests (Piano et al., 2004). Previous studies conducted at NARC Islamabad have highlighted that probiotics, particularly lactic acid bacteria, are essential components of human nutrition due to their various health benefits, such as improving digestion, supporting weight management, and maintaining immunological balance.

Materials and Methods

This study was carried out at the National Agriculture Research Centre (NARC) Islamabad.

Samples collection and culturing

Ten yogurt samples were aseptically collected from ten different local shops within the NARC region. Each sample was labeled from S1 to S10, corresponding to shops Sh1 to Sh10. The samples were transported to the laboratory under refrigerated conditions and processed immediately. Using sterile techniques and standard operating procedures (SOPs), each yogurt sample was serially diluted in phosphate-buffered saline (PBS) and plated on de Man, Rogosa, and Sharpe (MRS) agar selective for lactic acid bacteria. Plates were incubated aerobically at 37 °C for 48 hours. Colonies exhibiting a pH range between 6.2 and 6.5 were selected for further subculturing on fresh MRS agar and incubated again at 37 °C for 48 hours. Colonies that were Gram-positive and catalase-negative were retained for subsequent morphological and biochemical characterization

Identification of Bacterial Isolates

Bacterial isolates were identified based on their morphological characteristics and biochemical properties. Colonies were randomly selected and examined under a microscope following Gram staining to determine cell shape, arrangement, and Gram reaction.

Gram staining

Gram staining was performed according to the method described by Coico (2006). Bacterial smears were heat-fixed and stained with 0.5% crystal violet for one minute, followed by iodine treatment for one minute. Decolorization was carried out using 95% ethanol for 30 seconds, and a final counterstain with safranin was applied for one minute. Slides were examined microscopically at magnifications of 10x, 40x, and 100x. Gram-positive bacteria retained the crystal violet stain and appeared purple, while Gram-negative bacteria appeared red. (Coico, 2006).

Biochemical characterization

Biochemical assays (catalase, oxidase, and citrate tests) were conducted to further elucidate the biochemical features of the bacteria.

Catalase test

This examination indicates the existence of the enzyme catalase, which facilitates hydrogen peroxide's (H_2O_2) oxygen release. It is employed in the differentiation of bacteria that generate the catalase enzyme (Reiner, 2010). In order to perform catalase test, one colony was carefully mixed with hydrogen peroxide on a sanitized slide. If gas bubbles formed on the surface of the culture material, the test was declared positive (Reiner, 2010).

Oxidase test

Oxidase test, technique for identifying the presence of cytochrome C oxidase, sometimes referred to as cytochrome a3, an enzyme that is present during aerobic respiration. The 1% Kovac's oxidase reagent was applied to a small piece of filter paper, which was then allowed to air dry. Using a sterile loop, a well-isolated colony of bacterial strains was transferred onto filter paper from a newly cultured (18–24 hours) bacterial plate. For every colony under test, colour variations were examined. In ten to fifteen seconds, the hue changes to dark purple following an oxidase positive test. When oxidase negative organisms are present, the colour either stays the same or responds more slowly than two minutes (Shields & Cathcart, 2010).

Citrate Agar Test

The purpose of this experiment is to ascertain whether an organism can use citrate as an energy source. (MacWilliams, 2009). A mild inoculum that was meticulously separated from the colony's core was used to inoculate the slant. At a temperature of 35 to 37 degrees, incubate aerobically for up to 24 hours. A color shift from green to blue was seen along the slope (MacWilliams, 2009).

Indole Test

The indole test was conducted to evaluate the ability of bacteria to break down tryptophan to indole. Isolates were inoculated into tryptophan broth and incubated at 37 °C for 48 hours. After incubation, a few drops of Kovács reagent were added. The appearance of a red or pink ring at the surface indicated a positive result; a yellow ring indicated a negative result.

Methyl Red (MR) Test

This test was used to detect stable acid production from glucose fermentation. Isolates were inoculated into MR-VP broth and incubated at 37 °C for 48 hours. After incubation, 5 drops of methyl red indicator were added. A red color indicated a positive result (stable acid production), while a yellow or orange color indicated a negative result.

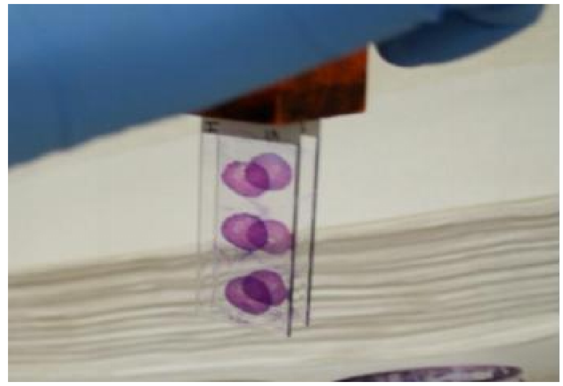

Figure: 1 Gram staining slide

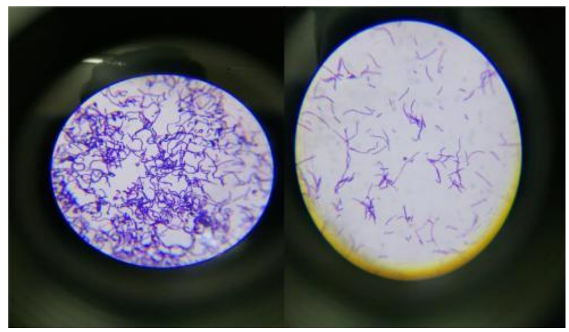

Figure: 2 Microscopic examinations of lactobacillus

RESULTS

Gram Staining and Microscopic examination of Lactobacillus

After Gram Staining and microscopic examination, it was observed that the colonies were purple in color and rod-shaped which indicated that these were Gram-positive bacteria.

Isolation of lactobacillus, and biochemical identifications

Lactobacillus Bulgaricus and Streptococcus thermophilus were identified from ten yogurt samples from ten different shops. The bacteria were isolated on MRS agar at 37°C at aerobic condition. These two strains, Streptococcus thermophilus and Lactobacillus bulgaricus, were isolated. In the tables below, 'S' indicates the sample number, and 'sh' denotes the corresponding shop.

Table 2. Morphological identification of *Lactobacillus bulgaricus* on MRS agar.

Sample	Gram staining	Shape	Form	Colour	Elevation	Opacity
S1/ sh1	Gram positive	Rod	Straight	White	Raised	Opaque
S2/ sh2	Gram positive	Rod	Straight	White	Raised	Opaque
S3/ sh3	Gram positive	Rod	Straight	White	Raised	Opaque
S4/ sh4	Gram positive	Rod	Spiral	Yellow	Convex	Opaque
S5/ sh5	Gram positive	Rod	Straight	White	Raised	Moist
S6/ sh6	Gram positive	Rod	Straight	Paler	Raised	Opaque
S7/ sh7	Gram positive	Rod	Straight	Paler	Convex	Opaque
S8/ sh8	Gram positive	Rod	Straight	Whitish	Convex	Opaque
S9/ sh9	Gram positive	Rod	Straight	Paler	Raised	Moist
S10/ sh10	Gram positive	Rod	Straight	Yellowish	Raised	Opaque

Table 3. Morphological identification of *Streptococcus thermophilus*.

Sample	Gram staining	Shape	Form	Colour	Elevation	Margin
S1/ sh1	Gram positive	Spherical	Irregular	Creamy white	Convex	Entire
S2/ sh2	Gram positive	Spherical	Irregular	Creamy white	Convex	Entire
S3/ sh3	Gram positive	Spherical	Irregular segments	Creamy white	Convex	Entire
S4/ sh4	Gram positive	Spherical	Irregular segments	Whitish	Convex	Entire
S5/ sh5	Gram positive	Circular	Irregular	Whitish	Convex	Entire
S6/ sh6	Gram positive	Circular	Irregular segments	Creamish	Convex	Entire
S7/ sh7	Gram positive	Circular	Irregular	Creamy	Convex	Entire

			segments	white		
S8/ sh8	Gram positive	Circular	Irregular segments	Creamy white	Convex	Entire
S9/ sh9	Gram positive	Circular	Irregular segments	Cream	Convex	Entire
S10/ sh10	Gram positive	Circular	Irregular segments	Creamy white	Convex	Entire

Table 4. Biochemical characterization of *Lactobacillus bulgaricus*

Sample	Catalase	Oxidase	Indole	Citrate	Methyl Red
S1/ sh1	Negative	Negative	Negative	Negative	Negative
S2/ sh2	Negative	Negative	Negative	Negative	Negative
S3/ sh3	Negative	Negative	Negative	Negative	Negative
S4/ sh4	Negative	Negative	Negative	Negative	Negative
S5/ sh5	Negative	Negative	Negative	Negative	Negative
S6/ sh6	Negative	Negative	Negative	Negative	Negative
S7/ sh7	Negative	Negative	Negative	Negative	Negative
S8/ sh8	Negative	Negative	Negative	Negative	Negative
S9/ sh9	Negative	Negative	Negative	Negative	Negative
S10/sh10	Negative	Negative	Negative	Negative	Negative

Tabel:5 Biochemical characterization of *Streptococcus thermophilus.*

Sample	Catalase	Oxidase	indole	Citrate	Methyl Red
S1/ sh1	Negative	Negative	Negative	Negative	Negative
S2/ sh2	Negative	Negative	Negative	Negative	Negative
S3/ sh3	Negative	Negative	Negative	Negative	Negative
S4/ sh4	Negative	Negative	Negative	Negative	Negative
S5/ sh5	Negative	Negative	Negative	Negative	Negative
S6/ sh6	Negative	Negative	Negative	Negative	Negative
S7/ sh7	Negative	Negative	Negative	Negative	Negative

S8/ sh8	Negative	Negative	Negative	Negative	Negative
S9/ sh9	Negative	Negative	Negative	Negative	Negative
S10/ sh10	Negative	Negative	Negative	Negative	Negative

DISCUSSION

Scientists have long been concerned by supplemental imitations medications that are used with everyday products. Nowadays, a variety of commonplace materials and methods are employed to cure illnesses. One of these strategies is the use of probiotics (Balcázar et al., 2006). Bifid bacteria and lactobacilli are common colonic flora that help prevent colonic infections, reduce body fat, boost immunity, lower the risk of colon cancer, and play an important part in human health. Lactic acid and carbon-based acids are produced by probiotic bacteria, which also lower atmospheric pH and prevent the growth of other bacteria. Bacitracin, an antibacterial chemical produced by these bacteria, can be regenerated as natural preservatives (Çaglar et al., 2006). Dairy products have been identified as useful for human health due to years of research. LAB and lactobacilli from traditional foodstuffs have been widely identified and accessible by a number of scientists in recent years, and they have studied their likely aggressive effects on a number of diseases (De Keersmaecker et al., 2006). Lactobacilli and other bacteria are examples of microorganisms that can reduce infections, including viable exclusion, which impacts an individual's health. Probiotics rely heavily on lactobacilli derived from inflammatory diets. One of the best-known foods that contains probiotics is yogurt (Cremonini et al., 2002). The current study focused on identifying Lactobacillus from local yogurt in order to achieve that goal. *Lactobacillus Bulgaricus* and *Streptococcus thermophilus* were identified from ten yogurt samples from ten different stores. The organisms were isolated on MRS agar at 37°C with oxygen present. These two strains *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were isolated based only on morphology and biochemical testing.

Both food fermentation and the production of bacteriocins are heavily influenced by Lactobacillus (Fioramonti et al., 2003).

Because of their capacity to support the gastrointestinal tract, LAB, including *Lactobacillus acidophilus* and Bifid bacterium linguine, are implicated in yogurt and other inflammatory foods and may be taken consistently as probiotics (Haskard et al., 2001). In order to address the issue of lactose intolerance in certain adults, *L. acidophilus* is mostly found in yogurt (Fonden et al., 2000).

Gram previously identified Lactobacillus species as reliable and useful for catalase-negative markings (Fonden et al., 2000). But according to a different study, Lactobacilli are a varied group of rod-shaped, grain-positive, catalase-negative bacteria that differ in both their physiology and genetic makeup. The oral hole is one of the strains of Lactobacillus that have been described in a number of investigations. The Lactobacillus class is a recognized food fermenter on a global scale. Inflammation of various monosaccharides and disaccharides is caused by Lactobacillus bacteria (Guarner & Malagelada, 2003). The probiotic Bacteria are adept in causing inflammation in unrelated carbohydrates, and they are thought to produce lactic acid. For those who are lactose intolerant and unable to digest lactose because they lack the enzyme galactosidase, this is quite helpful. The people who are lactose intolerant may therefore benefit from eating items that are worsened below the research. In order to increase the amount of milk, Lactobacillus breaks down casein, which leads to the production of dereliction products (Harish & Varghese, 2006).

CONCLUSION

The findings of this study confirm that probiotics, particularly *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, are beneficial to human health. These microorganisms play a key role in enhancing digestive health, modulating gut microbiota, and contributing to the prevention and treatment of gastrointestinal disorders. Probiotic strains are generally considered safe, well-tolerated, and suitable even for individuals with weakened immune systems. Yogurt, as a widely consumed fermented dairy product, serves as a valuable source of probiotic bacteria. Commercial yogurt is typically produced using a combination of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, both of which were successfully isolated and identified in this study from locally sourced samples.

The results suggest that the selected *Lactobacillus* strains hold promise for future probiotic development and could contribute to functional food formulations. With continued research and proper validation, these strains may offer a natural alternative to antibiotics, supporting efforts to combat antibiotic resistance. Furthermore, their application may extend beyond human health, with potential benefits in veterinary and animal nutrition as well.

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