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Comparative Assessment of Isolated Probiotic Strain versus Standard Probiotic Strain: Exploring Efficacy, and Health Benefits

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Abstract - *Probiotics are live microorganisms, such bacteria or yeast, that sustain a balanced microbial environment, especially in the digestive tract, and hence contribute significantly to the promotion of host health. Probiotic bacteria, such as lactobacillus and Bifidobacterium strains, are frequently found in foods like pickles, idli batter, and cow milk. These bacteria may have health advantages. These advantages include better immunity, better digestion, and better gut health. But not every product made with these sources has live, active probiotic cultures, so it's important to read the labels carefully.*

The purpose of this study is to evaluate an isolated probiotic strain in comparison to a reference probiotic strain. The strain that was isolated was found to be a Gram-positive, rod-shaped lactic acid bacterium (LAB), and it was obtained from samples of pickles, idli batter, and cow milk. After characterizing the isolate to assess its probiotic potential, a side-by-side comparison with a reference probiotic strain was performed.

Key Words*: *Probiotics, Microorganisms, Bacteria, Yeast, IoT, Digestive tract, Balanced microbial environment, Host health.

1. INTRODUCTION

The word "probiotic," which comes from the Greek "pro bios," which means "for life," describes live microorganisms that are beneficial to humans and animals alike, such bacteria or yeast. By fostering a balanced microbial habitat, especially in the gut, these microbes are essential to preserving health. Although yeasts and enterococci have also been used, probiotic strains from the genera *Lactobacillus*, Streptococcus, and Bifidobacterium are among the most often used. These strains' selection criteria frequently give priority to characteristics thought to be critical to their efficacy, such as origin, in vitro adherence to intestinal cells, and survival throughout gastrointestinal tract (GIT) transit.

Probiotic bacteria can be found in both dairy and non-dairy foods. The three primary types linked to dairy products are *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacteria*. The creation of new items supplemented with probiotic bacteria, such as pickles, idli batter, and cow's milk, has the potential to grow the industry and satisfy consumer demand.

Reducing the indiscriminate use of antibiotics, especially in animal agriculture, has become more important as a result of worries about antibiotic resistance among human infections. This has prompted legislative actions, such as Europe's 2006 full prohibition of antibiotics that promote growth in animal feed. Probiotics, sometimes referred to as direct-fed microbials, are becoming more widely acknowledged in agriculture as viable substitutes for antibiotics in terms of promoting growth and managing particular enteric infections.

The study has garnered interest because to the quest for new probiotics that can be utilized in medical, industrial, and agricultural contexts. With an emphasis on probiotics from cow's milk, idli batter, and pickles, this study seeks to close knowledge gaps and offer insights that will be helpful to the agricultural industry. The ability of probiotic strains to withstand gastrointestinal conditions, such as low pH and bile tolerance, as well as the generation of antimicrobial chemicals, are selection criteria.

Four primary goals serve as the foundation for this research: 1) isolating probiotic strains from specific samples; 2) characterizing the biochemical makeup of the isolated probiotic strains; 3) evaluating the in vitro survival traits of the gastrointestinal tract; and 4) conducting a comparative analysis with probiotics that are sold commercially.

2. LITERATURE REVIEW

The word "probiotic" was first used by Nobel Prize laureate Ellie Metchnikoff, who connected Bulgarians' long life expectancy to their consumption of fermented milk products

that included rod-shaped bacteria. The name is derived from the Greek word "pro bios," which means "for life." Lilly and Stillwell formally invented the term "probiotic" in 1965. Over time, several interpretations of probiotics have been put forth; Parker (1974) defined them as "organisms and substances which contribute to intestinal microbial balance." Probiotics are living bacteria that, when consumed in appropriate amounts, offer health advantages beyond those of basic diet, according to Guarner and Schaafsma (1998).

Later, Sanders (2003) clarified the term, defining probiotics as live bacteria that offer the host health advantages when given in sufficient quantities. A wide variety of organisms are included in probiotics, including as lactobacilli, Bifidobacterium, Bacillus, Saccharomyces, and Enterococcus. Supplemental meals, dairy products, and fermented foods are major sources of these bacteria.

Lactobacillus is a genus of bacteria that produce lactic acid (LAB). It contains more than 100 species, many of which are employed as probiotics in diverse food products. Initially identified by Tissier between 1899 and 1900, bifidobacteria represent a significant category of probiotics frequently present in the gastrointestinal tract, especially in breastfed infants. Enterococci, which are members of the LAB group, are also used in probiotic applications. The most frequently associated species with probiotics are *E. faecalis* and *E. faecium*.

Numerous health advantages of probiotics include the prevention of intestinal infections, immune system modulation, possible prevention of some malignancies, and adjustment of blood cholesterol levels. They achieve these effects by modulating immunological responses, producing organic acids and antimicrobial peptides, and engaging in competitive colonization.

Probiotics are used in the food sector, pharmaceuticals, agriculture, and human health. Probiotics are being investigated more and more in the field of human health for their potential therapeutic benefits as well as preventative measures against a range of illnesses. Probiotics are added to feed as supplements in agriculture to enhance animal health and food safety. Probiotics are being developed as biological medications in the pharmaceutical sector, with particular properties and dosages.

In conclusion, probiotics offer a viable approach to enhancing health and wellbeing in both people and animals. Their wide range of uses emphasize their significance across industries and the need for more study to realize their full potential.

3. METHODOLOGY

3.1 Assembling of Instances

Three samples of raw cow's milk, two samples of idli batter, and one sample of pickles were gathered in a hygienic manner from various parts of Baramati. Within three hours, aliquots of the materials were gathered into sterile vials and brought to the lab for examination. The Post-Graduation Research Centre (PGRC) of Shardabai Pawar Mahila Arts, Commerce, and Science College, located in Shardanagar, Malegaon Bk., Tal-Baramati, Dist. - Pune, 413115, Maharashtra, India, is the location of the probiotic strain study.

3.2 Media and Chemicals

The following medium and substances were used:

- Peptone water
- Broth made of glucose phosphate
- Simons citrate agar
- Minimal media
- MHA press

3.3 Media

Selective isolation of lactic acid bacteria (LAB) was achieved using modified De Man, Rogosa, and Sharpe agar (MRS) medium. To improve the media's specificity for LAB isolation, L-cysteine was added and the pH was increased to 6.5.

3.4 Separation of Probiotic LAB from the Gathered Material

The following procedures were followed in order to isolate probiotic LAB:

1. One milliliter of each sample is aseptically added to nine milliliters of sterile saline solution.
2. The samples were diluted in series (10^-1 to 10^-7).
3. use the spread plate method to plating 1 ml aliquots from the dilutions onto MRS agar enriched with 0.05 g/L cysteine (MRS-cys) in triplicate.
4. MRS-cys agar plates were incubated anaerobically for 48 hours at 37°C.
5. choosing lone colonies from plates with high dilution.
6. Using MRS-cys agar for streaking, verify purity.
7. Purified colonies are kept at -20°C in MRS-cys broth that has been supplemented with 10% glycerol.

3.5 Determining the Probiotic LAB Genus

+ +3.5.1 Identification of Morphology and Features of Colonies

After choosing isolated colonies on MRS-cys medium plates, the following morphological traits were noted:

1. Gram Staining: To ascertain the isolates' gram response.
2. Using endospore staining, one can identify endospores.
3. Swarming Motility Test: To evaluate the isolates' motility.

3.5.2 Identification by Biochemistry

For genus-level identification, the following techniques were used in biochemical identification tests:

1. Test for Catalase
2. Tests for Indole, Methyl Red, Voges Proskauer, and Citrate are included in the Oxidase IMViC test
3. Test for Arginine Hydrolysis
4. Carbon Dioxide Production through the Fermentation of Glucose

3.6 Description of the Isolated Strain

The following criteria were used to characterize the isolated strains:

1. Acid Resistance
2. Tolerance to Bile Salts
3. Tolerance for Phenols
4. NaCl Tolerance
5. Intolerant to lactose
6. Susceptibility to antibiotics Examine Antimicrobial Properties

4. RESULT

4.1 Isolation of Probiotic Strain

Using modified MRS media, probiotic bacterial strains were effectively extracted from samples of pickles, idli batter, and cow milk.

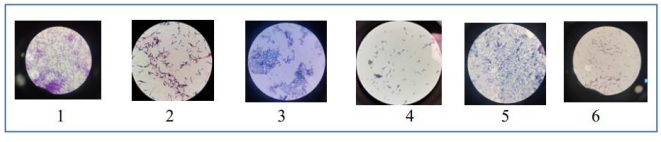


Fig 1: Isolated Strains

4.2 Determining the Isolated Strain

The isolated isolates were non-motile and showed signs of gram-positive status. Indole, methyl red, Voges-Proskauer, citrate, catalase, oxidase, arginine hydrolysis, and CO2 generation were all shown to have negative findings in biochemical testing. Tests on the fermentation of carbohydrates revealed heterofermentative traits, which may have led to the strains' identification as Lactobacillus sp.

Isolate	Size	Shape	Colour	Margin	Elevation	Opacity	Consistency	Gram Nature	Motility
1	2mm	Circular	Creamy white	Entire	raised	Opaque	sticky	Gram positive rod	Non motile
2	1mm	Circular	Creamy white	Entire	raised	Opaque	sticky	Gram positive rod	Non motile
3	3mm	Circular	Creamy white	Entire	raised	Opaque	sticky	Gram positive rod	Non motile
4	2mm	Circular	Creamy white	Entire	raised	Opaque	sticky	Gram positive rod	Non motile
5	2mm	Circular	Creamy white	Entire	raised	Opaque	sticky	Gram positive rod	Non motile
6	2mm	Circular	Creamy white	Entire	raised	Opaque	sticky	Gram positive Cocci	Non motile

Table 1 : Colony Characteristics

Name of test	Result
Indole test	Negative
Methyl red test	Negative
Voges-Proskauer test	Negative
Citrate test	Negative
Catalase	Negative
Oxidase	Negative
Arginine hydrolysis test	Negative
CO_2 production test	Negative

Table 2 : IMViC Test

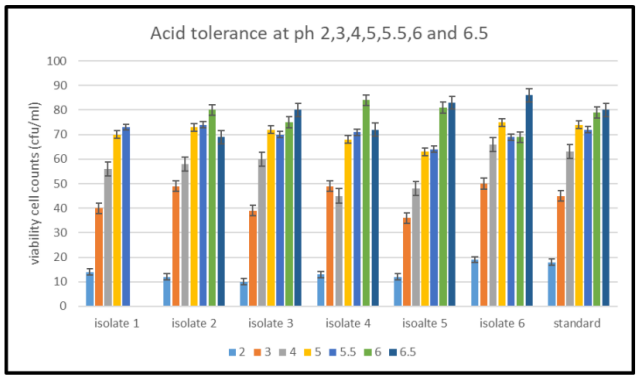
sugar fermentation test (sugars)	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Glucose	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)
Sucrose	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)
Maltose	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)
mannitol	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G+)	(A+)(G-)	(A+)(G-)
Fructose	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)
Lactose	(A+)(G-)	(A+)(G+)	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)
Galactose	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)	(A+)(G-)

A+ = Acid production G+ = Gas production A-= No acid production G-= no gas production

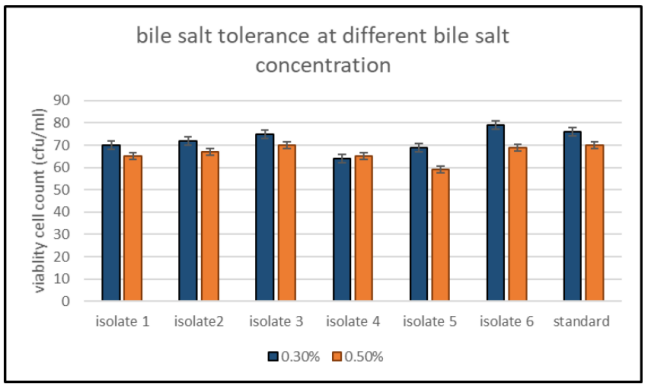
Table 3 : Carbohydarte Fermentation Test

4.3 Probiotic Strain Characterization

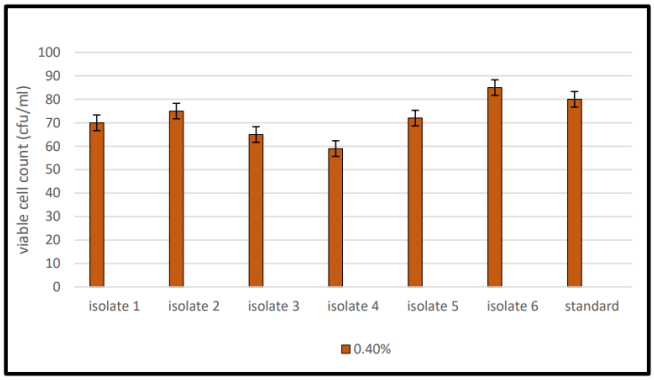
The isolated isolates exhibited tolerance to phenol, bile salts, and NaCl concentrations as well as optimal development at acidic pH values. They displayed resistance to both streptopenicillin and streptomycin. Furthermore, isolates 1, 2, and 6 showed a notable reduction in E. Coli growth.



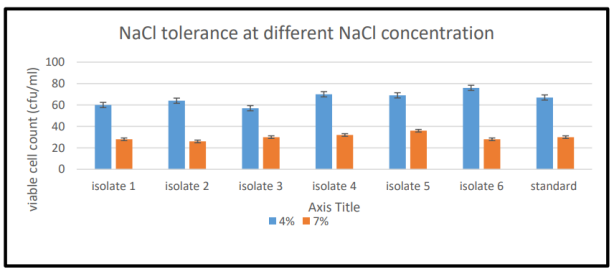
Graph 1: Survival of LAB isolates during 3 hours of incubation at pH 2, 3, 4, 5, 5.5, 6, 6.5



Graph 2: Bile Salt Tolerance Test



Graph 3: Phenol Tolerance Test



Graph 4: NaCl Tolerance Test

5. CONCLUSION

It was determined that the lactic acid bacteria strains obtained from samples of pickles, idli batter, and cow milk were most likely members of the Lactobacillus genus. Their characterization showed promise as probiotic bacteria, exhibiting resistance to bile salt, phenol, and NaCl, among other inhibitory chemicals, and acidic stressors. Antimicrobial activity studies demonstrated the medications' capacity to suppress test microorganisms, whereas tests for antibiotic susceptibility revealed resistance to specific antibiotics.

In vitro, the experimental strains of Lactobacillus showed encouraging probiotic properties, such as high tolerance to bile and pH and the capacity to stop the growth of pathogens. These results validate their use in the food and medical industries and point to their potential as antibiotic substitutes. Nevertheless, additional research, like as in vivo studies, is required to completely comprehend their effectiveness in promoting human

I'm looking at ways to use these isolates right now. To isolate and discover Lactobacillus spp. strains and other probiotic bacteria with potential health advantages, however, further research is needed. Further investigation in this field may lead to the creation of probiotic products with improved ability to prevent infections.

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