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Isolation of *Lactobacillus* Species from Different Types of Milk and Evaluate their Antagonistic Activity Against Pathogens

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Abstract:- Animal milk has been consumed by humans for a very long time. In the food industry, the probiotic *Lactobacillus* is regarded as a good substitute to lower the risk of food-borne illnesses. The bacteria *Lactobacillus* aids in the body's nutrient absorption, digestion, and defense against pathogens. Determination of *Lactobacillus* from unfermented and reconstituted Milk. The initial and biochemical tests allowed for the identification of the organisms. The two pathogens, identified as *Escherichia coli* and *Staphylococcus aureus* were isolated in light of the findings. The purpose of these two pathogens, selection was antagonistic research. With the cross-stretched method, the antagonistic activity test was conducted. The outcome demonstrates that there is more inhibition displayed by the *Lactobacillus* grown in buffalo milk. In an antagonistic test, the probiotic organism inhibits the test pathogens.

Keywords:- (*Lactobacillus, Milk, Probiotic, Pathogens, Antagonistic Activity)*

I. INTRODUCTION

A nutrient-dense liquid food called milk is made by mammals' mammary glands. Before they can digest solid foods, it serves as their primary food source for young mammals. First milk, or early lactation milk, has antibodies that strengthen the immune system and lower the risk of numerous ailments. Lactose and protein are found in milk.

A cup (244 grams) of whole cow's milk has 146 calories, 8g of protein, 8g of fat, 2.8% of calcium, 24% of vitamin D, 26% of riboflavin (B2), 18% of vitamin B12, 10% of potassium, 22% of phosphorus, and 13% of selenium. Milk is a good source of zinc, magnesium, thiamine (B1), and vitamin A. It is rich in hundreds of different fatty acids, such as Omega 3 fatty acids and conjugated linoleic acid (CLA). Higher levels of advantageous antioxidants, like vitamin E and beta carotene, which fight oxidative stress and reduce inflammation, can be found in organic and grass-fed cow's milk. Since it contains all nine of the essential amino acids needed for optimal bodily function, milk is referred to as a "Complete Protein." Casein and whey protein are the two different forms of protein found in milk. They are both regarded as premium proteins.

Numerous nutrients, including different amounts of fat and water (soluble vitamins, minerals, trace elements, and salts), are known to be present in cow milk. Lactose is the primary carbohydrate found in milk. While breast milk contains 7% lactose, milk contains 4.8% lactose (12 to 12.5 g per cup). Milk is a diverse blend of twenty different proteins. Human Ig E reacts to milk in a variety of ways. Ig E antibodies directed against lactoferrin and bovine serum albumin, two milk proteins. Children who are lactose intolerant frequently have a medical history of malnourishment, surgery, or gastrointestinal infections. There are variations in the specific components of milk protein that trigger an allergic response.

Nutritious buffalo milk is utilized to make dairy products like ice cream, butter, yogurt, and cheese. It is milked for commercial purposes in certain countries. Although there are many different kinds of buffalo, they are the world's main source of milk production. The majority of milk is produced by river buffaloes. About 80% of the world's buffalo milk is produced in India and Pakistan, with China, Egypt, and Nepal producing more buffalo than cattle. Buffalo milk has a rich, creamy texture that is ideal for making yogurt and butter cream. It is also high in fat and protein. Compared to whole milk, buffalo milk has higher levels of protein, fat, and lactose.

Approximately 2 percent of the world's yearly milk production comes from goats. Goats that are bred only for milk production exist. Compared to cow's milk, goat's milk has a slight sweetness. This is contingent upon various processing methods, packaging, and pasteurization procedures. Goats are better at converting dietary carotene to vitamin A. Goat's milk fat globules float instead of remaining on top. In order to extract the cream, a cream separator is needed. Additionally, it contains a lot of medium- and short-chain fatty acids. Consequently, the process of digestion is accelerated and simplified. Lactose is the name for the sugar that is found in milk. Extreme milk-related symptoms are caused by lactose intolerance. Goat milk is suitable for those who are lactose intolerant because it contains less lactose than milk.

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The bacteria lactobacillus aids in the body's nutrient absorption, digestion, and defense against pathogens. coloring while preparing and storing food. The vibrantly colored liquid known as milk is extracted from the mammary glands of cows. He went on to say that before other foods are digested, milk is one of the main food sources for young mammals. Lactose and protein are two more nutrients found in milk. Furthermore, milk and other dairy products naturally contain lactic acid bacteria (LAB). This is so because all the nutrients needed for lactic acid bacteria to reproduce are already present.

II. METHODOLOGY

➢ *Collection of Samples:*

Various types of milk samples, such as fermented goat milk, buffalo milk, and cow milk, were gathered from the local market.

Fig 1 Raw Cow Milk

Fig 2 Raw Buffalo Milk

Fig 3 Fermented Goat Milk

➢ *Isolation and Identification of LAB from Different Milk Samples:*

The samples were gathered and then serially diluted to the necessary concentrations. They were spread out and placed on MRS agar and nutrient agar. The plates were incubated under the proper circumstances. Following incubation, a count and tabulation of the colonies' numbers was performed.

➢ *Microscopic Examination (Gram's Staining):*

Gram staining is used to differentiate the gram positive and gram - negative organisms. A loop full of overnight broth culture was subjected to Gram Staining procedure and the result were recorded.

➢ *Biochemical Test*

- *Motility:*

Motile organisms are those that have locomotory organs such as cilia, flagella, or pseudopodia. A cover slip containing an isolate loop was used to test motility using the hanging drop technique.

- *Indole Test:*

This is incubated at 37°C for 48 or 98 hours before being tested in peptone water culture. This experiment shows how tryptophan is converted to indole. Kovac's indole reagent was added following incubation. Cherry red formation at the top layer was viewed favorably.

- *Methyl Red Test:*

This test is used to find out if acid is produced when glucose is fermented. After adding a loopful of inoculum to the MR-VP broth, the mixture was incubated for 48 hours at 37°C. Five drops of methyl red indicator were added and carefully mixed after incubation. A pronounced red hue was regarded favorably.

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- *Voges Proskauer Test:*

The production of acetyl methyl carbinol pyruvic acid, an intermediate step in its conversion to 2:3 butylene glycol, is required for this test. For 48 hours, a loopful of inoculums was inoculated at 37℃. Barrit's reagent was added and thoroughly mixed following incubation. The transition from pink to crimson red was viewed favorably.

- *Citrate Utilization Test:*

Citrate serves as the only carbon source in common citrate medium. This test indicates the user's ability to use this substance. Simmon citrate agar slant was streaked with a loopful of inoculums, and it was incubated for 24 to 48 hours at 37℃. Positive results are indicated by the media changing from green to Prussian blue after incubation.

- *'Catalase Test:*

It illustrates how the organisms produce the enzyme hydrogen peroxidase. On the spotless glass slide, a loopful of inoculums was placed, and a few drops of hydrogen peroxide were added to the culture. It was noted as a positive development that there was rapid effervescence.

- *Oxidase Test:*

The cytochrome oxidase, which catalyzes the oxidation of reduced cytochrome by oxygen, is responsible for this reaction. Within 10 to 30 minutes, colonies that are oxidase positive turn maroon, purple, and black. The oxidase disc was put on a sanitized slide, and after adding two or three drops of culture, it was determined whether or not the disc had formed a blue color.

➢ *MRS Agar:*

Bacterial growth medium, named for its creators, Sharpe and de Mon Rogosa. For laboratory research, this medium encourages the luxuriant growth of *Lactobacilli.* Because of the sodium acetate it contains, many competing bacteria are inhibited from growing.

➢ *Test Organisms:*

In vitro, antagonistical studies were carried out on two bacterial strains *Escherichia coli, Staphylococcus aureus.* Bacterial strains were grown on nutrient broth.

➢ *Antagonistic Activity of LAB Against Test Pathogens:*

After being grown in MRS broth, the identified *Lactobacillus sp.* were incubated for 48 hours at 37C. The broth culture was centrifuged for 20 minutes at 5,000 rpm to obtain a cell-free supernatant, which was then used for an antagonistic test against *Staphylococcus aureus* and *Escherichia coli.* A solitary streak in the middle of the Mueller Hinton Agar plate serves as the seed for the *Lactobacillus* strain. The first streak was streaked perpendicularly to the test organisms. For a duration of 24-78 hours, the plates were incubated at 37°C, and the inhibition was noted.

➢ *Determination of Antimicrobial Activity by Disc Diffusion Method:*

The Agar well diffusion method was used to conduct the modified antibacterial test (Collins et al., 1995). Using an organism swaber, the test microorganisms were evenly distributed after being inoculated on Mueller Hinton Agar (MHA). A sterile well puncher was used to create 8mm-diameter wells on MHA. Sterile forceps were used to carefully remove the agar blocks.

On nutrient broth, the isolated *Lactobacillus* strains were cultured. After the pellets were moved to the wells, the broth was centrifuged. Before the growth of the microorganisms was commended, the plates were left to stand for one hour at room temperature to allow the substances to diffuse. The zones of inhibition on the plates were measured after they were incubated for 22–72 hours at 37℃.

III. RESULTS

The microbiome was isolated from raw cow and buffalo milk as well as fermented goat milk using MRS agar plates that were incubated under the right conditions. The outcomes were tabulated and observed following the incubation period. Results of the motility test and microscopic examination of the gram staining were recorded and tallied.

Determination of *Lactobacillus* from unfermented and reconstituted Milk. The preliminary and biochemical tests allowed for the identification of the organisms. We isolated two pathogens, namely *Escherichia coli* and *Staphylococcus aureus* based on the findings. The purpose of these two pathogens, selection was antagonistic research.

With the cross-stretched method, the antagonistic activity test was conducted. The outcome demonstrates that there is more inhibition displayed by the *Lactobacillus* grown in buffalo milk. In an antagonistic test, the probiotic organism inhibits the test pathogens.

Table 1 Colony Morphology:

S.no	Samples	Media	Characteristics
1.	Raw cow Milk	MRS Agar	Small, rod -shaped Gram positive, thin, creamy white colonies.
2.	Raw Buffalo milk		Rod, Gram - positive, Mucoid, thick, yellow color, group of colonies.
3.	Fermented goat milk		Rod, Gram - positive, Pale yellow, Group of colonies.

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Table 2 Morphological and Biochemical Characterization of Bacteria

S.no	Biochemical tests	Raw cow milk	Raw buffalo milk	Fermented goat milk
1.	Indole, MR, VP, Citrate, Catalase and oxidase	Negative	Negative	Negative
2.	Lactose fermentation	Positive	Positive	Positive
3.	Motility	Non- motile	Non-motile	Non- motile

Table 3 Antagonistic Activity of *Lactobacillus* Against *Escherichia coli*:

S.no	Name of the organism	Samples	Name of test pathogen	Result
1.	*Lactobacillus*	Cow milk	*Escherichia coli*	14 mm
2.		Buffalo milk		18 mm
3.		Goat milk		13 mm

Table 4 Antagonistic Activity of *Lactobacillus* Against *Staphylococcus aureus*

S.no	Name of the organism	Samples	Name of test pathogens	Result
1.	*Lactobacillus*	Cow milk	*Staphylococcus aureus*	12 mm
2.		Buffalo milk		14 mm
3.		Goat milk		11 mm

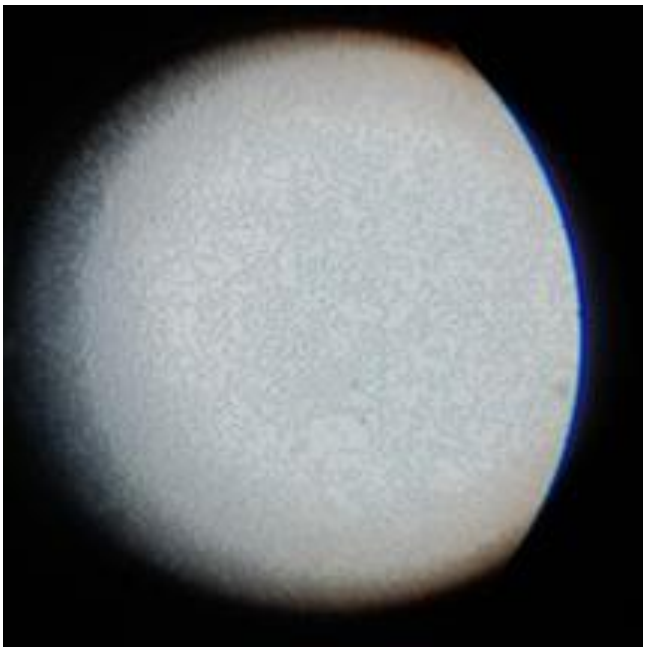

Fig 4 Gram Staining

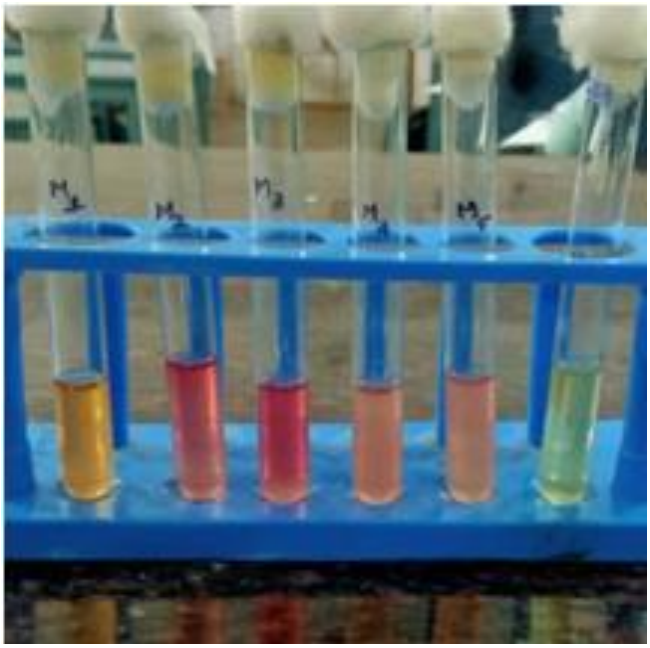

Fig 6 Methyl Red Test

Fig 5 Indole Test

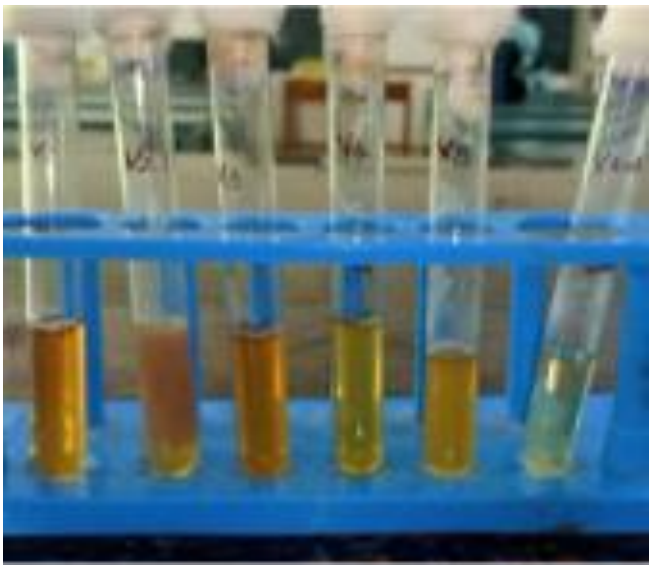

Fig 7 Voges Proskauer Test

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Fig 8 Citrate Utilization

Fig 9 Catalase Test

Fig 10 Colonies on MRS Agar Plates

Fig 11 Antagonistic Activity of *Lactobacillus* Against Test *Escherichia coli* and *Staphylococcus aureus*

IV. SUMMARY AND CONCLUSION

Three different types of milk samples were gathered; two were raw milk and one was fermented milk. The organism in MRS agar was isolated from both raw and fermented milk. It was possible to isolate the bacteria. Following that, isolated organisms are identified using biochemical testing and microscopic analysis. It was verified that Lactobacillus sp. Grew on MRS agar.

To assess *Lactobacillus* antagonistic activity against two test pathogens: *Escherichia coli*, a Gram - negative bacteria, and *Staphylococcus aureus*, a Gram - positive bacteria. The cross-streak method was used to carry out the antagonistic activity. The antagonistic activity of *Lactobacillus* against the two pathogens was demonstrated. It prevents *Staphylococcus aureus* and *Escherichia coli* from growing. Compared to other strains, the *Lactobacillus* grown in buffalo milk exhibits a high level of inhibition.
Antimicrobial activity of *Lactobacillus* against test organisms, including *Staphylococcus aureus* and *Escherichia coli*. Well diffusion method is used to carry out the method. The outcome demonstrates that zone inhibition is absent. because the centrifugation process breaks the lactobacilli's cell wall. Inhibition against the pathogens is absent.

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The results of this investigation showed that milk exhibited strong antagonistic activity against a variety of bacterial pathogen strains. These results bolster the notion that milk may have health benefits and that it is advised as a probiotic. In the current study, Milk's antagonistic activity against the microorganisms was investigated, and the presence or absence of inhibition zones served as a measure of its potency.

Based on the findings of this investigation, food preservation and human health are significantly impacted by the antagonistic effects of substances produced by bacteria on a variety of microorganisms. It is possible to grow these bacteria in order to produce a variety of foods and medications. Additionally, they can be employed in the creation of novel functional foods. Consequently, it is advised to incorporate more probiotic-containing dairy products into daily meals as well as identify and produce foods that have the highest and most potent levels of lactobacilli.

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