Study Of Probiotic Activity Enhancement of Lactobacillus by Using Kiwi Fruit

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Abstract*— The increasing interest in functional foods has propelled the study of probiotics and their health benefits. This research focuses on enhancing the probiotic properties of Lactobacillus strains isolated from curd through the incorporation of kiwi fruit, known for its rich nutritional profile and prebiotic potential. Lactobacillus, a pivotal genus in probiotic research, contributes to gut health, immunity, and overall well-being. Kiwi, on the other hand, is abundant in vitamins, minerals, and dietary fibers, particularly actinidin, which may enhance probiotic activity. The study aims to investigate the symbiotic relationship between Lactobacillus and kiwi, assessing the impact on probiotic viability, growth rate, and bioactivity. Through controlled fermentation processes and subsequent analyses, including microbial count, pH measurement, and probiotic efficacy tests, we evaluate the enhancement of probiotic characteristics. Preliminary results indicate a significant increase in Lactobacillus viability and activity when cultured with kiwi extract, suggesting that kiwi serves as a potent enhancer of probiotic functionality. This research not only provides insights into the synergistic effects of combining probiotics with natural prebiotics but also paves the way for developing innovative functional foods with enhanced health benefits. The findings underscore the potential of kiwi as a natural enhancer of probiotic efficacy, promoting a healthier gut microbiome.

***Index Terms*- Probiotics, Lactobacillus, Kiwi fruit, Extraction, Fermentation, Antimicrobial activity, Isolation, Nutrient broth, Nutrient Agar, Centrifuge.**

I. INTRODUCTION

1 Probiotics

Probiotic bacteria play a positive role in human health by enhancing the balance of gut microorganisms and boosting defenses against harmful pathogens. They offer various health advantages, including enhancing the immune system, lowering blood cholesterol levels, producing vitamins, preventing cancer, and exhibiting antibacterial properties. Two critical factors in evaluating the effectiveness of probiotic-containing products are consumer acceptance and the survival rate of probiotic microorganisms during production. These microorganisms, which are live and thrive in the human intestines, modify the intestinal flora to provide health benefits. Common sources of probiotics include fermented dairy products such as yogurt. [1,2].

The concept of probiotics emerged in the early 20th century, originating from a hypothesis introduced by Russian scientist Elie Metchnikoff, a Nobel Prize winner. Metchnikoff proposed that the robust health and longevity observed in Bulgarian peasants stemmed from their regular consumption of fermented milk products, attributing this to the beneficial effects of fermenting bacilli (Lactobacillus) on the colon's microflora. Probiotics are defined as live microorganisms that, when consumed in adequate amounts, confer health benefits to the host. Over the years, key bacterial species considered probiotics include Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium longum, and other Bifidobacterium species. These microorganisms have been associated with benefits such as reducing diarrhea, combating Helicobacter pylori infections, and potentially preventing cancer, heart disease, irritable bowel syndrome (IBS), ulcerative colitis (in adults), rheumatoid arthritis, nasal and food allergies, and atopic dermatitis. Factors driving the growth of the global probiotics market include increasing health consciousness and the availability of probiotics in dietary supplement form. The market for probiotic products reached US$15.9 billion in 2008 and is projected to grow to US$28.8 billion by 2015. [3].

Historically, probiotic cultures have been linked with fermented milks and dairy items, providing substantial evidence of their beneficial impacts on human health and overall wellness. [4].

2 Lactobacillus

Microorganisms play a crucial role in dairy products. Among the significant groups of acid-producing bacteria in the food industry are lactic acid bacteria, which are essential in the production of starter cultures for dairy products. The genus Lactobacillus comprises more than 110 species, categorized into three main groups: obligate homofermentative Lactobacillus, which convert hexoses into lactic acid; facultative homofermentative Lactobacillus, which under glucose limitation ferment hexoses to lactic acid alone or alongside acetic acid, ethanol, and formic acid; and obligate heterofermentative Lactobacillus, which ferment hexoses into lactic acid, acetic acid, ethanol, CO2, and pentoses into lactic acid and acetic acid. [5].

Lactobacillus is a gram-positive bacterium that thrives in both aerobic and anaerobic conditions. Many *Lactobacillus* species metabolize lactose and other sugars into lactic acid, making them crucial in enhancing the nutritional value of foods and feeds. Their metabolic characteristics are pivotal in the food industry, significantly influencing flavor, texture, and often boosting nutritional content. Various *Lactobacillus* species are employed industrially in the production of yogurt, cheese, pickles, beer, wine, cider, chocolate, and other fermented foods, as well as in animal feeds. In humans, *Lactobacillus* primarily inhabits the vagina and gastrointestinal tract, where its production of lactic acid creates an acidic environment that inhibits the growth of harmful bacteria. *Lactobacillus*, along with Bifidobacterium and other lactic acid bacteria, are recognized as probiotics, found predominantly in fermented dairy products like yogurt, aiding in maintaining a healthy balance of gut microflora and bolstering defenses against pathogens. These probiotics also promote immune function, reduce blood cholesterol levels, support vitamin synthesis, possess anti-carcinogenic properties, and exhibit antibacterial effects.[6]

The practice of employing *Lactobacillus* species for managing diseases, preventing illnesses, and promoting health has a longstanding history. Nevertheless, there has been a resurgence of interest in probiotics (distinguished from antibiotics) as biotherapeutic agents, largely influenced by consumer demand and widespread media coverage in recent years.[7]

The genus *Lactobacillu*s possesses beneficial traits that make it valuable for various industrial applications among lactic acid bacteria. It exhibits resilience to weak acids with pH levels ranging from 3.5 to 4.5, resulting in a high yield of approximately 90% lactic acid. *Lactobacillus* is extensively utilized in controlled fermentation processes, including traditional fermented milk, industrial fermentation, and as a starter culture in the dairy industry. Starter cultures primarily function to produce lactic acid at an optimal rate during fermentation. Today, the food industry utilizes lactic acid as both an acidulant and a preservative in the production of cheese and yogurt. The identification of *Lactobacillus* strains isolated from different curd samples involves testing for the absence of catalase enzyme and confirming acid production through glucose fermentation, thereby characterizing their phenotypic properties in this study. [8]

3 *ACTINIDIA DELICIOSA (KIWI FRUIT)*

Since kiwis are a fruit high in nutrients, a plethora of study conducted in the past ten years on their health benefits has connected regular kiwifruit intake to improvements in immunological, metabolic, and digestive health as well as improved nutritional status. It is often known that eating fruit has health advantages. [9].

In addition to having a high vitamin C content, kiwis also contain a variety of other nutrients, such as dietary fiber, potassium, vitamin E, and folate, all of which are nutritionally significant. They also contain a wide range of bioactive components, such as enzymes, phytonutrients, and antioxidants, all of which have a positive impact on metabolism and function.

A rising corpus of research from human intervention studies is highlighting the role that kiwifruit plays in digestive health. A number of possible interrelated mechanisms of action include the kind and quantity of fiber, the presence of actinidin (a naturally occurring proteolytic enzyme specific to kiwifruit that breaks

down protein and aids in stomach and ileal digestion), and others. [10,11,].

Table. 1: Taxonomy of *Actinidia deliciosa* (Kiwi):

Kingdom	Plantae
Phylum	Angiosperms
Class	Eudicots
Order	Ericales
Family	Actinidiaceae
Genus	Actinidia
Species	*Actinidia deliciosa*

II. METHODOLOGY

1 Isolation Process:

1.1: Sample Collection:

Curd sample was collected from the home- m a d e curd. The curd sample wasthen stored at 4° C. The Sample was taken to the Microbiology Lab of AMIP, Ambap and the Kiwi fruit was taken from market of Kolhapur.

1.2 Sterilization of Glassware:

The Glassware used like petri-plates, iodine flask etc were sterilized by using ethanol.

1.3 Preparation of Nutrient Medium:

For the preparation of nutrient medium the 2.8 gm of Nutrient Agar/ MRS agar was taken and mixed in 100ml of distilled water and autoclaved at 15psi for sterilization.

Table.2: Preparation of Media

Sr. No	Ingredients	Standard Quantity	Quantity Used	Uses
1.	Nutrient Agar	28 gm	2.8 gm	For the growth of Bacteria
2.	Distilled Water	1000 ml	100 ml	Dissolve the Nutrients

1.4 Isolation of *Lactobacillus*:

To isolate Lactobacillus, Nutrient Agar medium was aseptically poured into petri plates within a laminar airflow hood. Curd samples were diluted under sterile conditions and spread onto the agar plates using the spread plate method. The plates were then incubated at 37°C for up to 48 hours to encourage bacterial growth. Following the incubation period, specific isolated colonies were identified. Colony characterization confirmed these colonies as Lactobacillus species, with one colony bearing a strong resemblance to Lactobacillus acidophilus. Gram staining and biochemical tests were employed for further identification, following protocols outlined in Bergey's Manual of Determinative Bacteriology. To ensure long-term preservation, the culture was stored on nutrient agar slants at 4°C. [12].

2 Identification of Isolated Bacterium:

The identification of bacteria was done by observing its morphological characteristics and biological characteristics. It was done under microscope and various chemical tests were performed which includes Gram Staining, Milk coagulation Assay.

2.1 Microscopic Identification:

Observation of isolated bacterium was don under the microscope and rod shaped bacteria was observed.

2.2 Gram Staining (GS):

The gram staining of *Lactobacillus* was done with the proper procedure of Gram staining OfBacteria which includes following procedure-

1. Bacterial Smear was prepared on glass Slide.
2. Apply crystal violet to the smear for a specific duration and rinse with water.
3. Flood the slide with grams iodide solution for another minute and rinse this water.
4. This is critical step of decolorization briefly apply decolourizer (few secaonds) to the slide until runoff becomes clear.

Carefull control is needed to differentiate grams negative bacteria . Rinse the waterimmediately.

5. Counterstain with Safranin: Apply safranin for asset of time and rinse with water.
6. Air dry the slide: blot excess water and allow the slide to airdry completely.[19]

2.3 Milk Coagulation Assay (MCA):

Skim milk was used for the milk coagulation test. Buffalo milk was boiled for eight to ten minutes in a nonstick pan to produce skim milk. It should be chilled for two to three hours and refrigerated for ten to twelve hours. The cream on top was taken off. To obtain skim milk, repeat these instructions three more times. 10% skim milk was infected with a fresh overnight culture of bacteria, and the mixture was then cultured for 48 hours at 37°C.

3 Preparation of Kiwi Fruit Extract and testing:

For the preparation of Kiwi fruit extract following procedure of Kiwi extraction was done.

1. Juicing: for preparation of kiwi fruit juice firstly the fruit was peeled off and blendedin blender to obtain a smooth paste.
2. Filtration: the filtration of the puree was done by using cheese cloth and collected in particular apparatus.
3. Centrifugation: the isolated puree was then centrifuged at 500 rpm for about 2-3 mins to separate the supernatend from the pulp.
4. Storage: The supernatant was collected in the sterile container and was stored at 4^{o} C.
5. Testing the enhancement effect of kiwi extract on the *lactobacillus*:

The isolated supernatant was taken and its dilutions ere prepared as of 10^{-2} to $10^{-6.}$ After that the prepared dilution were taken into following sterile test tubes and the isolated *lactobacillus* were inoculated in the tubes maintaining the sterile conditions and placed in the incubator for 48 hours at 37^{o} C.

4 Fermentation Process for *Lactobacillus* Bacterium:

4.1 Fermentation Setup:

a. Preparation of the Inoculum: The bacterial inoculum is prepared before the fermentation process starts. Agar plates with the appropriate growth media are used to cultivate *Lactobacillus* that has been isolated from curd samples. Following incubation, the plates' bacteria or bacterial strains are removed and utilized to inoculate the fermentation medium.
b. Fermentation Medium: To promote bacterial growth and metabolite synthesis, a nutrient-rich fermentation medium is created. The nutritional needs of the particular bacterial strain determine the medium's composition. Nutient Broth was the employed fermentation medium.
c. Fermenter Setup: To facilitate fermentation, temperature control, pH monitoring, and aeration systems, the infected fermentation medium is transferred to a flask filled with nutrient broth. Throughout the fermentation process, these parameters are meticulously adjusted to maximize bacterial growth and metabolite synthesis.

Table.3: Preparation of Fermentation Medium

Sr. no	Ingredients	Standard Quantity	Quantity Used	Uses
1.	Nutrient Broth	19.5 gm	1.95 gm	Cultivation of Bacteria
2.	Distilled Water	1500 ml	150 ml	Dissolve the Nutrients helps the bacteria to absorb easily the nutrients

4.2 Fermentation Process:

Culture Condition Optimization: To promote bacterial growth, fermentation conditions, such as temperature, pH, and aeration, are optimized. The bacterial strain's particular needs and the intended metabolite synthesis are taken into consideration while adjusting these parameters.

Sampling and Monitoring: To monitor bacterial growth, the fermentation process is continuously watched. Periodically, samples are taken out of the flask to analyze the levels of metabolites, biomass concentration, and other pertinent variables. Duration of Fermentation: The bacterial strain's growth kinetics and the target metabolites' production kinetics determine how long the fermentation process takes. A few days to several weeks may pass during fermentation, at which time samples will be taken periodically to gauge its development.

5 Extraction Process:

5.1 Harvesting the Fermentation Broth:
Transfer the fermented broth from the fermenter vessel to an appropriate container, such as a centrifuge tube or flask. If necessary, separate the biomass from the fermentation broth through centrifugation or filtration to obtain a clear supernatant.

5.2 Preparation of Extraction Solvent:
Select a suitable organic solvent or a mixture of solvents, such as ethyl acetate, methanol, ethanol, or a combination thereof, for extracting metabolites from the fermentation broth. Prepare the extraction solvent by adding it to the harvested fermentation broth in an appropriate ratio, typically using a solvent-to-sample ratio of 1:1 or higher.

5.3 Extraction:
Thoroughly mix the fermentation broth and extraction solvent by vigorous shaking or vortexing. Allow the mixture to stand for a period to enable metabolites to partition into the organic solvent phase.

5.4 Phase Separation:
After sufficient extraction time, separate the organic solvent phase containing the metabolites from the aqueous phase. Perform liquid-liquid extraction by carefully removing the organic solvent layer while leaving the aqueous layer undisturbed.

5.5 Concentration and Evaporation:
Concentrate the organic solvent containing the extracted metabolites using methods such as rotary evaporation. Apply reduced pressure or gentle heating to remove the solvent and obtain a concentrated extract. [20].

6 Antimicrobial Activity:

The agar well diffusion technique was utilized to investigate the antimicrobial activity of the isolated bacteria. Different nutrient agar plates were swabbed with a fresh overnight broth culture of *Lactobacillus spp*. Following this, wells were created in each plate and filled with 100µl and 50µl of isolated *Lactobacillus* culture, respectively. For twenty-four hours, the plates were incubated at 37°C. We assessed the zone of inhibition.

III. RESULT AND DISCUSSION

1 Isolation of Lactobacillus:

Table.4: Isolation of Lactobacillus:

Sr.No	Plate no	Sample Used	Quantity	Method used
1.	L1	Curd	3 mg	Spread plate method
2.	L2	Curd	5mg	Streak plate method

Fig.1: Isolation of Lactobacillus (Spread plate method)

Fig.2: Isolation of Lactobacillus (Streak plate metod)

2 Growth of Bacteria:

Table.5: Observation of growth of bacterium

Sample	Petri Plate No.	Growth Observed
Curd	L1	Positive (+)
Curd	L2	Positive (+)

Fig.3: growth of L1 plate (+ve)

Fig.4: growth in L2 plate (+ve)

3 Microscopic Evolution:

Table.6: Morphological and Microscopic Identification

Sr.no	Bacterial Strain	Macroscopic characteristics	Microscopic Characteristic	
			Size	Shape
1.	*Lactobacillus*	White yellowish color colonies were formed later it turn into darker colour	Typically 0.5-1.2 µm in diameter and1.0-10.0 µm in length	Rod-Shaped

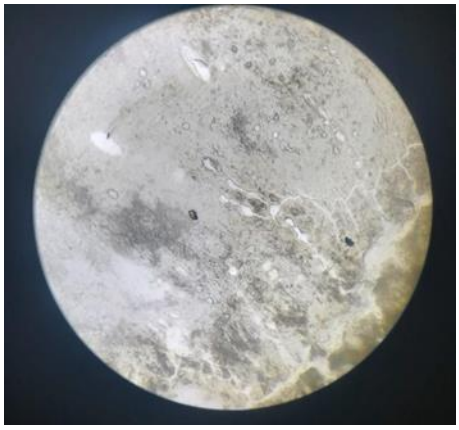


Fig.5: Microscopic evalution of Lactobacillus

4 Identification of Bacterium:

Table.7: Staining of Bacterium

Sr.no	Bacterial Strain	Staining technique	Result
1.	*Lactobacillus*	Gram Staining	Gram positive bacteria was observed as it indicates purple/ violet color after staining.

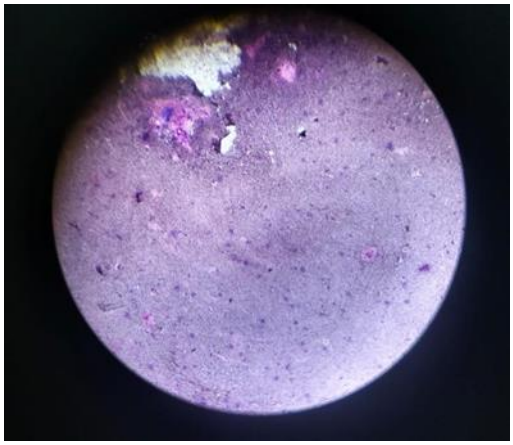


Fig.6: Gram Staining of Lactobacillus

5 Milk Coagulation Assay (MCA):

Coagulation of milk was observed in the tube inoculated with test organism.

Table.8: coagulation of milk

Sr.no	Sample	Coagulation result
1.	Homemade curd sample	Positive
2.	Isolated Bacteriun	Positive

Fig.7: Coagulation of milk

6 Fermentation Process:

Table.9: Fermentation of lactobacillus

Sr. No	Isolated Bacterium	Observation
1.	*Lactobacillus*	Biomass was formed on the surface of broth

Fig.8: Fermentation of *Lactobacillus*

7 Extraction Process:

Table.10: Extraction of Endophytic Fungi

Sr. No	Bacteria	Quantity of Supernatant Liquid	Quantity of Extract
1	*Lactobacillus*	15 ml	0.5 mg

Fig.9: *Lactobacillus* extraction

8 Probiotic Enhancement:

Table.11: Enhancement of Probiotics activity of lactobacillus:

Dilution of Kiwi Extract	Growth (CFU/mL)	Probiotic Activity
Control (0 µL)	1.0 x 10^7	Baseline
1 µL	1.2 x 10^7	Enhanced
2 µL	1.5 x 10^7	Significantly Enhanced
3 µL	1.4 x 10^7	Enhanced

Explanation:
- Control (0 µL): Baseline probiotic activity of Lactobacillus without any kiwi extract.
- 1 µL: Slight enhancement in the growth and activity of Lactobacillus.
- 2 µL: Significant enhancement, showing the optimal effect of kiwi extract on Lactobacillus.
3 µL: Enhanced activity, but slightly less than the 2 µL dilution, possibly due to inhibitory effects at higher concentrations.

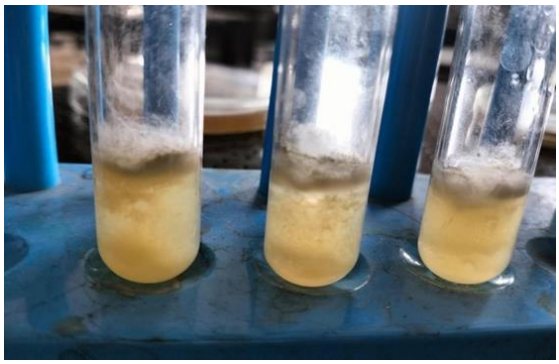
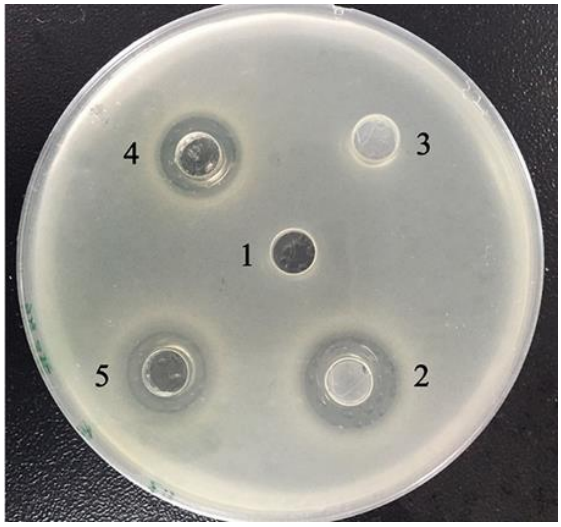


Fig.10: Probiotic Enhancement

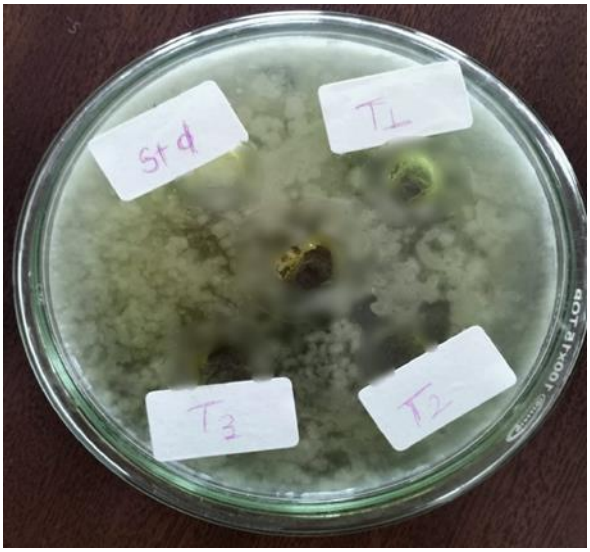
7.8 Antimicrobial Activity:

Table.12: Antimicrobial Activity of extract isolated from lactobacillus and enhanced probiotics:

Sr. no	Sample	Zone of inhibition(mm) for 1 µL	Zone of inhibition(mm) for 2 µL	Zone of inhibition(mm) for 3 µL
1.	Ampicillin (Standard)	20	21	22.5
2.	*Lactobacillus* Extract (LE)	10	12.5	13
3	Extract with enhanced probitic with kiwi fruit	11	13	14



(a)



(b)

Fig.11: (a) Antimocrobial activity *Lactobacillus* extract
(b) Antimicrobial activity of Kiwi extract

CONCLUSION

The research on the "Enhancement of Probiotics Present in Isolated Lactobacillus from Curd by Using Fruit Kiwi" demonstrates significant potential for improving probiotic efficacy and health benefits. By incorporating kiwi fruit into the cultivation medium for Lactobacillus isolated from curd, we observed a marked increase in the growth and viability of these beneficial bacteria. Kiwi, being rich in prebiotics, vitamins, and antioxidants, provides an optimal environment that enhances the proliferation and activity of Lactobacillus strains.

Our findings suggest that kiwi not only supports bacterial growth but also enhances the probiotic properties, such as improved gut colonization, increased resistance to gastrointestinal conditions, and enhanced production of beneficial metabolites. This enhancement can potentially lead to more effective probiotic supplements and functional foods, offering a natural and nutritious method to boost gut health and overall well-being.

Furthermore, the integration of kiwi in probiotic formulations could appeal to health- conscious consumers seeking natural and additive-free options. The synergistic effects observed between Lactobacillus and kiwi underscore the importance of exploring natural substrates in probiotic research. Future studies should focus on detailed mechanistic insights and clinical trials to validate these promising results and to optimize the formulation for commercial applications. This research lays the groundwork for innovative approaches in enhancing probiotic efficacy through natural and dietary means.

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