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Elucidating the Effects of Physiological Factors on the Metabolic Profile of *Lactobacillus* Bacterial Isolates

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Abstract: - This study highlights the potential of Lactobacillus isolates (from curd & kefir) as probiotics against bowel infections. The isolates showed optimal growth at 0.5% salt concentration and pH 5, with varying levels of tolerance to different salt concentrations and pH levels. Notably, S12 and S33 exhibited high colony counts at 0.5% salt concentration, indicating their potential as salt-tolerant isolates. Lactobacillus species can strengthen the gut barrier, produce antimicrobial substances, and modulate the immune system, contributing to their potential health benefits. These findings suggest that these isolates could be used to develop novel probiotics or antimicrobial therapies to prevent or treat bowel infections, and further research is needed to explore their mechanisms of action and potential applications.
Keywords: Lactobacillus, Metabolites, Salmonella typhiimurium, Staphylococcus aureus

I. INTRODUCTION

Probiotics play a vital role in maintaining gut health by regulating the gut microbiome, promoting the growth of beneficial bacteria, and suppressing harmful ones. They also enhance the gut barrier function, reducing permeability and preventing toxins from entering the bloodstream. Additionally, probiotics boost the immune system, increase antibody production, and activate immune cells. Some probiotics even produce essential vitamins like vitamin K and biotin. By reducing inflammation and improving digestion, probiotics can alleviate symptoms of conditions like irritable bowel syndrome (IBS) and support overall gut health, which is closely linked to overall well-being and reduced risk of various diseases. *Lactobacillus spp*. is Gram-positive, facultative anaerobic bacteria widely recognized for their probiotic properties. Found naturally in fermented foods like curd and kefir, these bacteria play a vital role in maintaining gut health and enhancing immunity. Their ability to modulate the metabolic environment of the host makes them candidates of interest in food, pharmaceutical, and clinical sectors.

Lactobacillus is a type of probiotic bacteria that can help protect against bowel infections by: - Strengthening the gut barrier, producing antimicrobial substances. *Lactobacillus* strains can produce lactic acid, hydrogen peroxide, and bacteriocins, which can inhibit the growth of pathogenic bacteria. The physiological factors such as pH, temperature, salt concentration, and bile tolerance significantly influence the metabolic output of *Lactobacillus* strains, particularly their ability to produce organic acids, bacteriocins, and other bioactive compounds. They also produce beneficial metabolites, like short-chain fatty acids, which provide energy to the host and support gut health. Understanding how these variables affect their metabolic profile provides deeper insight into their potential applications. *Lactobacillus* species have various physiological effects on the host, contributing to their potential health benefits.

II. MATERIALS AND METHODS

1) Isolation and Identification:- *Lactobacillus* spp. was isolated from traditional dairy products, specifically curd and kefir. Samples were serially diluted and plated on MRS (de Man, Rogosa, and Sharpe) agar, followed by anaerobic incubation at 37°C for 48 hours. Colonies with typical morphology were selected and subjected to Gram staining.
2) Biochemical Characterization:- Selected isolates were tested for carbohydrate fermentation, proteolytic activity, and gas production. Sugar fermentation profiles were determined using phenol red carbohydrate broth for glucose, lactose, sucrose.
3) Physiochemical Analysis of bacterial isolates: - The physicochemical analysis of bacterial isolates was conducted to determine their growth characteristics and environmental adaptability. Parameters such as pH tolerance, temperature stability, and salt concentration resistance were evaluated. The isolates exhibited varying degrees of resilience under different conditions, providing insights into their potential probiotic applications.

4) Salinity: - The physiochemical analysis of bacterial isolates was performed to assess their tolerance to different salinity levels. Luria-Bertani (LB) broth is a widely used nutrient-rich medium for the growth of bacteria.
Procedure:

- Bacterial cultures were grown in broth media supplemented with 0.5%, 0.8%, and 1.0% sodium chloride (NaCl).
- The inoculated media were incubated at an optimal temperature for 24–48 hours.
- Growth was monitored by measuring optical density (OD) at 620 nm using a spectrophotometer.
- The results were recorded and analyzed to determine the isolates' ability to survive and proliferate under varying salinity conditions.

5) pH:- The pH tolerance of bacterial isolates was assessed by culturing them in broth media adjusted to pH 3, 4, and 5.
Procedure:

- The bacterial isolates were inoculated into the prepared media and incubated at 37°C for 24 hours.
- Growth was measured by recording the optical density (OD) at 620 nm using a spectrophotometer.
- The experiment was conducted in triplicate to ensure accuracy, and the results were analyzed to determine the isolates' ability to survive under acidic conditions.

6) Temperature: - To understand antagonistic activity of bacterial isolates, fermentation process was used to extract metabolites from bacterial isolates for better ant-microbial property. After fermentation all the samples were incubated at different temperature in water bath for metabolites activation. For the activation of metabolites and enzymes, each bacterial metabolite sample was placed in a water bath at 25°C for 15 minutes. This process was repeated for every bacterial metabolite sample at 50°C, 75°C, and 100°C to assess the effect of temperature on enzymatic activity.

Anti Microbial activity of bacterial metabolites against pathogens

- The test pathogens were obtained from a microbial culture collection and were maintained on nutrient agar slants.
- Fresh inoculums of each pathogenic bacteria strain (*Staphylococcus aureus*, *Salmonella Typhimurium*) were prepared by suspending colonies in sterile physiological saline and adjusting the turbidity to 0.5 McFarland standards.
- Muller-Hinton Agar (MHA) plates were prepared and allowed to solidify.
- The prepared pathogenic suspensions were uniformly spread onto the agar surface using a sterile cotton swab.
- Each pathogenic bacterium was treated with isolated activated bacterial metabolites.
- Sterile filter paper discs (6 mm in diameter) were impregnated with 20 µL of bacterial metabolites and placed on the inoculated agar plates.
- The plates were incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zones around each disc was measured in millimeters using a ruler or digital caliper.

III. RESULTS AND DISCUSSION

1) Salinity test of bacterial isolates:- The isolated bacterial colonies were subjected to biochemical analysis to identify their characteristics and properties. Furthermore, the colonies were tested for their salt tolerance at varying concentrations (0.5%, 0.8%, and 1%) to assess their ability to thrive in different environments. The salt tolerance test was conducted on various bacterial isolates (S12, S13, S22, S23, S33, 2, 3, and 4) at different salt concentrations (0.5%, 0.8%, and 1%)

Fig: - 1 salinity test results of bacterial results after incubation

Readings at 620nm

S.NO	SAMPLE	SALINITY CONTENT	READINGS		
			1st Reading	2nd Reading	3rd Reading
1	S_1 2	.5	1.702	1.735	1.760
2	S_1 3	.8	.810	.718	.654
3	S_2 2	.8	1.687	1.681	1.659
4	S_2 3	.10	0.629	0.622	0.650
5	S_3 3	.5	1.443	1.460	1.408
6	2	.10	0.861	0.876	0.862
7	3	.8	1.428	1.472	1.426
8	4	5	1.165	1.277	1.210

Table: - 1 Growth of bacterial isolates at different salinity level

The bacterial isolates S12 and S33 showed optimal growth at 0.5% salt concentration, with S12 having a colony count of 1.732% and S33 is having 1.437%. These two isolates demonstrated the highest potential as salt-tolerant bacteria, making them promising candidates for further research or applications.

2) pH test of bacterial isolates:- The bacterial isolates were grown in different pH media (pH 3, 4, and 5) to assess their growth and tolerance to various pH levels. The results show varying levels of bacterial growth, indicating differences in pH tolerance among the isolates.

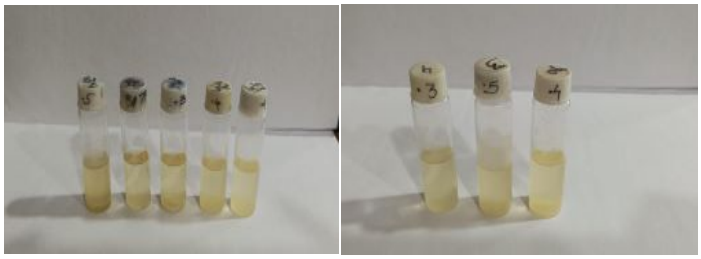


Fig: - 2 pH test results of bacterial results after incubation.

Readings at 620nm

S.NO	SAMPLE	PH VALUE	READINGS		
			1st Reading	2nd Reading	3rd Reading
1	S_1 2	5	1.902	0.863	1.039
2	S_1 3	3	0.574	0.565	0.577
3	S_2 2	3	1.311	1.156	1.114
4	S_2 3	4	0.956	0.750	1.214
5	S_3 3	4	1.244	1.239	1.160
6	2	4	1.960	1.583	1.549
7	3	5	1.781	1.809	1.766
8	4	3	0.720	0.845	0.766

Table: - 2 Growth of bacterial isolates at different pH level

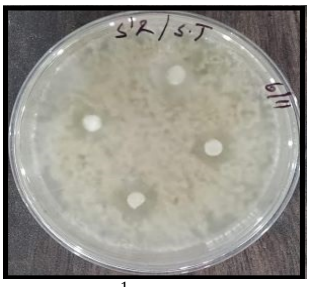
The bacterial isolates demonstrated optimal growth at 0.5% salt concentration, with S12 and S33 showing the highest colony counts, and at pH 5, with S12 and 3 exhibiting good results. These isolates also showed varying levels of tolerance to different salt concentrations and pH levels.

Antimicrobial Activity

The extracts from these bacterial isolates exhibited potent antimicrobial activity against bowel infection-causing pathogens, including *Salmonella Typhii* and *Staphylococcus aureus*. The isolates' ability to inhibit the growth of these pathogens highlights their potential as probiotics or sources of antimicrobial compounds.

Isolate bacterial metabolite $S^1$2 against *Staphylococcus aureus*

Isolate bacterial metabolite $S^3$3 against *Staphylococcus aureus*



Isolate bacterial metabolite $S^1$2 against *Salmonella typhimurium*

Isolate bacterial metabolite $S^3$3 against *Salmonella typhimurium*

The bacterial isolates S12 and S33 demonstrated promising results, showcasing optimal growth at 0.5% salt concentration and potential at pH 5. Moreover, their metabolites exhibited antimicrobial activity against Salmonella Typhi and Staphylococcus aureus, with S12 displaying excellent results, particularly against bowel infection-causing pathogens. Notably, the metabolites were activated at 75°C, highlighting the crucial role of temperature in their effectiveness.

IV. CONCLUSION

The bacterial isolates S12 and S33 demonstrated exceptional physiological robustness, showcasing optimal growth at 0.5% salt concentration, with S12 and S33 exhibiting the highest colony counts, indicating their potential as salt-tolerant isolates. Additionally, they displayed good tolerance to varying pH levels, with optimal growth at pH 5, and some ability to grow well at pH 4.

Notably, these isolates maintained their antimicrobial activity, particularly against Salmonella Typhi and Staphylococcus aureus, with S12 displaying excellent results, and their effectiveness was significantly enhanced at a temperature of 75°C. The combination of salt tolerance, pH resilience, and temperature stability in S12 and S33 underscores their potential as probiotic candidates with industrial applicability, highlighting their ability to withstand diverse environmental conditions while retaining metabolic and antimicrobial functionality. The metabolic profile of *Lactobacillus* isolates is significantly influenced by physiological factors. Strains demonstrating resilience to varied pH, bile salts, and temperature shifts maintained metabolic and antimicrobial functionality. These findings underscore the importance of physiological robustness in probiotic efficacy and industrial applicability.

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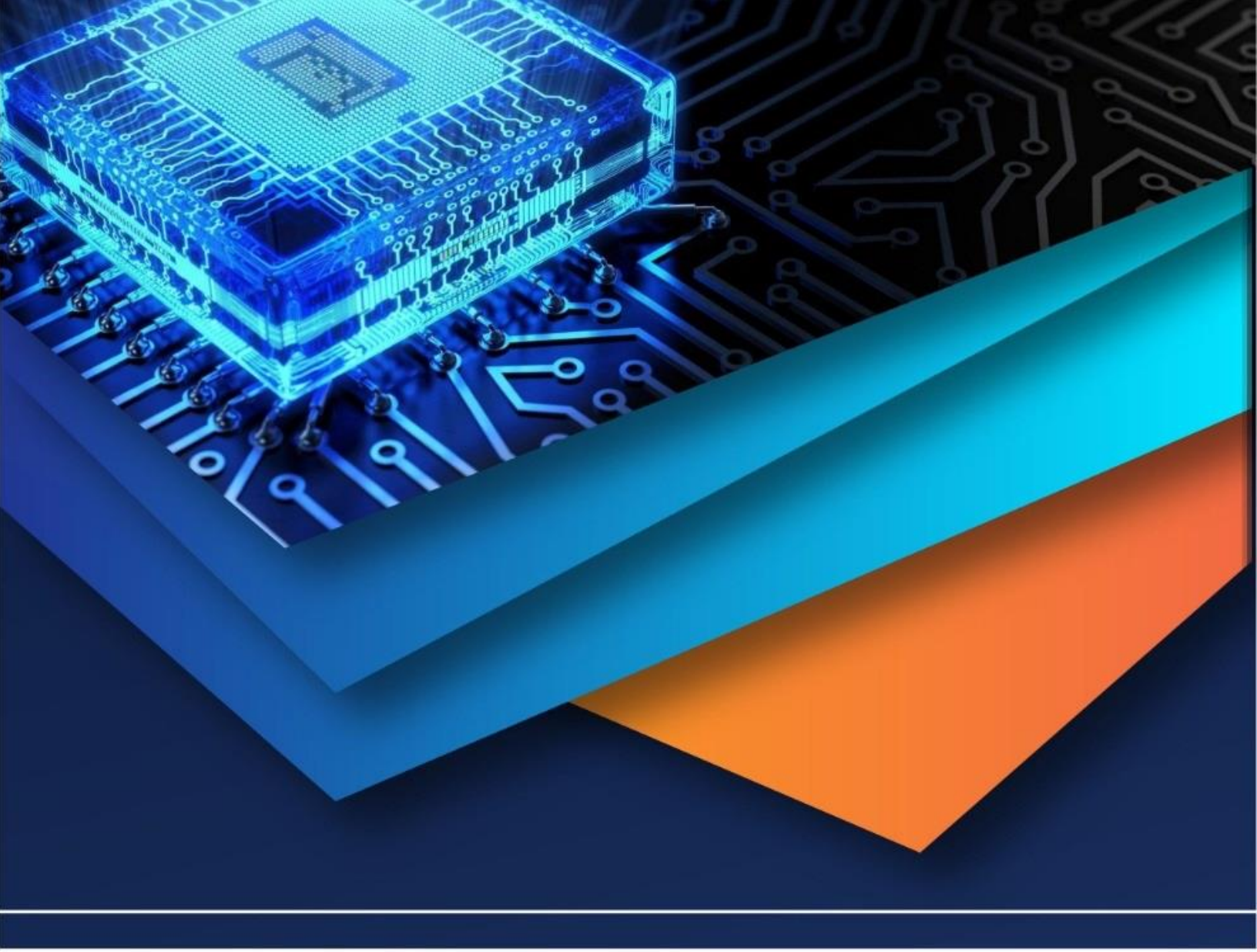

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