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Utilisation of Lactic Acid-Producing Bacteria from Fermented Rice Water and Milk as a Biofertilizer for Plant Growth Promotion

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Abstract: *The intensive use of chemical fertilizers can contribute to soil degradation, nutrient imbalance, water pollution, and declines in beneficial soil microbial communities. Biofertilizers based on beneficial microorganisms may provide a more sustainable approach to improving soil fertility and crop productivity. This study evaluated lactic acid-producing bacteria (LAB) obtained from fermented rice water and milk for their potential use as a biofertilizer. Fermented rice water was prepared and enriched with milk, after which LAB were isolated using culture-based methods and characterized by colony morphology, Gram staining, catalase testing, biochemical assays, and carbohydrate-fermentation profiles. The biofertilizer was evaluated under controlled pot conditions using fenugreek (Trigonella foenum-graecum) and compared with untreated control and market-fertilizer treatments. Soil pH, nutrient status, organic carbon, moisture content, plant growth parameters, biomass, and antimicrobial activity were assessed. The isolates were reported as Gram-positive and catalase-negative, with the ability to ferment tested sugars. LAB-treated soil showed improved nitrogen content, organic carbon, organic matter, loamy texture, and moisture retention relative to the reported control and market-fertilizer conditions. Fenugreek plants receiving the LAB treatment showed higher root and shoot development, leaf production, fresh biomass, and dry biomass. The LAB preparation also demonstrated inhibitory activity against selected bacterial and fungal test organisms in an agar-well-diffusion assay. These findings indicate that LAB derived from fermented rice water and milk may serve as an inexpensive microbial input for sustainable crop production. However, molecular identification of isolates, standardized formulation procedures, replicated statistical analysis, and field-scale validation are required before agricultural recommendations can be made.*

Keywords: lactic acid bacteria; biofertilizer; fermented rice water; fermented milk; fenugreek; soil fertility; plant growth promotion; sustainable agriculture.

I. INTRODUCTION

Increasing agricultural production has often depended on substantial applications of synthetic fertilizers. Although these inputs can increase crop yields, their excessive or prolonged use may adversely affect soil quality, nutrient balance, water systems, and beneficial soil microorganisms (Avis et al., 2008; Vessey, 2003). Sustainable nutrient-management strategies are therefore needed to maintain crop productivity while reducing environmental pressure. Microbial biofertilizers are one potential alternative because they can improve nutrient availability and soil biological activity through natural processes (Bintsis, 2018).

Plant growth-promoting microorganisms can support crop development through several mechanisms, including nutrient mobilization, organic-matter decomposition, nitrogen-related processes, phosphorus solubilization, production of growth-associated metabolites, and suppression of some plant-associated pathogens (Lynch et al., 2016; Gajbhiye & Kapadnis, 2016). Lactic acid bacteria are particularly relevant because they produce lactic acid and other metabolites

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during carbohydrate fermentation. These bacteria are commonly associated with fermented products, including milk and fermented rice-water preparations (Govindaraj et al., 2021; Sathe et al., 2007).

LAB may contribute to agricultural systems through the production of organic acids, antimicrobial compounds, enzymes, and other bioactive metabolites. Organic-acid production can alter the local chemical environment and potentially increase the availability of some nutrients, particularly phosphorus and micronutrients. In addition, LAB-associated antimicrobial activity may be relevant to the suppression of selected microorganisms (Desbois & Smith, 2010). However, laboratory antimicrobial activity should not be interpreted as proof of field-level plant-disease control.

Fermented rice water and milk are accessible substrates for microbial enrichment because they provide carbohydrates and other nutrients that may support LAB growth (Bassyouni et al., 2022). The use of such locally available materials could offer a low-cost approach to preparing microbial formulations for agricultural use. This study therefore investigated LAB obtained from fermented rice water and milk and examined their characteristics, antimicrobial activity, and potential effects on soil properties and fenugreek growth under controlled conditions.

The objectives were to: (i) isolate and characterize LAB from fermented rice water and milk; (ii) assess selected biochemical characteristics and lactic-acid production; (iii) evaluate antimicrobial activity against selected bacterial and fungal organisms; (iv) compare the effects of LAB biofertilizer, market fertilizer, and no-fertilizer control treatments on soil properties; and (v) assess plant-growth and biomass responses in fenugreek.

II. MATERIALS AND METHODS

2.1 Study location and experimental approach

The work was conducted under controlled laboratory conditions at the Microbiology Laboratory, Kamla Nehru Mahavidyalaya, Nagpur, India. The experimental work included fermentation of rice water and milk, isolation and characterization of LAB, preparation of an LAB-based formulation, antimicrobial testing, soil analysis, and pot-based plant-growth assessment.

A completely randomized design was reported with three treatments: untreated control (T1), market fertilizer (T2), and LAB biofertilizer (T3). Each treatment was conducted in triplicate. Fenugreek was the primary crop specified for the controlled plant-growth study. Root length, shoot length, number of leaves, and fresh and dry biomass were recorded during the experimental period.

2.2 Preparation of fermented rice water and milk

Rice grains were soaked in distilled water and maintained at room temperature for 24 h to obtain fermented rice water. Fresh milk was then added to the fermented rice-water preparation, followed by a further 48 h incubation to enrich LAB growth. In the longer thesis protocol, 100 mL milk was combined with 400 mL fermented rice water, incubated until visible fermentation characteristics developed, and filtered to obtain the liquid preparation. The final formulation was diluted at 1:10 before plant application.

2.3 Isolation and characterization of LAB

The fermented sample was serially diluted up to 10^{-6} using sterile distilled water. Suitable dilutions were cultured on de Man, Rogosa and Sharpe agar and incubated at 37°C for 24–48 h under anaerobic conditions. Distinct colonies were selected and purified through repeated streaking. Colony shape, colour, size, and margin were recorded as part of morphological characterization.

Gram staining was used to determine cellular morphology and Gram reaction. Catalase testing, IMViC tests, and carbohydrate-fermentation assays were conducted as biochemical screening procedures. The isolates were reported to be Gram-positive and catalase-negative, which is consistent with the intended selection of LAB-like isolates (Sathe et al., 2007). The isolates also fermented dextrose, lactose, maltose, sucrose, and fructose, producing acid as indicated by colour changes in the test medium.

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2.4 Preparation of LAB biofertilizer

Selected LAB isolates were inoculated into sterile broth and incubated at 37°C for 24–48 h to obtain active cultures. The resulting culture broth was used as the LAB biofertilizer preparation. The liquid preparation was applied to plants or soil following dilution, as described in the fermentation protocol (Patel et al., 2021; Pinto et al., 2022).

2.5 Soil and plant-growth assessment

Soil samples from each treatment were assessed for physicochemical properties. Soil pH was measured using a digital pH meter. Nitrogen was determined by the Kjeldahl method, phosphorus by the Olsen method, potassium by flame photometry, organic carbon by the Walkley–Black method, and moisture by the gravimetric method.

Fenugreek seeds were sown in pots containing soils assigned to the three treatments. Plant-growth measurements included root length, shoot length, leaf number, and biomass. Fresh and dry weights were used as indicators of biomass accumulation. Soil texture, nutrient content, organic-carbon status, and moisture retention were also compared among the treatments (Fhoula et al., 2013).

2.6 Antimicrobial activity

The antimicrobial activity of LAB isolates was assessed using the agar-well-diffusion method. Selected bacterial and fungal organisms were used as test organisms, and inhibition zones around wells were measured after incubation (Cortes-Zavaleta et al., 2014). The study reported activity against *Escherichia coli*, *Staphylococcus aureus*, and fungal organisms, including variable inhibition of *Aspergillus* spp.

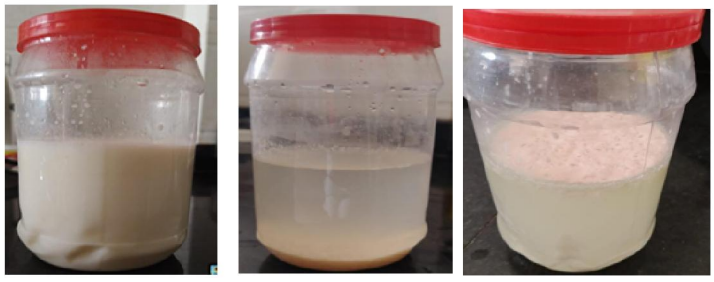
2.7 Data analysis

The available study documents state that experimental data were analyzed using mean values. Because the reported material does not provide standard deviations, confidence intervals, analysis-of-variance outputs, or exact *p*-values, the present interpretation is descriptive. Future work should use appropriate statistical testing, including analysis of variance and post-hoc comparisons, to determine whether observed treatment differences are statistically significant

III. RESULTS

3.1 Fermentation of rice water and milk

Fermented milk, fermented rice water, and the combined fermented rice-water–milk preparation showed visible changes consistent with fermentation, including turbidity, sedimentation, and surface foaming. These materials were used as the source for isolation of lactic acid bacteria-like organisms and preparation of the experimental biofertilizer.



(A) (B) (C)

Figure 1. Fermentation substrates used for the preparation of the LAB-based biofertilizer: (A) fermented milk, (B) fermented rice water, and (C) fermented rice water supplemented with milk.

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3.2 Isolation and preliminary characterization of LAB

Microbial colonies were recovered from the fermented preparation on culture media. The colonies were reported as small, off-white, circular to irregular, and visibly distinct on agar plates. These isolates were subsequently purified and subjected to preliminary phenotypic and biochemical characterization.

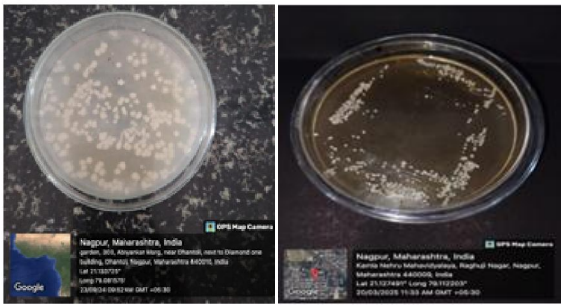


Figure 2. Isolation of presumptive lactic acid bacteria from the fermented rice-water and milk preparation: (A) colony growth obtained by pour-plate culture and (B) isolated colonies obtained by streak-plate culture.

The reported viable counts increased during storage, from 5×10^5 CFU/g on day 1 to 6×10^5 CFU/g on day 7 and 7.5×10^5 CFU/g on day 14. A second count series was also reported as 1×10^5, 2×10^5, and 3.5×10^5 CFU/g at days 1, 7, and 14, respectively.

Storage period	Reported viable count (CFU/g)
Day 1	5×10^5
Day 7	6×10^5
Day 14	7.5×10^5

Table 1. Viable bacterial counts in the fermented preparation during storage.

3.3 Microscopic and biochemical characterization

Microscopic examination after Gram staining showed Gram-positive rod-shaped and coccal cells. The isolates were reported to be catalase-negative, as indicated by the absence of bubble formation after the addition of hydrogen peroxide. These features are consistent with preliminary characterization of LAB-like isolates, although molecular identification is required for species-level confirmation.

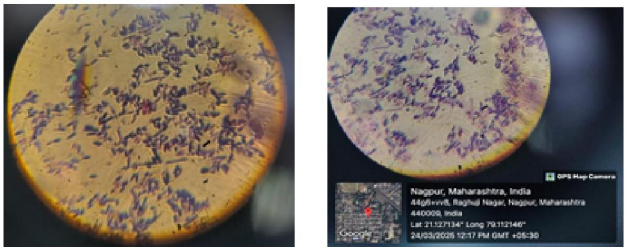


Figure 3. Microscopic appearance of Gram-stained presumptive lactic acid bacteria isolated from fermented rice water and milk.

Gram-positive rod-shaped and coccal cells were observed (Figure 3).

3.4 Lactic acid production and antimicrobial activity

Titrable acidity measurements showed lactic acid values of 0.92%, 0.94%, and 0.97% across three trials. The corresponding pH values were 7.8, 8.0, and 8.3. The reported pH values should be checked before journal submission because they are higher than typically expected for a lactic-acid-fermented preparation.

Trial	Volume of	Normality of	Volume of	Lactic acid	pH

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	NaOH (mL	NaOH	sample (mL	(%)	
1	10.3	0.1	10	0.92	7.8
2	10.5	0.1	10	0.94	8.0
3	10.8	0.1	10	0.97	8.3

Table 2. Titrable acidity and pH of the fermented LAB preparation.

The LAB preparation showed small-to-moderate inhibitory activity against *Escherichia coli*, greater inhibition against *Staphylococcus aureus*, and variable activity against fungal organisms, including *Aspergillus* spp. Exact inhibition-zone diameters were not reported; therefore, the findings should be described qualitatively rather than as statistically confirmed differences.



Figure 4. Antimicrobial activity of the LAB preparation determined by the agar-well-diffusion method.

3.5 Effects of treatment on soil properties

The LAB-biofertilizer treatment showed soil pH values between 7.0 and 7.2 during the four-week observation period. In comparison, control soil remained between pH 6.4 and 6.5, whereas soil receiving market fertilizer remained between pH 6.0 and 6.1.

Week	Control	LAB biofertilizer	Market fertilizer
1	6.5	7.2	6.1
2	6.5	7.1	6.1
3	6.4	7.1	6.0
4	6.4	7.0	6.0

Table 3. Soil pH under control, LAB-biofertilizer, and market-fertilizer treatments.

The biofertilizer-treated soil was reported as loamy, while control soil was described as sandy and market-fertilizer-treated soil as clayey. The biofertilizer treatment also showed the highest reported available nitrogen content (363.55 kg/ha), organic carbon (1.06%), and organic matter (1.83%).

Treatment	Soil texture	Available nitrogen (Kg)	Available phosphorus (Kg)	Available potassium (Kg)	Organic carbon	Organic matter (%)
Control	Sandy	175.60	2.4	59.60	0.20	0.35
LAB biofertilizer	Loamy	365.55	4.80	25.40	1.06	1.83
Market Fertilizer	Clayey	200.70	4.77	48.40	0.33	0.57

Table 4. Soil texture and nutrient characteristics under the three treatments.

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3.6 Fenugreek biomass and soil-moisture response

After 30 days, the LAB-biofertilizer treatment produced the highest reported fresh biomass (320 g) and dry biomass (85 g) in fenugreek. The market-fertilizer treatment produced 300 g fresh biomass and 78 g dry biomass, while the control produced 250 g fresh biomass and 60 g dry biomass.

Treatment	Growth duration (Days)	Fresh weight (g)	Dry weight (g)	Reported Observation
Control	30	250	60	Normal growth
LAB biofertilizer	30	320	85	Enhanced root & shoot growth
Market Fertilizer	30	300	78	Faster early growth

Table 5. Biomass response of fenugreek under different fertilizer treatments.

Source: Adapted from Table 28 of the thesis.

Soil moisture was highest in the LAB-biofertilizer treatment (65.74%), compared with market-fertilizer soil (40.85%) and control soil (35.67%). This pattern may have contributed to the improved growth response observed in the biofertilizer treatment.

Treatment	**Initial weight (g)**	**Dry weight (g)**	**Moisture content (g)**	**Moisture content (%)**
Control	31.09	20.00	11.09	35.67
LAB biofertilizer	40.90	14.00	26.90	65.74
Market Fertilizer	30.43	18.00	12.43	40.85

Table 6. Soil-moisture content under the three treatments.

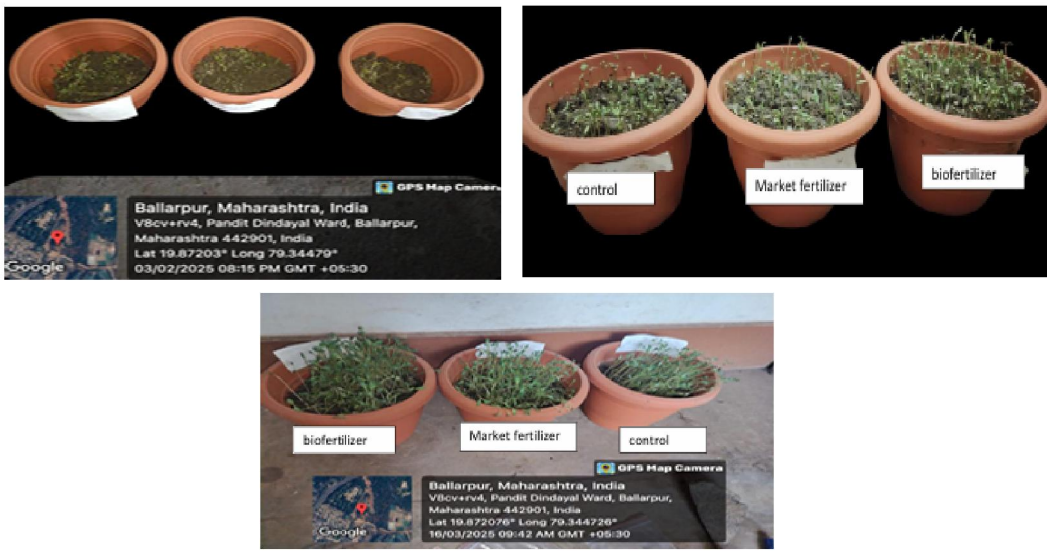


Figure 5. Experimental application of the LAB-based biofertilizer to fenugreek plants and visual progression of seed germination.

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IV. DISCUSSION

This study indicates that fermented rice water enriched with milk can serve as a source of culturable LAB-like organisms. The culture-based findings—Gram-positive morphology, catalase-negative reaction, carbohydrate fermentation, and acid production—are consistent with preliminary LAB characterization. Nevertheless, phenotype-based identification alone is insufficient to identify isolates at the species level, particularly because other bacteria may share some of these properties. Molecular identification is essential before the formulation can be standardized or developed for wider agricultural use.

The soil results suggest that the LAB treatment may have improved nutrient status, organic-carbon content, organic matter, and moisture retention. Such effects could result from microbial decomposition of organic materials, changes in nutrient cycling, production of organic acids, or interactions between introduced microorganisms and native soil communities. However, these mechanisms were not directly measured in the present study and should therefore be treated as plausible explanations rather than confirmed causal pathways.

The higher growth and biomass responses in LAB-treated fenugreek indicate that the formulation may have improved the growing environment or nutrient availability. The reported moisture content and loamy soil texture may also have contributed to plant performance through improved water retention and soil aeration. As plant response can be influenced by seed quality, pot size, soil quantity, irrigation, initial nutrient status, and environmental conditions, future experiments should report and control these factors in greater detail.

The antimicrobial findings are promising but should be interpreted cautiously. Agar-well-diffusion assays demonstrate inhibition under laboratory conditions; they do not establish efficacy against plant disease in soil, under greenhouse conditions, or in agricultural fields. Additional studies should test defined pathogens, compare cell-free supernatant with live-cell treatments, determine whether inhibition is pH-dependent, and evaluate whether the preparation can reduce disease severity in inoculated plants. A key limitation is the lack of complete statistical reporting.

V. CONCLUSION

LAB obtained from fermented rice water and milk showed characteristics consistent with preliminary LAB identification, including Gram-positive morphology, catalase-negative reactions, carbohydrate fermentation, and acid-production capacity. The LAB-based preparation was associated with improved reported soil fertility indicators, moisture retention, fenugreek growth, and biomass compared with untreated control and market-fertilizer treatments. The preparation also demonstrated antibacterial and antifungal activity in laboratory assays.

The findings support the potential of fermented rice-water and milk-derived LAB as a low-cost biofertilizer candidate. Before use beyond preliminary controlled experiments, future research should include molecular identification of isolates, formulation-stability studies, dose optimization, well-replicated statistical analysis, pathogen-challenge experiments, and long-term field trials across different crops and soil types.

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