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STUDY OF THE CROP-BENEFICIAL ENZYME ACTIVITY OF *Lactobacillus* ISOLATES FROM CURD, PAPAYA AND BANANA ON SPINACH CROP

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ABSTRACT

This study evaluated the crop-beneficial enzymatic activity of *Lactobacillus* isolates from curd, papaya, and banana on spinach. Ten presumptive *Lactobacillus* isolates were obtained from homemade curd, papaya, and banana samples. Selected isolates were tested for morphological and biochemical characteristics. All isolates were rod-shaped, gram-positive, and negative for catalase, citrate utilization, and gelatin hydrolysis. Most isolates exhibited phosphate solubilization and phytase activity. A polybag experiment with 10 treatments and three replicates, using 25% and 50% concentrations of cell-free supernatant, was conducted to assess the effect of these isolates on spinach growth. Treatment T₉ (RB-1) performed well across some parameters, including plant height, fresh and dry biomass, root and shoot length and chlorophyll content, compared with controls. Nutrient analysis revealed increased nitrogen, phosphorus and potassium uptake, especially in treatments with isolates CS-4, MP-3, CS-1, and RB-1. The results highlight the significant potential of specific *Lactobacillus* isolates to enhance spinach growth and nutrient uptake. This research underscores the potential of specific *Lactobacillus* isolates to promote growth and nutrient bioavailability in spinach, supporting their use as biofertilizers in sustainable agriculture.

Key words : *Lactobacillus*, Crop-beneficial enzymes, Spinach (*Spinacia oleracea*), Cell-free supernatant (CFS).

Introduction

Lactobacillus is a common bacterium found on various fruits and vegetables, including papaya and banana. Many *Lactobacillus* species have been found in both fresh and fermented papaya (Sam *et al.*, 2024). *Lactobacillus* can also be isolated from overripe fruit and other parts of the banana plant, such as the pseudostem and peel (Chen *et al.*, 2017; Yang *et al.*, 2022). *Lactobacilli* are used as human probiotics and are also effective in managing plant diseases and promoting plant development. *Lactobacilli* accelerate the decomposition of organic matter in the soil, resulting in a faster release of nutrients required for plant growth. *Lactobacillus* produces lactic acid and other organic acids that improve nutrient solubility, particularly for phosphate, iron, and zinc, and slightly lower soil pH. It also solubilizes minerals like phosphate, increasing their availability to

plants. While *Lactobacillus* does not fix nitrogen on its own, its enzymatic activity increases the availability of nitrogen by interacting with nitrogen-fixing bacteria. Applying *Lactobacillus* isolates increased wheat growth under drought stress, indicating their potential to enhance plant resistance to abiotic stress (Sinha *et al.*, 2024). *Lactobacillus agilis*, *Lactobacillus plantarum* and *Lactobacillus acidophilus* promoted wheat growth in the face of heat and drought stress (Khan *et al.*, 2024). *Lactobacillus* species acted as biocontrol agents against banana blood disease (Sam *et al.*, 2024). The ability of metabolite-rich media, commonly referred to as cell-free supernatant (CFS), to promote plant development in the absence of microbes has become a focus of recent studies. Cell-free supernatant (CFS) and other chemicals produced by plant growth-promoting microorganisms (PGPM) improve plant growth in both stressed and non-stressed environments (Naamala *et al.*, 2023). Spinach

(*Spinacia oleracea*) grows rapidly, making it an excellent model for examining the effects of microbial inoculants on plant growth. The current study was carried out to isolate, characterize, and examine *Lactobacillus* isolates from non-rhizosphere sources such as curd, papaya, and banana as PGPM (plant growth-promoting microorganisms) for spinach as the test crop, due to the growing need for developing microbial fertilizers using lactic acid.

Materials and Methods

Isolation of Bacteria

Ten samples of homemade curd were collected from different regions of Chhattisgarh, Andhra Pradesh and Telangana. Thirteen isolates originally isolated by Mahima (2023) from different parts of the papaya plant (papaya leaves, petiole, seed, raw and mature papaya) were provided by the Department of Microbiology, IGKV Raipur (Chhattisgarh), for this study. Two isolates were collected from banana samples from the local market in Raipur. Bacteria were isolated using serial dilution and plating on de Man, Rogosa and Sharpe (MRS) agar. Plates were incubated at 37°C for 24-48 hours. Individual colonies were purified by successive transfers on MRS medium and maintained on MRS agar slants at 6-8°C.

Morphological and biochemical characterization

Colony characteristics, including margin, shape, and color, were observed on MRS agar. Gram staining was performed on a one-day-old bacterial culture. Nine viable and purified isolates were subjected to biochemical tests, including the catalase test, triple sugar iron (TSI) test, gelatin liquefaction test, citrate test and starch hydrolysis test. These tests were conducted following standard procedures.

Screening for Plant Growth-promoting traits

Phosphate Solubilization : Presumptive isolates were grown on Pikovskaya (PKV) agar medium containing tricalcium phosphate. A clear halo zone after incubation for two days at 30°C indicated phosphate solubilization (positive).

Phytase activity : Presumptive isolates were spot inoculated onto modified Chalmers Agar containing 1% sodium phytate and incubated for 48 hours at 30°C. A clear halo after counterstaining with cobalt chloride and molybdate/vanadate solution indicates phytase production.

Antibiotic susceptibility : Presumptive isolates were tested against kanamycin (5 µg/disc) and ampicillin (10 µg/disc) to assess antibiotic susceptibility by measuring the inhibition zones.

Preparation of Cell-free Supernatant : Cultures were grown in MRS broth at 37°C for 5 days. After centrifugation (10,000 x g, 15 min, 4°C), supernatants were sterile-filtered and diluted to 25% and 50% for use in spinach seed treatments.

Polybag experiment : A pot experiment was conducted using a Completely Randomized Design (CRD) with 10 treatments and 3 replications for 25% and 50% each. Spinach (*Spinacia oleracea*) seeds were primed by soaking in CFS from selected *Lactobacillus* isolates at 25% and 50% concentrations, then sown. Control seeds were treated with sterile MRS broth. Growth parameters, such as plant height, were recorded at 15, 30, and 45 days after sowing (DAS). Chlorophyll (SPAD values), biomass, root and shoot length, and nutrient content (N, P, K) were recorded at 45 days after sowing (DAS).

Statistical analysis

The experiment was conducted in a completely randomized design (CRD) with 10 treatments and three replications. Data obtained at 25% and 50% concentrations were analyzed separately using one-way ANOVA.

Results

Isolation and Morphological characterization

The nine selected isolates (CS-1, CS-4, CS-5, CS-7 from curd; RP-1, MP-3, PL-2, PL-3 from papaya; RB-1 from banana) showed white to creamy white colonies with regular, smooth margins. Gram staining of all nine bacterial isolates was positive (all nine isolates showed a purple color). Microscopic examination showed rod-shaped, non-motile cells.

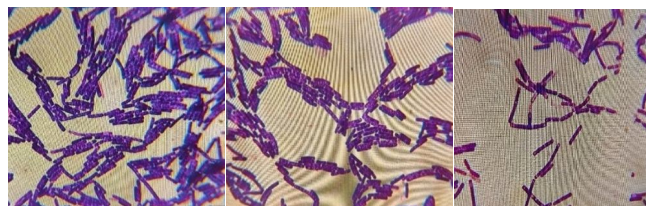


Fig. 1 : Gram staining of *Lactobacillus* isolates from curd, papaya, and banana.

Biochemical characterization

All nine selected isolates tested negative for catalase, gelatin hydrolysis, and citrate utilization. Isolates from papaya and banana (RP- 1, MP- 3, PL- 2, PL- 3, RB- 1) tested positive for starch hydrolysis. All selected isolates fermented glucose, sucrose, and lactose in the TSI test without H₂ S production. All isolates, except RP- 1, tested positive for phytase activity, and all except CS- 5, PL- 3, and RP- 1 showed phosphate solubilization.

Table 1 : Plant height (cm) of 25% and 50% concentration treatments at different DAS.

	25%			50%		
	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS
T ₁ control	4.46 ^b	7.10 ^d	8.16 ^c	5.20 ^b	9.00 ^{abc}	10.96 ^c
T ₂ CS-5	5.46 ^a	9.16 ^a	10.20 ^{bc}	6.23 ^a	9.83 ^{ab}	13.16 ^{ab}
T ₃ CS-1	5.06 ^{ab}	7.60 ^{bcd}	10.33 ^{bc}	5.86 ^{ab}	10.03 ^a	14.46 ^{ab}
T ₄ CS-4	5.30 ^a	8.86 ^{ab}	11.23 ^{ab}	5.26 ^b	9.73 ^{ab}	14.06 ^{ab}
T ₅ PL-3	5.56 ^a	7.90 ^{abcd}	10.03 ^{bc}	5.26 ^b	10.36 ^a	12.56 ^{bc}
T ₆ RP-1	5.33 ^a	8.63 ^{abc}	11.06 ^{abc}	6.30 ^a	9.16 ^{ab}	12.43 ^{bc}
T ₇ MP-3	5.00 ^{ab}	7.40 ^{bcd}	9.16 ^{bc}	5.66 ^{ab}	7.36 ^c	14.70 ^a
T ₈ PL-2	5.00 ^{ab}	8.36 ^{abc}	11.33 ^{ab}	5.66 ^{ab}	9.13 ^{ab}	13.96 ^{ab}
T ₉ RB-1	5.30 ^a	8.20 ^{abcd}	13.50^a	6.06 ^a	8.86 ^{abc}	15.03^a
T ₁₀ CS-7	5.03 ^{ab}	7.16 ^{cd}	11.68 ^{ab}	5.56 ^{ab}	8.40 ^{bc}	13.50 ^{ab}
SE(m)	0.17	0.44	0.85	0.22	0.48	0.62
C.D @ 5%	0.52	1.23	2.55	0.65	1.42	1.84

Antibiotic susceptibility test

All nine isolates were tested for antibiotic susceptibility using two antibiotic discs: kanamycin (5 mcg/disc) and ampicillin (10 mcg/disc). All isolates except CS- 1 and CS- 7 were susceptible to both antibiotics. CS- 5 showed the highest susceptibility to ampicillin, with an inhibition zone of 37 mm. RP-1 showed the highest susceptibility to kanamycin, with an inhibition zone of 18 mm.

Effect on Spinach growth

At both 25% and 50% supernatant concentrations, all *Lactobacillus* treatments significantly improved spinach growth compared to controls. The 50% CFS concentration was more effective than the 25% concentration. Treatment T₉ (RB-1) consistently showed the

Table 2 : Fresh and dry weight of spinach (g).

	Root weight (g)				Shoot weight (g)			
	Fresh		Dry		Fresh		Dry	
	25 %	50 %	25 %	50 %	25 %	50 %	25 %	50 %
T ₁ control	13.80 ^c	16.46 ^c	3.33 ^c	3.63 ^b	80.76 ^b	98.33 ^c	3.63 ^c	4.13 ^c
T ₂ CS-5	17.26 ^{ab}	19.23 ^{bc}	4.07 ^{bc}	7.26 ^a	94.66 ^a	105.66 ^{bc}	4.66 ^{ab}	7.63 ^{ab}
T ₃ CS-1	16.16 ^{abc}	20.30 ^{bc}	4.16 ^{abc}	6.96 ^a	96.33 ^a	109.66 ^{abc}	4.66 ^{ab}	6.83 ^b
T ₄ CS-4	15.33 ^{bc}	20.56 ^{ab}	4.60 ^{ab}	7.13 ^a	98.00 ^a	110.33 ^{abc}	4.76 ^{ab}	6.86 ^b
T ₅ PL-3	17.66 ^{ab}	21.26 ^{ab}	4.46 ^{ab}	6.80 ^a	94.33 ^a	116.00 ^{ab}	4.60 ^{ab}	7.70 ^{ab}
T ₆ RP-1	18.00 ^{ab}	21.23 ^{ab}	4.46 ^{ab}	7.53 ^a	92.86 ^a	120.00 ^a	4.36 ^{bc}	7.96 ^{ab}
T ₇ MP3	16.80 ^{abc}	21.73 ^{ab}	4.48 ^{ab}	7.66 ^a	91.66 ^a	116.33 ^{ab}	4.23 ^{bc}	7.50 ^{ab}
T ₈ PL-2	16.40 ^{abc}	22.43 ^{ab}	4.59 ^{ab}	7.16 ^a	95.16 ^a	114.66 ^{ab}	4.60 ^{ab}	7.40 ^b
T ₉ RB1	19.03^a	24.33^a	5.06^a	8.40^a	100.00^a	122.00^a	5.36^a	9.20^a
T ₁₀ CS7	15.60 ^{bc}	18.70 ^{bc}	4.16 ^{abc}	7.00 ^a	90.40 ^{ab}	115.00 ^{ab}	4.43 ^{bc}	6.70 ^b
SE(m)	0.90	1.18	0.28	0.54	3.07	4.19	0.25	0.52
C.D @ 5%	2.69	3.50	0.83	1.61	9.10	12.40	0.77	1.56

Table 3 : Root and shoot length of spinach (cm).

Treatments	Root length (cm)		Shoot length (cm)	
	25 %	50 %	25 %	50 %
T ₁ control	4.16 ^d	5.00 ^b	13.50 ^b	19.33 ^d
T ₂ CS-5	8.83^a	9.16^a	14.83 ^b	21.33 ^{cd}
T ₃ CS-1	7.10 ^{abc}	8.33 ^a	15.56 ^b	24.03 ^{abc}
T ₄ CS-4	6.86 ^{abc}	7.83 ^a	13.66 ^b	22.33 ^{bcd}
T ₅ PL-3	6.53 ^{abcd}	7.66 ^a	14.66 ^b	23.56 ^{abcd}
T ₆ RP-1	6.63 ^{abc}	7.00 ^{ab}	16.33 ^b	21.33 ^{cd}
T ₇ MP-3	5.66 ^{cd}	7.16 ^{ab}	16.33 ^b	26.00 ^{ab}
T ₈ PL-2	6.66 ^{abc}	8.16 ^a	15.50 ^b	22.33 ^{bcd}
T ₉ RB-1	8.26 ^{ab}	9.16^a	19.66^a	27.00^a
T ₁₀ CS-7	6.33 ^{bcd}	8.66 ^a	15.66 ^b	23.00 ^{abcd}
SE(m)	0.70	0.68	0.94	1.32
C.D @ 5%	2.10	2.02	2.80	3.93

best performance.

Plant height measurement at different DAS

Plant height increased across all treatments at 15, 30, and 45 days after sowing (DAS) (Table 1). At 15 DAS, the maximum plant height was recorded in T₅ (PL-3) at 5.5 cm (25%) and in T₂ (CS- 5) at 6.233 cm (50%). At 30 DAS, the maximum plant height was recorded in T₂ (CS- 5) at 9.167 cm (25%) and in T₃ (CS- 1) at 10.367 cm (50%). At 45 DAS, the maximum plant height was recorded in T₉ (RB-1) at 13.5 cm (25%) and 15.033 cm (50%).

Biomass of spinach after harvesting

Fresh biomass (Table 2) indicated that the maximum root fresh weight (24.333 g) and shoot fresh weight (122 g) were recorded in T₉ (RB- 1) at a 50% concentration,

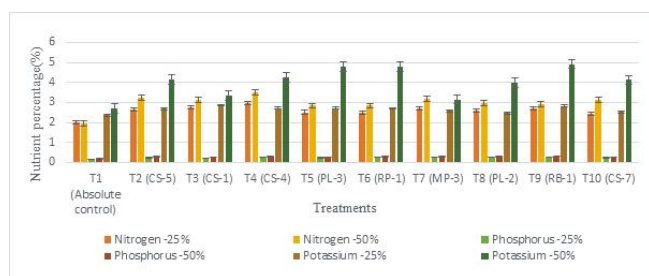


Fig. 2 : Effect of 25% and 50% treatments on nitrogen, phosphorus and potassium uptake in spinach.

Table 4 : SPAD meter readings of spinach (45 DAS).

Treatments	SPAD meter readings	
	25 %	50 %
T ₁ control	28.25 ^e	35.10 ^b
T ₂ CS-5	34.60 ^{cd}	44.60 ^a
T ₃ CS-1	37.10 ^{abcd}	42.46 ^a
T ₄ CS-4	37.90 ^{abc}	42.70 ^a
T ₅ PL-3	34.00 ^d	41.06 ^{ab}
T ₆ RP-1	36.05 ^{bcd}	42.03 ^a
T ₇ MP-3	39.30 ^{ab}	42.06 ^a
T ₈ PL-2	38.20 ^{abc}	40.30 ^{ab}
T ₉ RB-1	39.90^a	45.90^a
T ₁₀ CS-7	37.00 ^{abcd}	41.73 ^a
SE(m)	1.12	1.79
C.D @ 5%	3.32	5.32

while the minimum values were noted in the control. Similarly, at 25 % concentration, the maximum root fresh weight (19.033 g) and shoot fresh weight (100 g) were recorded in T₉ (RB-1), while the minimum values were noted in the control.

Dry biomass exhibited the same trend, with the maximum root dry weight (8.4 g) and shoot dry weight (9.2 g) observed in T₉ (RB-1) at a 50% concentration. Similarly, at 25 % concentration, maximum root dry weight (5.067 g) and shoot dry weight (5.367 g) are documented in T₉ (RB-1), while minimum values were noted in the control.

Root and shoot length of spinach after harvesting

Root and shoot lengths are listed in Table 3. At 25% concentration, the maximum root length was 8.833 cm in T₂ (CS-5) and the maximum shoot length was 19.667 cm in T₉ (RB-1), with the minimum in the control. Similarly, at 50% concentration, the maximum root length was 9.167 cm in both T₉ (RB-1) and T₂ (CS-5), and the maximum shoot length was 27 cm in T₉ (RB-1), with the minimum in the control.

Chlorophyll content

Chlorophyll content, evaluated by SPAD values (Table

Table 5 : N, P, K percentages of 25% and 50% treatment concentrations of cell-free supernatant of *Lactobacillus* isolates.

Treatments	25 %			50 %		
	N%	P%	K%	N%	P%	K%
T ₁ control	1.99 ^g	0.16 ^c	2.36 ^d	1.94 ^c	0.18 ^c	2.70 ^f
T ₂ CS-5	2.62 ^{cd}	0.23 ^{ab}	2.68 ^{abc}	3.22^{ab}	0.28 ^{ab}	4.13 ^{abc}
T ₃ CS-1	2.74 ^b	0.21 ^b	2.86^a	3.12 ^{ab}	0.25 ^b	3.35 ^{cef}
T ₄ CS-4	2.98^a	0.24 ^a	2.72 ^{ab}	3.50 ^a	0.29 ^a	4.26 ^{ab}
T ₅ PL-3	2.51 ^{ef}	0.23 ^{ab}	2.70 ^{abc}	2.83 ^b	0.26 ^{ab}	4.80 ^a
T ₆ RP-1	2.49 ^f	0.24 ^a	2.69 ^{abc}	2.83 ^b	0.27 ^a	4.79 ^a
T ₇ MP-3	2.72 ^b	0.25^a	2.57 ^{bcd}	3.16 ^{ab}	0.28 ^{ab}	3.15 ^f
T ₈ PL-2	2.58 ^{de}	0.24 ^a	2.47 ^{cd}	2.97 ^b	0.28 ^{ab}	3.97 ^{bcd}
T ₉ RB-1	2.69 ^{bc}	0.24 ^a	2.82 ^a	2.90 ^b	0.30^a	4.92^a
T ₁₀ CS-7	2.45 ^f	0.23 ^{ab}	2.51 ^{bcd}	3.13 ^{ab}	0.27 ^{ab}	4.12 ^{abcd}
SE(m)	0.02	0.007	0.07	0.13	0.01	0.24
C.D @ 5%	0.08	0.02	0.20	0.39	0.03	0.73

4), was highest in T₉ (RB-1) with 39.9 (25%) and 45.9 (50%) and lowest in the control.

Nutrient Uptake in Spinach

The observations are listed in Table 5 and Fig. 2. The maximum nitrogen content for 25% concentration treatments was significantly higher in T₄ (CS-4), 2.98%, and for 50% concentration treatments was significantly higher in T₂ (CS-5), 3.227%. The maximum phosphorus content for 25% concentration treatments was significantly higher in T₇ (MP-3), 2.51% and for 50% concentration treatments, it was significantly higher in T₉ (RB-1), 0.302%. The maximum potassium content for 25% concentration treatments was significantly higher in T₃ (CS-1), 2.86% and for 50% concentration treatments, it was significantly higher in T₉ (RB-1), 4.92%.

Discussion

Morphological and biochemical characterization confirmed that all isolates belonged to *Lactobacillus* spp., based on their gram-positive, rod-shaped, non-motile morphology and characteristic biochemical responses. The absence of catalase activity and the ability to ferment multiple sugars support their classification as lactic acid bacteria. The improvement in spinach growth across all treatments suggests that metabolites in the cell-free supernatant (CFS) play an important role in plant growth promotion. The superior performance of 50% CFS treatment compared with 25% indicates a concentration-dependent response, with higher metabolite availability leading to greater plant growth stimulation. The consistent superior performance of isolate RB-1 across multiple growth parameters (plant height, biomass, chlorophyll content) suggests it may possess stronger functional traits

than the others, while highest uptake of nitrogen was shown by isolate CS-4. The increase in plant height and biomass can be linked to improved nutrient uptake and enhanced metabolic activity. This is in agreement with the findings of Zang *et al.* (2023), who reported that the chlorophyll content and biomass of mustard plants increased in *Lactobacillus plantarum* inoculated treatments compared to uninoculated treatments. Increased chlorophyll content (SPAD values) further indicates improved photosynthetic efficiency, which likely contributed to higher biomass accumulation. Similar findings have been reported by Uma *et al.* (2018) and Shrestha *et al.* (2014), who demonstrated that lactic acid bacteria enhance plant growth through improved physiological performance. The presence of microbial metabolites may stimulate root elongation and branching, thereby increasing the plant's ability to absorb water and nutrients. Similar findings were seen by Panetto *et al.* (2023), who reported that *L. acidophilus* has shown siderophore synthesis, IAA generation, phosphate solubilization and nitrogen fixation. Under field conditions, it increased root dry matter, and Turaeva *et al.* (2021) reported indole acetic acid (IAA) and gibberellic acid (GA) produced by *L. plantarum* applied to wheat seeds, higher germination rates and greater coleoptile growth is seen. The significant increase in nitrogen, phosphorus, and potassium content in treated plants indicates improved nutrient mobilization and uptake. Phytase-producing isolates likely contributed to the mineralization of organic phosphorus, making it available for plant uptake, while phosphate-solubilizing activity may have enhanced inorganic phosphorus availability. This is in agreement with findings of El Ifa *et al.* (2024), who reported that when barley plants grown in poor sandy soil and irrigated with a phytic acid containing solution were inoculated with each of the PGPR isolates, increase in root growth, plant height, and chlorophyll content were observed and similar findings were seen by Li *et al.* (2024), where the maize seedlings growth is enhanced by phosphate solubilizing characteristics of a lactic acid bacterium. The greater effectiveness of 50% CFS treatments in increasing nutrient content further supports the role of metabolite concentration in shaping plant response. These results align with Naamala *et al.* (2023) and Zang *et al.* (2023), who reported that microbial metabolites significantly enhance nutrient uptake and chlorophyll content in plants.

Conclusion

The present study demonstrates that selected presumptive *Lactobacillus* isolates from curd, papaya, and banana possess notable plant growth-promoting and

enzymatic abilities that can be harnessed to improve the growth and nutrient content of *Spinacia oleracea*. All isolates exhibited beneficial features, such as phosphate solubilization and phytase activity, although efficacy varied among the strains. The presumptive isolates CS-1, CS-4, MP-3 and RB-1 performed well. These results confirm that certain *Lactobacillus* strains can positively influence plant development, likely through the release of growth-promoting metabolites and enhancement of nutrient bioavailability. This research supports the integration of such beneficial microbes into plant production systems as a viable route toward eco-friendly and efficient biofertilizers. The evidence presented here encourages further exploration of *Lactobacillus*-based bio-inoculants for sustainable agriculture, particularly in the cultivation of leafy vegetables like spinach.

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