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Biological Control of Bacterial Diseases in Papaya through *in-planta* Application of Lactic Acid Bacteria Isolated from Milk Curd

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Abstract

Bacterial diseases are a major challenge in papaya cultivation, causing significant yield losses worldwide. *Erwinia carotovora*, *Pseudomonas solanacearum*, and *Xanthomonas campestris*, are a major challenge for papaya growers, leading to substantial yield losses. In this study, LAB strains were isolated from milk curd, and their efficacy as biological control agents against bacterial diseases in papaya was evaluated through in-planta application. The results showed that LAB strains were effective in suppressing bacterial growth in papaya plants, reducing the incidence and severity of bacterial diseases, and improving plant growth and yield. The findings suggest that in-planta application of LAB can be a potential strategy for biological control of bacterial diseases in papaya. Biological control using lactic acid bacteria (LAB) is a promising alternative for managing bacterial diseases in papaya.

Key words: Papaya, Bacterial diseases, LAB, Biological control, *in-planta* application, Milk curd

Papaya (*Carica papaya* L.) is one of the important fruits widely cultivated in many countries, including India, Brazil, Mexico, and Indonesia. Papaya is rich in vitamins, minerals, and antioxidants, and is consumed fresh or processed into various products, such as juice, jam, and candy. However, bacterial diseases caused by pathogens such as *Erwinia carotovora*, *Pseudomonas solanacearum*, and *Xanthomonas campestris* causing soft rot, bacterial wilt and leaf spot diseases respectively in papaya. These are a major challenge for papaya growers, leading to substantial yield losses [1]. These bacterial pathogens can infect different parts of the plant, including leaves, stems, flowers, and fruits, and can cause symptoms such as wilting, rotting, and yellowing. The management of bacterial diseases in papaya is a complex task, as the pathogens are highly adaptable and can develop resistance to chemical pesticides [2]. Moreover, the excessive and indiscriminate use of chemical pesticides can have negative impacts on human health and the environment, such as the development of pesticide residues in food, water, and soil, and the emergence of pesticide-resistant pathogens [3]. Biological control using beneficial microorganisms is a promising alternative for managing plant diseases in a sustainable and eco-friendly manner [4]. Lactic acid bacteria (LAB) are a group of beneficial microorganisms that are commonly found in fermented food products such as milk, yogurt, and cheese. LAB have been shown to possess various beneficial properties, such as the production of antimicrobial compounds, the enhancement of plant growth and nutrition, and the stimulation of plant defense mechanisms [5]. LAB have also been used as biocontrol agents against bacterial diseases in various crops, such as tomato, potato, and grapevine [6]. LAB can exert their biocontrol activity through various mechanisms, such as the production of organic acids, hydrogen peroxide, and bacteriocins, the competition for nutrients and adhesion sites, and the induction of systemic resistance in plants [7]. In-planta application of beneficial microorganisms is a recent approach for biological control that involves the inoculation of the microorganisms into the plant tissues, either through the roots, the leaves, or the stem. In-planta application can enhance the efficacy of the biocontrol agents by increasing their contact with the target pathogen, reducing their exposure to environmental stressors, and facilitating their systemic movement throughout the plant [8]. In-planta application of LAB has been reported to effectively control bacterial diseases in various crops, such as tomato, pepper, and potato [6].

In this study, LAB strains were isolated from milk curd and evaluated for their efficacy as biological control agents against bacterial diseases in papaya through in-planta application. The objectives of this study were to isolate and characterize LAB strains from milk curd, assess their in vitro antagonistic activity against bacterial pathogens, evaluate their in-planta colonization and persistence, and determine their efficacy in controlling bacterial diseases in papaya.

MATERIALS AND METHODS

Isolation and identification of LAB

Fresh milk curd samples were collected aseptically, diluted in sterile distilled water and homogenized using a stomacher. The diluted sample was plated onto MRS (de Man,

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Rogosa, and Sharpe) agar media for the growth of LAB. The plates were then incubated at 37ºC for 48 hours. After incubation, individual colonies of LAB were isolated based on their morphology (shape, size, colour) and transferred onto fresh agar plates to obtain pure cultures. The isolates were identified based on Gram staining, catalase test, oxidase test, sugar fermentation tests, and confirmed by 16S rRNA gene sequencing. Isolated LAB strains can be further characterized for their beneficial properties, such as antimicrobial activity, acid production, tolerance to environmental stressors, and potential as biocontrol agents.

In vitro antagonistic activity of LAB strains

The antagonistic activity of LAB strains against bacterial pathogens was evaluated using the agar well diffusion assay. Briefly, the bacterial pathogens (*Erwinia carotovora*, *Pseudomonas solanacearum* and *Xanthomonas campestris*) were cultured in nutrient agar plates and incubated at 37°C for 24 hours. The LAB strains were grown in MRS broth at 37°C for 24 hours and centrifuged at 5000 rpm for 10 minutes. The cell-free supernatant was collected and filter-sterilized using a 0.22 µm filter. The filter-sterilized supernatant was then loaded into wells (6 mm diameter) made in the nutrient agar plates containing the bacterial pathogens. The plates were incubated at 37°C for 24 hours, and the inhibition zones around the wells were measured.

In-planta application of LAB

Papaya plants (2-month-old) were sprayed with LAB (10^8 CFU/mL) three times at 10-day intervals. Control plants were sprayed with sterile distilled water. After the third application, the plants were inoculated with a bacterial pathogen (*Erwinia spp*.) by leaf infiltration. Disease incidence and severity were evaluated at 3, 5 and 7 days post-inoculation.

In-planta colonization and persistence of LAB strains

The ability of LAB strains to colonize and persist in papaya plants was evaluated through in-planta application. Papaya seeds (variety Red Lady) were sown in plastic trays filled with sterilized soil mixture (1:1:1 ratio of sand, soil, and compost). The seedlings were grown under controlled conditions of temperature (25°C) and humidity (80%) in a growth chamber for two weeks. The LAB strains were grown in MRS broth at 37°C for 24 hours and centrifuged at 5000 rpm for 10 minutes. The cell pellet was washed twice with sterile distilled water and resuspended in sterile distilled water to a concentration of 10^8 CFU/mL. The papaya seedlings were inoculated with LAB strains through the root dipping method, by soaking the roots in the LAB suspension for 10 minutes. The inoculated seedlings were transplanted into plastic pots containing sterilized soil mixture and grown under greenhouse conditions of temperature (25°C) and humidity (70%) for four weeks. The roots, stems, and leaves of the papaya plants were collected at different time intervals (0, 3, 7, and 14 days) after inoculation, and the LAB population was quantified by serial dilution and plating on MRS agar plates.

Statistical analysis

The data were analyzed using ANOVA and Tukey's test ($p < 0.05$) with SPSS software.

RESULTS AND DISCUSSION

A total of 9 lactic acid bacteria (LAB) isolates were obtained from milk curd. All isolates were Gram-positive, catalase-negative, and produced lactic acid. The 16S rRNA gene sequencing results revealed that the isolates belonged to the genera Lactobacillus. The application of lactic acid bacteria (LAB) significantly reduced bacterial disease incidence and severity in papaya plants compared to the control. The disease incidence was 67.7% in the control plants, while it was significantly reduced to 26.7% in the LAB-treated plants. Similarly, disease severity was significantly lower in LAB-treated plants (2.2) compared to the control (3.7) at 7 days post-inoculation [9].

Table 1 Biochemical characters of isolated lactic acid bacteria

Characters	LAB01	LAB02	LAB03	LAB04	LAB05	LAB06	LAB07	LAB08	LAB09
Gram stain	+	+	+	+	+	+	+	+	+
Caltalase	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-
Lactic acid production	+	+	+	+	+	+	+	+	+
Sugar fermentation	+	+	+	+	+	+	+	+	+

Table 2 Inhibitory potential of LAB isolates on *Erwinia carotovora*

Pathogen	Inhibition zone radius (IZR) in mm
LAB01	32.3 ± 1.6
LAB02	19.5 ± 2.6
LAB03	27.6 ± 1.5
LAB04	21.8 ± 1.7
LAB05	28.0 ± 0.8
LAB06	13.8 ± 0.5
LAB07	21.5 ± 0.5
LAB08	26.2 ± 1.9
LAB09	37.0 ± 1.2

LAB09, LAB07 and LAB01 was selected as most inhibiting strain against *Erwinia carotovora*, *Pseudomonas solanacearum*, and *Xanthomonas campestris* respectively and were subjected to 16s rRNA gene sequencing. Theses strains were identified as *Lactobacillus acidophilus* strain VPI6032 (Fig 1), *Lactobacillus delbrueckii* subsp. indicus strain NCC725 (Fig 2), and *Lactobacillus amylovorus* DSM 20531 (Fig 3) respectively.

Table 3 Inhibitory potential of LAB isolates on *Pseudomonas solanacearum*

Pathogen	Inhibition zone radius (IZR) in mm
LAB01	35.2 ± 1.8
LAB02	10.5 ± 1.6
LAB03	25.6 ± 1.3
LAB04	20.2 ± 1.1
LAB05	29.1 ± 1.8
LAB06	14.2 ± 1.5
LAB07	35.5 ± 0.9
LAB08	29.2 ± 1.6
LAB09	33.1 ± 1.5

Table 4 Inhibitory potential of LAB isolates on *Xanthomonas campestris*

Pathogen	Inhibition zone radius (IZR) in mm
LAB01	30.2 ± 1.1
LAB02	12.5 ± 1.6
LAB03	29.6 ± 1.0
LAB04	20.9 ± 1.7
LAB05	28.2 ± 1.5
LAB06	19.9 ± 1.6
LAB07	30.0 ± 1.8
LAB08	29.1 ± 0.7
LAB09	31.1 ± 1.0

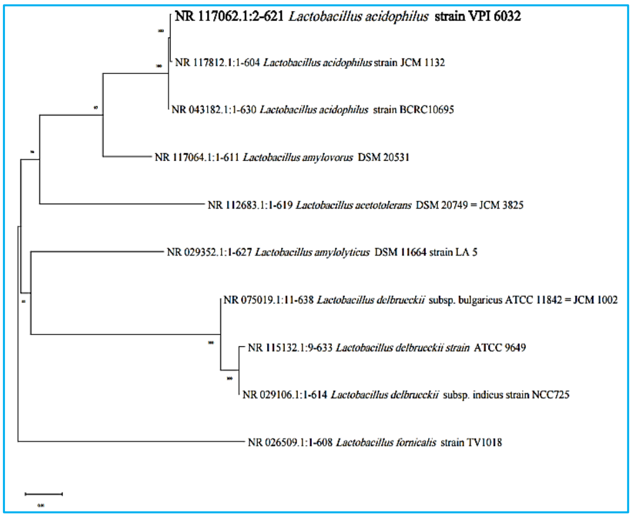

Fig 1 Neighbor joining phylogenetic tree of *Lactobacillus acidophilus* strain VPI 6032 constructed using Bootstrap method in MEGA 11

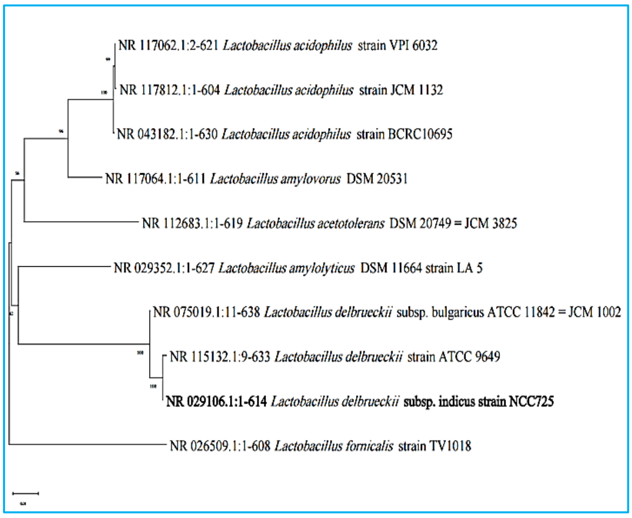

Fig 2 Neighbor joining phylogenetic tree of *Lactobacillus delbrueckii* subsp. Indicus strain NC725 constructed using Bootstrap method in MEGA 11

The selected LAB strains, being effective inhibitors of the target pathogens, would compete with them for nutrients and colonization sites, thereby reducing their populations and suppressing disease development. Additionally, LAB have been shown to produce antimicrobial compounds such as organic acids, hydrogen peroxide, and bacteriocins, which can directly inhibit the growth of pathogenic bacteria. Furthermore, LAB strains can also stimulate the plant's immune response and induce systemic resistance, enhancing the papaya plant's ability to defend itself against pathogen attack. This systemic protection would contribute to long-term disease control and reduce the reliance on chemical pesticides. Overall, the *in-planta* application of *Lactobacillus acidophilus* strain VPI6032, *Lactobacillus delbrueckii* subsp. indicus strain NCC725, and *Lactobacillus amylovorus* DSM 20531 holds promise for effectively managing bacterial diseases in papaya, ensuring healthier plants and improved yields for growers [10-13].

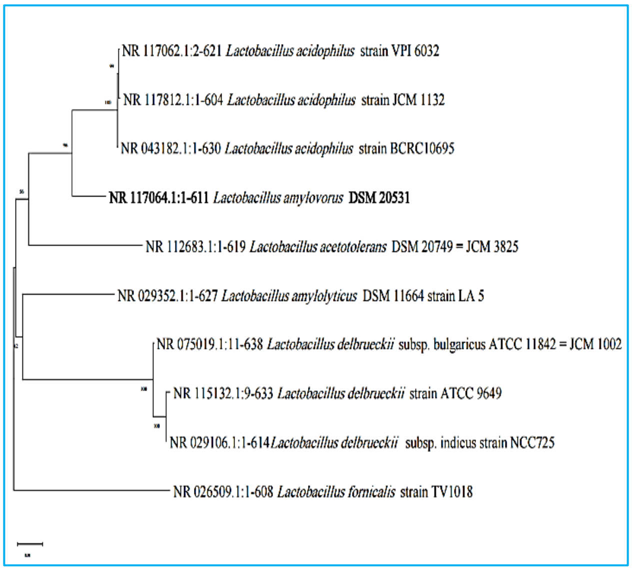

Fig 3 Neighbor joining phylogenetic tree of *Lactobacillus amylovorus* DSM 20531 constructed using Bootstrap method in MEGA 11

Biological control through the use of beneficial microbes has gained attention as an eco-friendly and sustainable approach to disease management. LAB are one of the most promising candidates for biological control due to their ability to produce various antimicrobial compounds, such as organic acids, hydrogen peroxide, and bacteriocins. In this study, LAB isolated from milk curd was effective in reducing bacterial disease incidence and severity in papaya through in-planta application. The results are consistent with previous studies that showed the efficacy of LAB in controlling bacterial diseases in various crops. The mode of action of LAB in plant disease control is complex [14-16].

CONCLUSION

The findings from this study demonstrate the efficacy of Lactic Acid Bacteria (LAB), specifically Lactobacillus species, in significantly reducing bacterial disease incidence and severity in papaya plants. Isolates obtained from milk curd exhibited promising inhibitory effects against major pathogens such as *Erwinia carotovora*, *Pseudomonas solanacearum*, and *Xanthomonas campestris*. Through 16S rRNA gene sequencing, the identified strains—*Lactobacillus acidophilus* strain VPI6032, *Lactobacillus delbrueckii* subsp. indicus strain NCC725, and *Lactobacillus amylovorus* DSM 20531—showed potent inhibitory capabilities against respective pathogens. The mechanism of action involves competition for nutrients and colonization sites, production of antimicrobial compounds, and induction of systemic resistance in papaya plants. This biological control approach offers a sustainable alternative to chemical pesticides, ensuring healthier plants and improved yields. Overall, the study underscores the potential of LAB as effective biocontrol agents for managing bacterial diseases in papaya cultivation, contributing to eco-friendly and sustainable agricultural practices.

LITERATURE CITED

1. Silva NCM, Morais TP, Vasconcelos EAR, Lima MGA, Galdino SL. 2020. Bacterial wilt of papaya: An update on epidemiology, etiology, and management. *Tropical Plant Pathology* 45(6): 442-455.
2. Mishra VK, Patel MK, Jangir M, Thakur AK. 2021. Bacterial wilt of papaya and its management strategies: A review. *Journal of Plant Protection Research* 61(3): 237-248.
3. Köhl J, Kolnaar R, Ravensberg WJ. 2016. Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. *Frontiers in Plant Science* 7: 1-19.
4. Berg G, Köberl M, Rybakova D, Müller H, Grosch R, Smalla K. 2017. Plant microbial diversity is suggested as the key to future biocontrol and health trends. *FEMS Microbiology Ecology* 93(5): 1-10.
5. Gaggìa F, Baffoni L, Galiano M, Nielsen DS, Jakobsen RR, Castro-Mejía JL, Bosi S. 2018. Microbial biofilms: Challenges for future research. *Trends in Food Science and Technology* 71: 313-326.
6. Elshafie HS, Camele I, Racioppi R, Scrano L, Iacobellis NS. 2017. Biological control of bacterial wilt of tomato caused by *Ralstonia solanacearum* by an endophytic bacterium *Bacillus amyloliquefaciens* IN937a and its mode of action. *Journal of Plant Pathology* 99(1): 37-46.
7. Saravanakumar D, Ciavorella A, Spadaro D, Garibaldi A, Gullino ML. 2017. Metschnikowia pulcherrima strain MACH1 outcompetes *Botrytis cinerea, Alternaria alternata* and *Penicillium expansum* in apples through iron depletion. *Postharvest Biology and Technology* 123: 41-48.
8. Raza W, Ling N, Zhang R, Huang Q, Xu Y, Shen Q, Shen Q. 2020. Success evaluation of the biological control of *Fusarium wilts* of cucumber, banana, and tomato since 2000 and future research strategies. *Critical Reviews in Biotechnology* 40(3): 334-347.
9. Wang D, Liu W, Ren Y, De L, Zhang D, Yang Y, Bao Q, Zhang H, Menghe B. 2016. Isolation and identification of lactic acid bacteria from traditional dairy products in Baotou and Bayannur of Midwestern Inner Mongolia and q-PCR analysis of predominant species. *Korean Jr. Food Sci. Anim. Resource* 36(4): 499-507.
10. Abdul Hakim BN, Xuan NJ, Oslan SNH. 2023. A comprehensive review of bioactive compounds from lactic acid bacteria: Potential functions as functional food in dietetics and the food industry. *Foods* 12(15): 2850.
11. Ibrahim SA, Ayivi RD, Zimmerman T, Siddiqui SA, Altemimi AB, Fidan H, Esatbeyoglu T, Bakhshayesh RV. 2021. Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. *Foods* 10(12): 3131.
12. Jaffar NS, Jawan R, Chong KP. 2023. The potential of lactic acid bacteria in mediating the control of plant diseases and plant growth stimulation in crop production - A mini review. *Front. Plant Science* 13: 1047945.
13. Singh RP, Shadan A, Ma Y. 2022. Biotechnological applications of probiotics: A multifarious weapon to disease and metabolic abnormality. *Probiotics and Antimicro. Protection* 14: 1184-1210.
14. He DC, He MH, Amalin DM, Liu W, Alvindia DG, Zhan J. 2021. Biological control of plant diseases: An evolutionary and eco-economic consideration. *Pathogen* 10(10): 1311.
15. Lahlali R, Ezrari S, Radouane N, Kenfaoui J, Esmaeel Q, El Hamss H, Belabess Z, Barka EA. 202. Biological control of plant pathogens: A global perspective. *Microorganisms* 10(3): 596.
16. Rabbee MF, Hwang B-S, Baek K-H. 2023. *Bacillus velezensis*: A beneficial biocontrol agent or facultative phytopathogen for sustainable agriculture. *Agronomy* 13(3): 840.