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Biochemical and Pathogenic Characteristics of Rhizobacteria with Insecticidal Activity Against Spodoptera litura

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ABSTRACT

The increasing demand for environmentally friendly alternatives to chemical pesticides has driven the exploration of rhizosphere bacteria as biocontrol agents. The rhizosphere is recognized as a hotspot for functionally diverse bacteria with biocontrol potential owing to intense plant-microbe interactions and selective ecological pressures. This study successfully isolated and characterized thermotolerant bacteria from acidic long bean and soybean soils to control the armyworm pest *Spodoptera litura*. Sixteen bacterial isolates were obtained through serial dilution and temperature selection (28°C to 90°C), two of which, KP284 and K504a, demonstrated potent entomopathogenic activity. These isolates induce rapid larval mortality (wet-lysis type) within 2–5 days after ingestion, which is a distinct and effective mode of action. Biochemical profiling revealed that both isolates were gram-negative, obligate aerobes, catalase- and oxidase-positive, with strong acid production during carbohydrate fermentation, and capable of growing under acidic to neutral pH conditions. Importantly, both isolates were non-pathogenic to plants and animals, ensuring their safety. Furthermore, the bacterial maintained viability in low-cost organic carrier media (molasses and rice washing water), supporting the formulation of a practical biopesticide. These findings highlight KP284 and K504a as promising candidates for sustainable pest management in tropical agriculture, representing a novel contribution to rhizobacterial biocontrol research.

Keywords: Biochemical test, biopesticides, organic carrier formulation, thermotolerant rhizobacteria

INTRODUCTION

Insect pests are a major constraint to global agricultural productivity and food security. Recent expert assessments indicate that pests and plant pathogens are associated with average yield losses of approximately 21–30 % across major staple crops, including wheat, rice, maize, potato, and soybean, with losses exceeding 40 % in certain regions (Savary *et al.* 2019). These substantial losses threaten agricultural sustainability and pose serious economic challenges to farming systems worldwide. Among insect pests, the armyworm *Spodoptera litura* is considered one of the most destructive species in tropical and subtropical regions because of its high feeding capacity and broad host range. Armyworms originate from the tropics and are considered dangerous pests because they can attack more than 80 types of crops, including rice, corn, soybeans, beans, cabbage, onions, and other vegetables. Armyworm pests can result in significant yield losses if not handled properly (Agastya *et al.* 2017; Septian *et al.* 2021; Damiri *et al.* 2022).

At present, armyworm pests are still controlled using chemical pesticides. The use of pesticides can support increased agricultural production, especially of food crops. However, the intensive use of chemical pesticides to control *S. litura* often causes negative impacts, such as pesticide residues in the soil that can harm non-target organisms, pest resistance, reach water sources and affect ecosystems (Agastya *et al.* 2017; Damiri *et al.* 2022). Pesticide residues may accumulate within the food chain, posing potential toxicological risks to both animal and human consumers. The negative impacts caused by the use of chemical pesticides have encouraged international agreements to restrict the use of chemicals in the production process, especially synthetic chemical pesticides in pest control in agriculture, plantations, and forestry, and to begin to transition toward the application of environmentally friendly pesticides (Fajrullah & Kristiana 2023).

Environmentally friendly pest control of *S. litura* can be achieved by utilizing biocontrol or biological agents to preserve the environment and promote sustainable agriculture. In contrast to chemical pesticides, biological agents can minimize the risk of pest resistance (Lalitha *et al.* 2022). Among microbial biocontrol agents, bacteria have been the most

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extensively studied because of their ability to produce bioactive compounds and adapt to diverse environments (Yusuf *et al.* 2024). Bacterial strains of biological agents are mostly obtained from plants, plant rhizospheres (Rahmatullah *et al.* 2025), and soil (Yuan *et al.* 2024). *Bacillus thuringiensis* var. kurstaki has been reported to be effective in causing up to 98% larval mortality of *S. litura* under laboratory conditions, indicating its high potential as a biocontrol agent (Thakur *et al.* 2023). Soil is a rich habitat for microorganisms, including those pathogenic to insects. Microorganisms that are often found and have the potential to control insect pests usually belong to groups of spore-forming bacteria that can be isolated from soil (Ali *et al.* 2024; Lisnawita *et al.* 2025).

The use of entomopathogenic bacteria isolated from soil is an effective and practical approach for controlling *S. litura*. Various bacterial species have been reported to efficiently suppress *S. litura* populations through specific modes of action while minimizing the risk of resistance development in the target pests. Among them, *Bacillus thuringiensis* is one of the most extensively studied bacterial biocontrol agents and has been proven effective against a wide range of insect pests, with well-defined insecticidal mechanisms and a favorable environmental safety profile (Sharma *et al.* 2024). Bacteria possess substantial efficacy in suppressing *S. litura* populations, thereby offering a strong basis for biopesticide development. This study aimed to isolate, explore, and characterize bacterial strains with entomopathogenic potential against S. litura and to conduct physiological or biochemical analyses of these strains. *Additionally, we evaluated* their viability in low-cost organic carrier media to assess their suitability as environmentally friendly pest control agents for future biopesticide formulations.

METHODS

The research was conducted from August to October 2019 at the Soil Biotechnology Laboratory of the Department of Soil Science and Environmental Resources, Bogor Agricultural University, Indonesia. The tools used in this research are Laminar Air Flow Cabinet, Autoclave, Erlenmeyer 250 ml and 100 ml, petri dish, ose needle, test tube, shaker, balance, micropipette, vortex, bunsen, waterbath, syringe, medium jar for caterpillar culture and tissue culture bottle for carrier incubation. The materials used were soil samples from soybean and long bean plantations, *S. litura* caterpillars, instant NA media, NaCl 0.85%, alcohol, distilled water, gauze, plastic wrap, microtips, blood agar media, tobacco plants, molasses, and rice washing water. The research procedures included soil sampling, determination of soil pH, isolation of bacteria, calculation of Total Plate Count, purification of bacterial isolates, morphological observations, pathogenicity and hemolysis tests, mortality tests on *S. litura* caterpillars, and preparation of inoculum/carriers.

Soil Sampling and Soil pH Measurement

Soil samples were collected from 2 cropping locations, namely soybean and long bean plants, from the Dramaga Campus of Bogor Agricultural University. Soil samples were collected from the rhizosphere using purposive sampling at five sampling points, composited into a single sample per unit, and replicated twice for each treatment. A sampling depth of 20 cm was selected because it represents the topsoil layer with high biological activity and the central zone of root–microorganism interactions in the rhizosphere. In each soil sample, the soil pH was determined using the glass electrode method (Khairiyah *et al.* 2022).

Isolation and Purification of Rizosphere Bacteria

Soil samples were weighed 10 g and dissolved in 90 ml of sterile physiological solution (0.85% NaCl). The solution was homogenized using an automatic shaker for 30 minutes at 80 rpm. Dilutions were carried out at 6 levels, namely 10^{-1} to 10^{-6}, each dilution sample was pipetted as much as 0.1 ml which was spread on NA (Nutrient Agar) media, and carried out in duplicate. The isolated samples were then incubated at room temperature (20-25 C) for 3-7 days (Firlandiana *et al.* 2024). The purification process of bacterial isolates aimed to obtain previously grown pure colonies. Bacterial colonies with different diversity in one sample were then purified on NA media. Furthermore, one bacterial colony was transferred to a slanted tube to obtain a pure bacterial isolate.

Pathogenicity Test of Rhizosphere Bacteria

Pathogenicity testing was performed by streaking distinct bacterial colonies with a sterile loop onto Blood Agar Plates (BAP). This test aimed to screen for biosafety by observing the ability of the bacteria to produce hemolysis. If hemolysis occurs, a clear zone appears around the colonies. There are three types of hemolysis: β-hemolysis (complete lysis of red blood cells around the colony), α-hemolysis (partial lysis with a greenish discoloration around the colony), and γ-hemolysis (no hemolysis) (Devi *et al.* 2019).

Macroscopic and Microscopic Characterization

The purified bacteria were then observed macroscopically and microscopically. Macroscopic characterization was performed by visually observing the morphology of the bacteria, including their shape, edge, elevation, size, appearance, optical properties, texture, and color. Microscopic (light microscope) characterization was performed using Gram staining to identify bacterial morphology. This coloring process uses four types of dyes: crystal violet, Lugol's iodine, 96% ethanol, and safranin. Gram-positive bacteria appear purplish blue, whereas Gram-negative bacteria appear red (Wulandari & Purwaningsih 2020).

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Mortality Test Against S. litura

Mortality tests were conducted using the oral method, which involves poisoning through the food route. To conduct this test, five *S. litura* larvae were fed soybean leaves that were treated with a suspension of the pathogen isolates. The leaf was dipped into the isolate suspension until the entire surface was wet. The leaves were then air-dried. After the solvent evaporated, the leaves were given to the test larvae. The cells were then incubated for 2 × 24 hours and compared with the control (without isolate treatment). Subsequently, the number of dead (lysed and dried) and live caterpillars was recorded (Hoesain *et al.* 2023).

Biochemical Test of Rhizosphere Bacteria

Biochemical tests performed include Gram staining (Tripathi & Sapra 2025), catalase test (Talaiekhozani 2022), carbohydrate fermentation test (Gunkova *et al.* 2021), oxygen demand test (Abrevaya *et al.* 2015), pH, temperature, saline, urease, and oxidase tests (Cheesbrough 1987). Before testing, bacterial isolates were rejuvenated on NA solid media until 24 hours old isolates were obtained.

Inoculation of Rhizosphere Bacteria into Carrier Media

Isolates that have passed pathogenicity tests, biochemical tests, and shown effectiveness in mortality tests against caterpillars will be further tested as inoculum or carrier material for the formulation of biopesticides. The carrier material used was liquid with two treatments: 5% molasses solution and a combination of 5% molasses with 10% rice washing water. Each carrier material was added with 10% isolates from liquid media (Fahruddin & Sulfahri 2019).

RESULTS AND DISCUSSION

Isolates Characteristics of Potential Bacteria as Anti Pests

Sixteen bacterial isolates were obtained from the rhizosphere soils of long bean and soybean plants collected from acidic environments. Soil pH analysis showed that the long bean rhizosphere soil was highly acidic (pH 4.08), whereas the soybean rhizosphere soil was moderately acidic (pH 5.43) (Table 1). Acidic soils are known to selectively favor stress-tolerant microbial populations with adaptive traits that may be relevant to biocontrol applications (Wang *et al.* 2022; He *et al.* 2022).

All isolates were initially subjected to biosafety screening, including plant pathogenicity and hemolysis assays, to evaluate their potential risks to plants, humans and animals (Table 4). The results indicated that most isolates exhibited pathogenic or hemolytic activity, whereas only two isolates, KP284 and K504a, showed negative reactions in both plant pathogenicity and hemolysis tests, indicating a favorable biosafety profile for these two isolates. Based on these results, KP284 and K504a were selected for further evaluation of their entomopathogenic potential against *S. litura.* Subsequent larval mortality assays demonstrated that both isolates were capable of inducing significant larval mortality, supporting their potential as safe and effective bacterial candidates for biological control.

The temperature treatment consisted of 4 levels: room temperature (28°C), medium temperature (50°C), and high temperatures (70°C and 90°C). Based on the results of the four temperature treatments, as shown in Table 2, the results of the calculation of the total bacterial population with the total plate count (TPC) method were highest at a dilution level of 10^6 CFU, namely at room temperature (28°C) and medium temperature (50°C). The purpose of the temperature treatment was to obtain specific bacterial isolates that could grow under mesophilic or thermophilic conditions, which are expected to play a role as potential bacteria against *S. litura* pests. The ability of

Table 1 Soil pH analysis of rhizosphere soil samples collected from long bean and soybean plantations at IPB Dramaga Campus, Bogor, Indonesia

Field locations	pH	Classification
Long Bean	4,08	Very acidic
Soybean	5,43	Acidic

Table 2 Total plate count of rhizosphere bacteria isolated from long bean and soybean soils under different temperature treatments

Soil Source	Temperature (°C)	Total Bacterial Population (CFU/g)
Long Bean	28	13.5×10^6
	50	10.65×10^6
	70	4.95×10^6
	90	3.75×10^6
Soybean	28	6.1×10^6
	50	10.45×10^6
	70	3.4×10^6
	90	2.65×10^6

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isolates to grow at elevated temperatures (50–90°C) reflects the thermotolerant or even thermophilic nature of certain strains, which is not commonly reported in rhizobacteria-based biocontrol studies. Most entomopathogenic bacteria, such as *B. thuringiensis*, have optimal growth at mesophilic temperatures (approximately 30–37°C). The genus *Bacillus*, commonly found among thermophilic bacteria, is known for its high heat resistance and ability to produce extracellular enzymes that remain active under extreme environmental conditions. The presence of heat-tolerant bacteria in this study expands the potential for field application in tropical or arid regions, where higher soil temperatures may limit bacterial viability. Thermotolerant strains are more resilient and suitable for long-term shelf life and transportation (Mong *et al.* 2022).

Character selection was carried out by observing the morphological characteristics of the colonies, and 16 isolates with morphological character differences in the categories of color, shape, upper surface, edge, and elevation were obtained. Based on Table 3, the successfully isolated bacterial colonies mostly had a yellowish white color, with some having milky white, white, and clear colors. The microscopic morphological observations of the bacteria revealed mostly molar-tooth shapes, along with circular, irregular, and bread crumb shapes. Macroscopically, KP284 (circular–concentric, eroded edges, umbonate) and K504a (molar-tooth, filiform, eroded edges) exhibit colony characteristics commonly associated with spreading colonies and exopolysaccharide/exoenzyme production. Morphological patterns such as filiform/erose have been linked to surface motility (swarming) and secretion of extracellular enzymes that increase virulence in insect-pathogenic bacteria (Salazar-Gutiérrez *et al.* 2017).

Pathogenicity and Hemolysis Test of Anti-pest Bacterial Isolates

Pathogenicity and hemolysis tests (Table 4) revealed that most rhizosphere isolates exhibited pathogenic potential toward plants or animals, as indicated by symptoms in the test hosts and the

Table 3 Morphological observation of bacterial isolates

Isolates code	Color	Shape	Upper surface	Edge	Elevation
KP284	Yellowish white	Circular	Concentric	Erose	Umbonate
KP286a	Milky white	Molar-tooth	Spreads irregularly	Undulate	Raised
KP286b	Milky white	Bredd crumb	Spreads irregularly	Lobate	Raised
KP505	Translucent	Molar-tooth	Filiform	Erose	Convex
KP705	Yellowish white	Irregular	L-shape	Entire	Convex
KP706	Translucent	Molar-tooth	Rhizoid	Undulate	Raised
KP904	Yellowish white	Molar-tooth	Round with wavy edges	Undulate	Raised
KP906	White	Circular	Round	Entire	Umbonate
K286a	White	Molar-tooth	Round with wavy edges	Undulate	Convex
K286b	Milky white	Molar-tooth	Spreads irregularly	Lobate	Raised
K504a	Translucent	Molar-tooth	Filiform	Erose	Convex
K504b	Translucent	Irregular	Specialized surface	Entire	Raised
K504c	Milky white	Circular	Round	Entire	Raised
K704	Yellowish white	Circular	Round	Entire	Raised
K904	Translucent	Molar-tooth	Filiform	Undulate	Umbonate
K906	White	Breadcrumb	Spreads irregularly	Lobate	Raised

Table 4 Pathogenicity and hemolysis test results

Isolate code	Plant pathogens	Human/animal pathogens
KP284	-	-
KP286a	-	+
KP286b	+	+
KP505	+	+
KP705	+	+
KP706	+	+
KP904	+	-
KP906	+	-
K286a	+	+
K286b	-	+
K504a	-	-
K504b	-	+
K504c	-	+
K704	+	+
K904	+	+
K906	+	+

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presence of hemolysis zones on Blood Agar medium. However, the two selected isolates, KP284 and K504a, did not exhibit pathogenicity toward plants or animals or hemolytic activity, highlighting their biosafety and suitability as potential biocontrol agents.

Hemolysis on Blood Agar is a widely used preliminary indicator of bacterial safety. Isolates that produce β-hemolysis (complete clearing) generally express strong hemolysins, which are often correlated with toxicity toward animals or humans (Devi *et al.* 2019). α-hemolysis (greenish zone) reflects partial hemolytic activity, while γ-hemolysis (no zone) is considered non-hemolytic and safe, since red blood cells remain intact. Thus, the γ-hemolytic profiles of KP284 and K504a reinforce their non-pathogenic nature. This aspect is crucial because biosafety toward non-target organisms is a primary criterion for selecting biocontrol agents. Certain entomopathogenic bacteria, such as Serratia marcescens and Pseudomonas fluorescens, despite being effective against insects, are known to produce hemolysins or toxins that may pose risks to humans and animals (Mora & Arioli 2014). Therefore, isolates showing hemolytic activity must be excluded at the early screening stages. Interestingly, KP284 and K504a caused significant mortality in Spodoptera litura larvae with a wet-lysed phenotype, despite the lack of hemolytic activity. This indicates that their entomopathogenic mechanism is likely not hemolysin-based but rather associated with the secretion of lytic enzymes, such as proteases and chitinases (Harrison & Bonning 2010; Lopes *et al.* 2021). These enzymes are known to degrade insect structural proteins and chitin, leading to tissue liquefaction, a hallmark observed in the mortality assays conducted in this study.

Toxicity of Bacterial Isolates to the *S. litura*

The results of the caterpillar mortality test using anti-pest bacterial isolates (Table 5). Among the isolated strains, two isolates, KP284 and K504a, demonstrated strong entomopathogenic activity against *S. litura*. These two isolates were capable of inducing larval mortality within 2–5 days through a 'wet-lysed' mechanism, distinct from the dry-dead condition observed in control treatments. This indicates the potential production of insecticidal metabolites or enzymatic actions that disrupt larval tissue integrity. Similar pathological signs have been reported in studies involving entomopathogenic bacteria, such *as Serratia marcescens* and *Xenorhabdus spp*. (Ramadhan & Hernowo 2013; Sharma *et al.* 2024). Other studies have revealed that *Bacillus thuringiensis* and *Beauveria bassiana* show maximum efficacy against *S. litura* larvae at the second instar (Maqsood *et al.* 2018).

The observed wet-lysed mortality suggests that the bacteria may produce lytic enzymes or toxins targeting the insect midgut, similar to *Cry* or *Vip* toxins in Bt-based biopesticides. Further investigation through molecular or metabolomic profiling could validate this mechanism and identify novel bioactive compounds. These findings are significant for the discovery of new classes of biopesticides. Lytic activity by bacterial metabolites has been linked to protease and chitinase production, both of which are crucial for breaching insect cuticles and gut lining (Lopes *et al.* 2021; Banerjee *et al.* 2022). The biochemical characteristics of the two most effective isolates, KP284 and K504a (Table 6), provide important clues regarding their mode of action, particularly related to their enzymatic potential. These properties are indicative of metabolically active bacteria that can survive and function effectively in the rhizosphere and insect gut. The catalase and oxidase positivity suggests that these isolates have robust reactive oxygen species (ROS) detoxification mechanisms, enabling them to survive oxidative stress during host invasion or colonization (Borisov *et al.* 2021). Additionally, the aerobic nature of these isolates supports their ability to produce extracellular enzymes, including proteases and chitinases, which require oxygen-dependent metabolic pathways (Masi *et al.* 2023).

Proteases and chitinases are two of the most important lytic enzymes involved in the pathogenesis of

Table 5 Number of caterpillar deaths by anti-pest bacterial isolates

Isolate codes	Number of dead pests					Description
	Day 1	Day 2	Day 3	Day 4	Day 5	
Control (1)	0	0	3	4	5	Dry - not lysed
Control (2)	0	2	4	5	5	Dry - not lysed
Control (3)	0	1	3	4	4	Dry - not lysed
KP284 (1)	0	2	3	3	4	Wet - lysed
KP284 (2)	0	2	5	5	5	Wet - lysed
KP284 (3)	0	0	4	5	5	Wet - lysed
K504a (1)	0	1	3	4	4	Wet - lysed
K504a (2)	0	2	4	4	5	Wet - lysed
K504a (3)	0	2	3	4	5	Wet - lysed

Note: Control (1) = , dst

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entomopathogenic bacteria. Proteases degrade insect structural proteins, whereas chitinases target chitin in the insect exoskeleton and peritrophic membrane lining the midgut. The wet-lysed phenotype observed in larvae mortality assays strongly indicates tissue degradation consistent with enzymatic activity (Harrison & Bonning 2010; Zhao *et al.* 2019). Chitinolytic activity, in particular, has been associated with larval liquefaction and disintegration, which are hallmark signs observed in the treated *S. litura* larvae.

Biochemical Characteristics of Selected Bacterial Isolates

The biochemical characteristics of the two selected bacterial isolates, KP284 and K504a, were evaluated using a series of standard tests (Table 6). Both isolates were identified as gram-negative, catalase-positive, and oxidase-positive. They are obligate aerobes capable of growing under aerobic conditions. Neither of the isolates exhibited motility or urease activity. In terms of carbohydrate metabolism, both isolates demonstrated strong acid production when fermented with glucose, lactose, and sucrose. This indicates their ability to utilize various carbon sources. Regarding environmental tolerance, both KP284 and K504a showed growth at elevated temperatures (34°C and 37°C) and limited or no growth at lower (2°C) and sub-zero temperatures (-20°C). Their growth was optimal in acidic to neutral pH ranges (pH 5–7), with both isolates tolerating salinity levels of up to 5% NaCl. However, growth was reduced or absent at 9% salinity levels.

Moreover, carbohydrate fermentation capabilities, including the production of acids from glucose, lactose, and sucrose, suggest a capacity for diverse nutrient utilization, which may correlate with broader metabolic capabilities, including enzyme production. Acidic by-products of fermentation could also contribute to the weakening of insect gut structures, potentiating the action of proteases and chitinases (Yasika & Shivakumar 2025).

Urease activity differed between the two isolates, with KP284 exhibiting positive urease activity and K504a being urease-negative. Urease-positive bacteria can hydrolyze urea into ammonia, which may influence microbial survival and competitiveness under certain environmental conditions (Mora & Arioli 2014). However, urease activity is not directly associated with insecticidal efficacy, and its mere presence does not imply increased pathogenicity toward non-target organisms. Importantly, both isolates were confirmed to be non-pathogenic to plants and non-hemolytic, indicating that urease activity in KP284 did not compromise its biosafety profile.

Table 6 Biochemical test results of isolates

Characteristics	K504a (Soybean land)	KP284 (Long bean land)
Morphological characteristics		
Cell shape	Rod-shaped (Bacilli)	Rod-shaped (Bacilli)
Gram stain	Gram-negative	Gram-negative
Motility	–	–
Biochemical characteristics		
Catalase	+	+
Oxidase	+	+
Urease	–	+
Oxygen demand	Obligate aerobes	Obligate aerobes
Temperature resistance (°C)		
34	+	+
27	+	+
2	–	–
-20	–	–
Carbohydrate fermentation (acid production)		
Glucose	+	+
Lactose	+	+
Sucrose	+	+
pH resistance		
pH 3	–	–
pH 5	+	+
pH 6	++	++
pH 7	++	++
pH 9	–	–
Salinity tolerance (%)		
2	+	+
3	–	–
5	–	–
10	–	–

Note: - = No growth; + = Moderate growth; and ++ = Much growth.

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Further enzymatic assays and molecular detection of chitinase (*chiA and chiB*) and protease (*prtA and vpr*) genes are warranted to confirm this enzymatic activity and strengthen the case for their use as biocontrol agents. Importantly, the two most effective isolates were confirmed to be non-pathogenic to plants and did not exhibit hemolytic activity, ensuring a good biosafety profile for potential agricultural use. Biosafety evaluation is critical for selecting biocontrol agents to avoid unintended impacts on non-target organisms and ecosystems.

Viability of Bacteria in Carriers

The viability of the two selected bacterial isolates, KP284 and K504a, was evaluated in two types of liquid carrier media: (1) 5% molasses (Figure 1) and (2) a combination of 5% molasses with 10% rice washing water (Figure 2). Both carrier formulations were designed to assess their potential as low-cost organic media for maintaining bacterial survival over time. Viability tests revealed that both isolates survived and maintained growth in both carrier treatments over the incubation period. Isolate KP284 showed a consistent increase in cell density in the molasses-only carrier, reaching optimal growth after several days of incubation in the molasses-only carrier. K504a exhibited slightly enhanced growth in the combined carrier (molasses and rice washing water), indicating that the additional organic nutrients from rice washing water may have supported better bacterial proliferation. This strategy offers a cost-effective and environmentally friendly approach to inoculum delivery in the field. These carriers not only support bacterial growth but also enhance shelf life and ease of application, which are crucial factors for adoption by farmers. Organic carriers, such as molasses, have been reported to stabilize bacterial populations in tropical storage, thereby extending the shelf life of biofertilizers. Molasses-based media have been proven to support the growth of various beneficial bacteria, such as *Bacillus* and *Azotobacter*, which are essential for promoting plant growth (Chakraborty 2020; Hindersah *et al.* 2020).

Compared to previous studies focusing primarily on *B. thuringiensis*, this study introduces local isolates with comparable or potentially novel modes of action. This

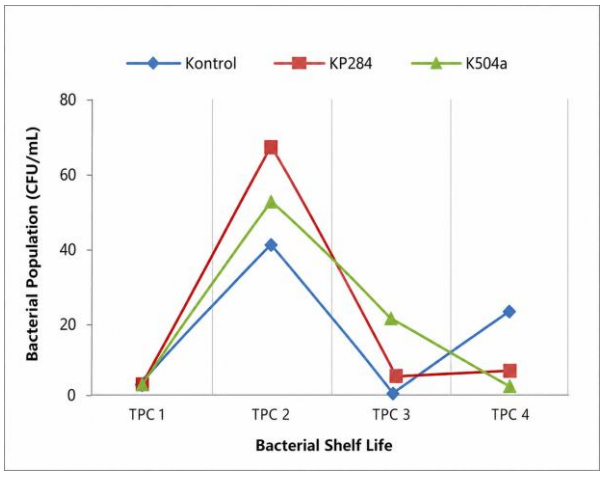

Figure 1 Viability of Anti Pest Bacteria on Carrier Molasses 5% + 95% Aquades.

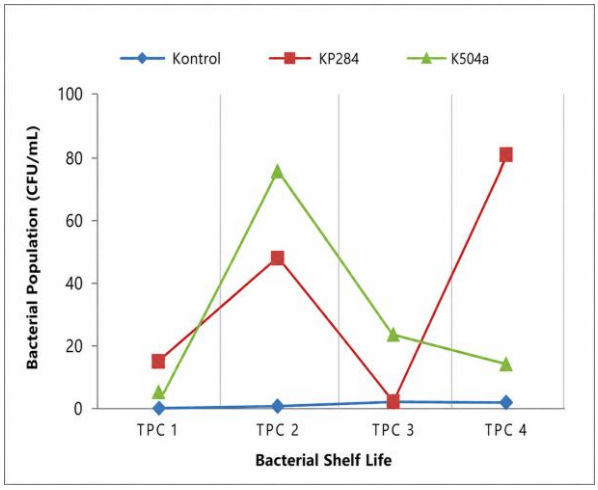

Figure 2 Viability of Anti Pest Bacteria on Carrier Molasses 5% + Rice Washing Water 10% + Aquades 85%.

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highlights the value of exploring indigenous microbial resources that may be better adapted to local environmental conditions and pest populations. Indigenous isolates offer the advantages of ecological compatibility and regulatory simplicity for local agricultural implementation (Grzywacz *et al.* 2014; Sabbahi *et al.* 2022). Future studies should focus on the molecular identification and genome-based characterization of KP284 and K504a to understand their modes of action. Field trials in different agroecological zones are also necessary to validate their efficacy and stability. This study provides a strong foundation for developing environmentally friendly, locally sourced biopesticides for integrated pest management (IPM) strategies. Integrated biopesticide development requires consideration of pathogen-host interactions, formulation compatibility, and deployment strategies (Behle & Birthisel 2023; Namdeo Aiwale *et al.* 2025; Sheoran *et al.* 2025).

CONCLUSION

Two native rhizobacterial strains of KP284 and K504a isolated from the acidic long bean and soybean rhizosphere soils, respectively, exhibit potent insecticidal activity against *Spodoptera litura*. They are non-pathogenic to plants and animals and lack hemolytic activity. Their ability to induce rapid larval death through a distinct wet-lysed phenotype. Moreover, their capacity to remain viable in simple, low-cost carriers such as molasses and rice washing water underscores their practicality for field-scale applications. The rhizobacteria can provide safe, effective, and locally adapted candidates for biopesticide development. These findings have potential application for eco-friendly solutions for pest management and strengthen the basis for integrating such isolates into sustainable agricultural practices in the future.

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