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Isolation and characterization of native *Bacillus thuringiensis* isolates from rice ecosystems of Bapatla district, Andhra Pradesh

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ABSTRACT

ustainable pest management involves the exploration of native microbial resources as eco-friendly alternatives to chemical pesticides. The present study investigated the occurrence of native *Bacillus thuringiensis* (*Bt*) in rice ecosystems of Bapatla district, Andhra Pradesh. Twenty soil samples collected from five rice-growing mandals were processed using the sodium acetate selective isolation technique. Preliminary characterization yielded fifteen Gram-positive isolates, of which fourteen showed colony characteristics comparable to the reference strain HD-1. Biochemical characterization confirmed eleven isolates as *B. thuringiensis*. The confirmed isolates were screened for pathogenicity against third instar larvae of *Spodoptera litura* by leaf-dip bioassay. Considerable variation in insecticidal activity was observed among the native isolates, with larval mortality ranging from **50.00 to 90.00 per cent.** Six lepidopteran-specific isolates (BV S , BV, S , BV, S, , BhV, S , CV, S, and VeV, S,) recorded superior pathogenicity. The study demonstrated the occurrence of diverse native *B. thuringiensis* populations in rice ecosystems and identified six isolates exhibiting specific activity against lepidopteran larvae, indicating their potential for biological pest management.

Keywords: : *Bacillus thuringiensis,* Leaf-dip bioassay, Native isolates, Rice ecosystems and *Spodoptera litura*

Bacillus thuringiensis Berliner (*Bt*) is a Gram-positive, spore-forming bacterium widely used as a microbial biocontrol agent against economically important insect pests. Its insecticidal activity is attributed to the production of parasporal crystal (Cry) proteins during sporulation, making *Bt* an important component of eco-friendly pest management. Native *Bt* populations occurring in agricultural soils exhibit considerable diversity in their morphological, biochemical and insecticidal characteristics. Isolation and characterization of indigenous *Bt* isolates are important for identifying promising strains with potential for biological control and future biopesticide development.

Information on the occurrence of native *Bt* isolates in the rice ecosystems of Bapatla district, Andhra Pradesh, is limited. Therefore, the present investigation was undertaken to isolate and identify native *B. thuringiensis* from rice ecosystems through morphological and biochemical characterization, followed by pathogenicity screening against *S. litura* to identify promising lepidopteran-specific isolates.

MATERIAL AND METHODS

Soil Sample Collection

The study was conducted during 2024–2026 at the Department of Entomology, Agricultural College, Bapatla, Andhra Pradesh. Twenty soil samples were collected during the pre-sowing period from ten villages representing five rice-growing mandals of Bapatla district, viz., Bapatla, Chirala, Repalle, Vemuru and Bhattiprolu (Table 1). Soil samples were collected from a depth of 10–15 cm following the quadrat method, packed in sterile polyethylene bags and stored at 4°C until further processing (Plate 1).

Isolation of *B. thuringiensis*

Native *B. thuringiensis* isolates were recovered using the sodium acetate selective isolation method described by Travers *et al.* (1987). One gram

of soil was inoculated into Luria broth supplemented with 0.25 M sodium acetate (pH 6.8) and incubated at 28°C on a rotary shaker (250 rpm) for 4 h. The culture was subjected to heat shock at 80°C for 3 min, serially diluted and plated on T3 medium (Plate 2). Plates were incubated at **35 ± 2°C** overnight, and colonies exhibiting a characteristic cream-coloured "fried egg" appearance comparable to the reference strain (*B. thuringiensis* subsp. *kurstaki* , HD-1) were selected (Plate 3).

Gram Staining, Morphological and Biochemical Characterization

The recovered bacterial isolates were subjected to Gram staining following the method of Cappuccino and Sherman (1992), and Gram-positive isolates were selected for further characterization (Plate 4). These isolates were examined for colony colour, shape, elevation, margin and the characteristic "fried egg" appearance, and compared with the reference strain, *B. thuringiensis* subsp. *kurstaki* (HD-1). Isolates showing morphological similarity to the reference strain were further confirmed by biochemical characterization using the RAPID HiBacillus™ Identification Kit (KB013). Identification was based on sugar fermentation patterns, substrate utilization and associated metabolic reactions, and the isolates were confirmed as *B. thuringiensis* based on the overall biochemical reaction profile.

Pathogenicity Screening

The biochemically confirmed isolates were evaluated against third instar larvae of *S. litura* using the leaf-dip bioassay described by Shelton *et al.* (1993). Castor leaf discs (9 cm diameter) were dipped in *Bt* suspensions of different concentrations (1 × 10x to 1 × 10^{12} CFU. mL^{-1}) containing 0.1% Tween 80 for 5 min and air dried (Plate 5). Twenty larvae were released per treatment with three replications, while distilled water-treated leaf discs served as the untreated control. Larval mortality was recorded at 24 h intervals up to seven days, and moribund larvae were considered dead. Based on pathogenicity, six highly virulent lepidopteran-specific isolates were selected.

Statistical analysis

The experimental data was analysed following a Completely Randomized Design (CRD). Percentage

Table 1. Details of soil samples collected from Bapatla district, AP

S.No.	Name of Mandal	Name of Village	Location Geo-coordinates	Total samples
1	Bapatla	Bapatla(V_1)	15.91906° N, 80.47781° E	2
		Appikatla(V_2)	15.96689° N, 80.50258° E	2
2	Repalle	Repalle(V_1)	16.02513° N, 80.84998° E	2
		Penumudi(V_2)	16.04031° N, 80.86186° E	2
3	Vemuru	Vemuru(V_1)	16.15999° N, 80.73678° E	2
		Jampani(V_2)	16.18564° N, 80.69615° E	2
4	Bhattiprolu	Oleru(V_1)	16.05411° N, 80.86326° E	2
		Pallekona(V_2)	16.04553° N, 80.81771° E	2
5	Chirala	Gavinivaripalem (V_1)	15.84794° N, 80.41714° E	2
		Thotavaripalem(V_2)	15.84032° N, 80.39460° E	2
	Total			20

V1- Village 1 V2 - Village 2



Plate 1. Soil samples collected from different lo cations



Plate 2. Shaker incubation of *B. thuringiensis* from soil samples

mortality data were subjected to appropriate transformation before analysis of variance (ANOVA), and statistical analyses were performed using R software (Version 4.5.3).

RESULTS AND DISCUSSION

Twenty bacterial isolates were recovered from 20 soil samples collected from rice ecosystems of five mandals of Bapatla district using the sodium acetate selective isolation technique. Following incubation on T3 medium, colonies showing creamy white colour with smooth surface and characteristic "fried egg" appearance were selected as presumptive *B. thuringiensis* isolates. The occurrence of *B. thuringiensis* in agricultural soils has been well documented by Delucca *et al.* (1981) and Kaur *et*

Table 2. Gram staining of native isolates of *B. thuringiensis*

S.No.	Mandal	Gram Positive Isolates	No. of Isolates
1	Bapatla	BV_1S_1, BV_1S_2, BV_2S_1, BV_2S_2.	4
2	Repalle	RV_2S_1.	1
3	Vemuru	VeV_1S_1, VeV_1S_2, VeV_2S_2.	3
4	Bhattiprolu	BhV_1S_1,BhV_1S_2, BhV_2S_1.	3
5	Chirala	CV_1S_1, CV_1S_2, CV_2S_1, CV_2S_2.	4
Total			15

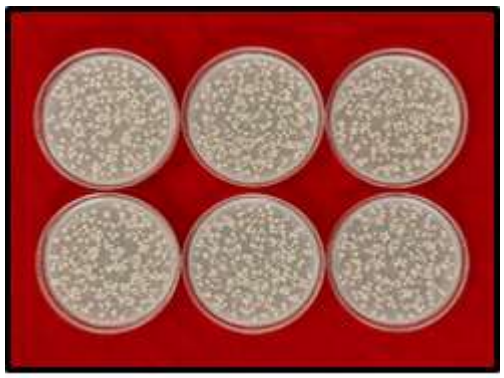


Plate 4. Isolation of *Bt* bacterial cultures (Fried egg colonies)



Plate 5. Gram staining of *Bt* isolates

al. (2006). Similarly, Devi *et al.* (2022) recovered 15 *Bt* isolates from crucifer-growing soils of Andhra Pradesh, while Nagaraju *et al.* (2023) and Gemmeda *et al.* (2023) also reported the widespread occurrence and diversity of native *Bt* isolates in different agroecosystems. The recovery of *Bt* isolates from all the surveyed mandals in the present investigation further confirms the abundance of native *B. thuringiensis* populations in rice-growing soils.

Gram staining revealed that fifteen of the twenty isolates were Gram-positive and appeared as purple-stained rod-shaped bacterial cells. The highest number of Gram-positive isolates was recorded from Bapatla and Chirala mandals (4 isolates each), followed by Bhattiprolu and Vemuru (3 isolates each), whereas Repalle recorded only one Gram-positive isolate. Similar Gram-positive rod-shaped morphology has been reported by Hassan *et al.* (2021), Devi *et al.* (2022) and Ganga *et al.* (2023), confirming Gram reaction as an important preliminary criterion for the identification of *B. thuringiensis*.

Among the fifteen Gram-positive isolates, fourteen recorded colony characteristics comparable to the reference strain HD-1 and were provisionally identified as *B. thuringiensis*. Most isolates produced creamy white colonies with characteristic "fried egg" morphology, although slight variations were observed in colony colour, shape, elevation and margin. Similar



Plate 6. Release of *S. litura* larvae



Plate 7. Mortality of *S.litura* larvae

Table 3. Per cent mortality of third instar larvae of *S. litura* with native isolates of *B. thuringiensis.*

*Concentration/Isolate	BV_1S_1	BV_2S_1	BV_2S_2	BhV_2S_1	CV_2S_2	VeV_2S_2	HD-1
1x 10^8 CFU. mL^{-1}	51.67-45.96 no	46.67 (43.09) pqr	61.67 (51.54) k	48.33 (44.04) opq	31.67-34.23 u	26.67 (31.07) v	56.67 (48.84) lm
1x 10^9 CFU. mL^{-1}	61.33 (51.54) k	56.67 (48.84) lm	71.67 (57.86) hi	55.00 (47.87) mn	43.33-41.16 rs	33.33 (35.25) u	68.33 (55.77) ij
1x 10^{10} CFU. mL^{-1}	71.67-57.86 hi	66.33 (54.53) j	82(64.89) de	66.33-54.53 j	45-42.13 qrs	38.33 (38.24) t	78.33 (62.29) ef
1x 10^{11} CFU. mL^{-1}	76.67-61.14 fg	71.67 (57.86) hi	86.67 (68.66) c	73.33 (58.93) gh	51.67-45.96 no	41.67 (40.20) st	85.00 (67.21) cd
1x 10^{12} CFU. mL^{-1}	82.00 (64.89) de	78.33 (62.29) ef	90.00 (71.57) b	76.67 (61.14) fg	60-50.77 kl	50.00 (45.00) op	93.33 (75.24) a
Control	0.00%						
	Isolates		Concentration		Isolate * Concentration		
SEm±	1.002		1.002		1.002		
CD (P=0.05)	2.826		2.826		2.82		
CV (%)	3.296						

morphological variability among native *Bt* isolates was reported by Mukhija and Khanna (2018), Karuppaiyan *et al.* (2022) and Gothandaraman *et al.* (2025), indicating the occurrence of diverse native *Bt* populations in agricultural soils.

Biochemical characterization confirmed eleven of the fourteen morphologically identified isolates as *B. thuringiensis*, whereas three isolates differed in one or more biochemical reactions and were excluded from further studies. The confirmed isolates were subsequently evaluated for their insecticidal activity against *S. litura*.

Pathogenicity screening of the eleven *Bt* isolates revealed considerable variation in insecticidal activity. Larval mortality increased with increase in concentration ($1 \times 10x$ to 1×10^{12} CFU. mL^{-1}) and ranged from 50.00 to 90.00 per cent at 1×10^{12} CFU. mL^{-1} (Table 3). The reference strain HD-1 recorded the highest larval mortality (93.33%), while among the native isolates, BV, S, recorded the highest mortality (90.00%), followed by BV S (82.00%), BV, S (78.33%) and BhV, S (76.67%). CV, S, (60.00%) and VeV, S, (50.00%) recorded comparatively lower mortality (Plate 6). Larvae treated with *Bt* suspensions showed reduced feeding, sluggish movement, cessation of feeding and gradual darkening before death, which are characteristic symptoms of *Bt* infection. Similar variability in pathogenicity among native *Bt* isolates has been reported by Reyaz *et al.* (2017), Harika (2020) and Gemmeda *et al.* (2023). Based on pathogenicity, six effective strains against *S.litura* namely BV S , BV, S , BV, S, , BhV, S , CV, S, and VeV, S, were identified. The results indicated considerable variation in the pathogenicity of native *B. thuringiensis* isolates against *S. litura*, reflecting the diversity of native *Bt* populations in rice ecosystems.

CONCLUSION

The present study confirmed the occurrence and diversity of native *Bacillus thuringiensis* isolates in the rice ecosystems of Bapatla district. Among the confirmed isolates, BV, S, , BV S , BV, S , BhV, S , CV, S, and VeV, S, showed promising pathogenicity against *Spodoptera litura*. These native isolates have potential for further evaluation and development as eco-friendly biocontrol agents for lepidopteran pest management.

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