pISSN: 0259-9996 – eISSN: 2783-3968

Journal of Entomological Society of Iran

2025, 45 (1), 121–138

Research Article

Biochemical, ecological and molecular characterization of *Xenorhabdus anantnagensis* associated with *Steinernema anantnagense* from India: evaluating nematode efficacy against *Helicoverpa armigera*

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Abstract. This study provides a molecular and phenotypic characterization of an entomopathogenic nematode-bacterium complex isolated from agricultural soil and nematode efficacy against *Helicoverpa armigera*. The nematode, identified as *Steinernema anantnagense* KP_CU, was characterized using the internal transcribed spacer (ITS) region of rRNA, revealing 100% similarity with the type population of *S. anantnagense*. Phylogenetic analysis confirmed its conspecific status within a clade including *S. kushidai, S. akhursti,* and *S. populi*. Concurrently, the associated bacterium, identified as *Xenorhabdus* sp. KP_CU, exhibited 100% similarity in its 16S rRNA sequence with *Xenorhabdus anantnagensis* XENO-2^T, suggesting conspecificity. Phenotypic characterization aligned the bacterium closely with *X. anantnagensis*, highlighting typical traits such as rod-shaped, gram-negative cells and absence of bioluminescence. Biochemical tests further supported this identification, distinguishing KP_CU from other *Xenorhabdus* species based on citrate utilization, gelatinase, lysine decarboxylase, urease, arginine dihydrolase, ornithine decarboxylase, glucose oxidation, cytochrome oxidase and indole production. Phylogenetic analysis based on 16S rRNA sequences placed *Xenorhabdus* sp. KP_CU within a monophyletic clade with *X. anantnagensis*, along with sister relationships to *X. japonica* and *X. vietnamensis*. The bacterial strains also exhibited larvicidal activity against *Galleria mellonella* and even at the lowest optical density (OD_{590} = 0.125) induced over 80% larval mortality within merely 24 h post-injection, emphasizing its elevated virulence. The strain KP_CU could kill the wax moth larvae with 38, 16 and 9 IJs at 24, 36 and 48 h, respectively. The nematode isolate KP_CU demonstrated high virulence against *H. armigera* larvae, with complete mortality achieved within 60 h across all tested inoculum levels. Mortality began at 36 h post-inoculation at 100 IJs/larva and was reached within 24 h at 200 IJs/larva. LD50 values decreased significantly from 38 IJs at 24 h to just 9 IJs at 48 h, indicating potent lethality. Additionally, progeny production showed a dose-dependent increase, though slightly reduced at higher doses, suggesting a trade-off between virulence and reproductive success. These results suggest that *S. anantnagense* KP_CU could hold potential as a biocontrol agent for *H. armigera* in agricultural settings in India.

Keywords: Entomopathogenic nematology, Insect pathology, Biocontrol, Phylogenetic analysis, 16S rRNA.

Article history
Received: 29 June 2024
Accepted: 13 September
Published: 01 January 2025

Subject Editor: Javad Karimi

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DOI: https://doi.org/10.61186/jesi.45.1.10

Introduction

Entomopathogenic nematodes (EPNs) of the genus *Steinernema* are globally important soil-dwelling insect pathogens, thriving in diverse soil habitats (Bhat *et al.*, 2020; Jaffuel *et al.*, 2018). These nematodes form symbiotic

relationships with bacteria of genus *Xenorhabdus,* residing in their alimentary canals-(Machado *et al.*, 2023). Together, they can kill insect pests within 24 to 48 h, making them highly relevant for biological pest control (Janardhan *et al.*, 2023). The entomopathogenic bacteria (EPB) reside in the intestines of the infective juvenile (IJ) stages of these EPNs. Upon entering an insect host, the IJs release into the insect's hemocoel (Askary *et al.*, 2022). The EPB depend on their nematode partners for transmission between hosts and protection from external environments (Bhat *et al.*, 2019; Machado *et al.*, 2024). While a single bacterial species can be associated with multiple EPN species, each EPN species typically harbors a specific bacterial species (Gulcu *et al.*, 2017).

Steinernema IJs typically enter the insect host through natural openings, and after penetration, regurgitate their associated bacteria (Bhat *et al.*, 2019; Machado *et al.*, 2022). The bacteria proliferate in the insect hemocoel, producing a range of toxins, secondary metabolites, hydrolytic enzymes (such as lipases, phospholipases, and proteases), and broad-spectrum antibiotics, leading to host death via septicemia (Machado *et al.*, 2023; Rana *et al.*, 2020). Additionally, these bacterial symbionts produce antimicrobial compounds that restrict or stop the growth of competing microbes within the host, creating conducive environment for nematode reproduction and development (Heena *et al.*, 2021). EPNs and their symbiotic bacteria, are considered safe biocontrol agents, posing no threats to humans, other mammals, vertebrates, or plants (Bhat *et al.*, 2019; Drema *et al.*, 2024). Their specificity to arthropods presents lower environmental risk compared to chemical plant protection agents (Akhurst & Smith, 2002). The biochemical capabilities of *Xenorhabdus* bacteria are particularly notable, enhancing their relevance in agriculture, biotechnology, and medicine (Bhat *et al.*, 2017; Machado *et al.*, 2023).

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae), commonly referred to as the cotton bollworm, is among the most destructive pests affecting global agriculture, inflicting significant damage on various economically critical crops (Rana *et al.*, 2021; Riaz *et al.*, 2021). This highly polyphagous species has an extensive geographic range, spanning Asia, Africa, Europe, and Australia. It is particularly notorious for attacking key crops, including cotton (*Gossypium hirsutum*), chickpea (*Cicer arietinum*), pigeon pea (*Cajanus cajan*), tomato (*Solanum lycopersicum*), and maize (*Zea mays*) (Bhat *et al.*, 2019; Riaz *et al.*, 2021). In India, infestations of *H. armigera* have caused severe yield losses in vegetable crops, resulting in annual economic damages reaching millions of dollars (Askary *et al.*, 2022). Historically, the control of *H. armigera* has predominantly depended on chemical insecticides, such as pyrethroids, organophosphates, and carbamates. However, the indiscriminate and excessive use of these pesticides has led to resistance development within the pest populations, rendering these chemical agents increasingly ineffective (Bhat *et al.*, 2020; Machado *et al.*, 2021). Furthermore, reduced susceptibility to transgenic Bt cotton has exacerbated its pest status, complicating control measures. In response to these challenges, alternative approaches, particularly using indigenous biological control agents like entomopathogenic nematodes, have emerged as sustainable, eco-friendly strategies for managing this tenacious pest (Loulou *et al.*, 2023).

This study focuses on the phenotypic, biochemical, ecological and molecular characterization of *Xenorhabdus* species isolated from *Steinernema* nematodes found in Indian agricultural soils. The isolated nematodes were also molecularly characterized. This partnership is particularly noteworthy due to the unique ecological niche and adaptive traits exhibited by these organisms. Finally, the isolated nematodes were tested in laboratory bioassays for pathogenicity and reproductive potential against *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae).

Materials and methods

Nematode isolation

Soil samples were collected from different agricultural fields in the Anantnag district of Jammu and Kashmir, India. The isolation of EPNs from these soil samples was performed using the soil baiting method (Bedding & Akhurst, 1975) with *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) as bait. The cadavers were rinsed with double distilled water (ddH_2O) and disinfected with 0.1% NaOCl (Rana *et al.*, 2020; Suman *et al.*, 2020). The IJs of the EPNs were recovered from the insect cadavers using the White Trap method (White, 1927) and labelled as KP_CU. The IJs were then sterilized with 0.1% NaOCl and stored in tissue culture flasks at 15°C until further use.

Molecular characterization of EPNs

The isolated EPNs were molecularly characterized using internal transcribed spacer (ITS) (ITS 1, 5.8S, ITS 2) rRNA markers. Genomic DNA was isolated from IJs using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden,

Germany). Initially, the IJs were thoroughly rinsed with Ringer's solution, followed by placement on sterile glass slides containing small aliquots of AE buffer (2 µL). The nematodes were then cut into fragments, which were transferred to sterile 0.5 mL Eppendorf tubes containing 17 µL of AE buffer and 2 µL of proteinase K. DNA extraction was performed following the manufacturer's instructions. Amplification of ITS rRNA genes was performed via polymerase chain reaction (PCR) using the primers 18S: 5′-TTGATTACGTCCCTGCCCTTT-3′ (forward) and 26S: 5′-TTTCACTCGCCGTTACTAAGG-3′ (reverse) (Vrain *et al*. 1992). The PCR mixture comprised 16.8 µL ddH2O, 2.5 µL 10X PCR buffer, 0.5 µL dNTP mix (10 mM), 1 µL of each primer, 0.2 µL DreamTaq Green DNA polymerase, and 3 µL of the DNA template (Leonar *et al.*, 2022). The PCR conditions involved initial denaturation at 95°C for 3 minutes, followed by 40 cycles of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 1 minute, with a final extension at 72°C for 15 minutes. The PCR products (5 µL) were separated by electrophoresis on a 1% TAE (Tris-acetic acid-EDTA) agarose gel stained with ethidium bromide (HiMedia, India) (Bhat *et al.*, 2023; Sebumpan *et al.*, 2022; Yadav *et al.*, 2022).The amplified products were then Sanger sequenced using the ABI 3730 (48 capillary) electrophoresis instrument by Bioserve Ltd. (Hyderabad, India), and the assembled sequence was submitted to GenBank.

Isolation and molecular characterization of bacterial strain

Symbiotic bacterial strain associated with the IJs of isolate KP_CU were isolated from the hemolymph of the *G. mellonella* infected with these EPNs. The infected larvae of *G. mellonella* were dissected using a sterile blade, and the haemolymph was streaked on fresh LB agar plates (Machado *et al.*, 2023). The agar plates were properly sealed with parafilm and kept in a BOD incubator for 24-48 h at 25-28°C. Colonies resembling *Xenorhabdus* were further streaked onto fresh LB agar to obtain pure cultures (Akhurst, 1980). Single bacterial colonies were isolated by transferring individual colonies onto new LB agar plates (Machado *et al.*, 2023). Colony morphology, texture, pigmentation, and production were assessed to identify bacterial primary forms. The isolates were sub-cultured and maintained on LB agar plates at 28°C (Loulou *et al.*, 2023).

The isolated bacterium was molecularly characterized using 16S rRNA studies. For this, genomic DNA was extracted from two- to three-day-old bacterial cultures using the GenElute Bacterial Genomic DNA Kit (Sigma–Aldrich, Switzerland), following the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGWTCCARCC-3′) (Sandström *et al.*, 2001). The PCR profiles used were: 1 cycle at 94℃ for 4 min; followed by 36 cycles at 94℃ for 30 s; 55℃ for 60 s, and 72℃ for 45 s; and a final extension at 72℃ for 10 min (Loulou *et al.*, 2022). The PCR products were separated by electrophoresis in a 1% TAE (Tris-acetic acid-EDTA) agarose gel stained with ethidium bromide, and the amplified PCR products were Sanger sequenced. The obtained sequences were assembled and submitted to GenBank.

Phenotypic and biochemical characterization of the bacteria

Phenotypic and biochemical tests were also performed to characterize the symbiotic bacterium associated with the isolated EPN. The isolated bacteria were cultured on sterile plates of nutrient bromothymol blue-triphenyltetrazolium chloride agar (NBTA) (containing of 25 mg/L of bromothymol blue, 4 mg/L of 2,3,5-triphenyltetrazolium chloride, and 20 g/L of nutrient agar) and MacConkey agar for 24-48 h to record their phenotypic characteristics by observing their adsorption properties towards bromothymol blue (BTB) and neutral red, respectively (Akhurst, 1980). Gram staining was performed using the following protocol: 1 min with Crystal Violet, 1 min with Iodine mordant, 30 sec with 95% ethanol, and 1 min with Safranin O. Optimal growth temperatures were determined by incubating the bacteria at 20°C, 24°C, 28°C, 30°C, 37°C, and 42°C. To assess salt tolerance, bacterial growth was evaluated in saline conditions at NaCl concentrations of 1% (standard LB medium), 2%, and 3%. The pH tolerance range was also investigated using LB medium adjusted to pH 3, 5, 7 (control), 8, and 9. All growth assays were performed in 15 mL conical centrifuge tubes containing 5 mL of LB broth inoculated with 0.1 mL of overnight bacterial cultures. These were incubated at 28°C with shaking at 180 rpm for 24 h, following the protocol of (Machado *et al.*, 2021).

Antibiotic susceptibility was tested on LB agar supplemented with 30 mg/L of tetracycline, vancomycin, or gentamicin. Bacterial suspensions were calibrated to a 0.5 McFarland standard using a DEN-1B McFarland densitometer (Biosan, Riga, Latvia) in 0.85% NaCl. A 100 µL aliquot of each calibrated suspension was plated

onto LB agar enriched with the respective antibiotics and incubated at 28°C for 24 h. All experiments were conducted in triplicate for reproducibility.

The isolated bacteria were further characterized by biochemical tests using the Hi-Media kit, KB003 Hi25 Enterobacteriaceae Identification Kit. This Kit is mainly prepared for the characterisation of Gram-negative Enterobacteriaceae species and was kept at 2-8°C. The kit comprises 24 biochemical tests, which include 13 conventional biochemical tests and 11 carbohydrates utilization tests. For biochemical tests, phase I bacteria cultured on NBTA media were transferred to 5000 µL heart infusion broth (Hi-media). The 24 wells of the kit were filled with 0.05 mL of aliquots from each bacterial culture that had been grown overnight at a temperature of 35-37°C. Fifty microliter aliquots from the overnight cultured bacteria were added to each of the 24 wells of the kit. Independent oxidase and catalase tests were conducted on 16-hour LB-bacterial cultures using oxidase reagent discs and 3% hydrogen peroxide (H2O2), respectively. The oxidase test was performed by rubbing a bacterial colony onto the provided oxidase disc, while the catalase test was executed via the slide method with H_2O_2. Results were recorded as positive (+) or negative (–) based on the observed color changes in the media, in accordance with the manufacturer's instructions. All biochemical tests were performed in duplicate to ensure accuracy.

Sequence alignment and phylogenetic analyses

The ITS rRNA sequence of the nematodes and the 16S rRNA sequences of the symbiotic bacteria were edited using BioEdit (Hall, 1999). These sequences were then compared with those in GenBank using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990) of the National Centre for Biotechnology Information (NCBI). Alignments of the nematodes and bacterial sequences, along with related sequences from *Steinernema* nematodes and *Xenorhabdus* species, were performed for the ITS and 16S rRNA regions using default MUSCLE parameters (Edgar, 2004) in MEGA 11 (Tamura *et al.*, 2021). *Steinernema glasseri* (Steiner, 1929) Wouts, Mracek, Gerdin & Bedding, 1982 (Rhabditida, Steinernematidae) and *Photorhabdus stackebrandtii* (An & Grewal, 2010) Machado et al., 2018 (γ-Proteobacteria: Enterobacteriaceae) were used as outgroup taxa for the EPNs and their EPB, respectively. Pairwise distances were calculated using MEGA 11 (Tamura *et al.*, 2021). The distances were computed using the Maximum Composite Likelihood (MCL) and number of differences in Mega 11. Phylogenetic trees of the ITS and 16S rRNA regions were constructed using the Maximum Likelihood method (Felsenstein, 1981), based on the Hasegawa-Kishino-Yano and Tamura-Nei parameter models, respectively, in MEGA 11 (Tamura *et al.*, 2021). Neighbour-Join and BioNJ methods were automatically applied to obtain the initial tree(s). The phylogenetic trees were edited using Interactive Tree of Life (iTOL v6) (Letunic & Bork, 2024).

Ecological characterization

Entomopathogenic properties of isolated *Xenorhabdus* species were carried out following the methodology of Machado *et al.*, (2024). To assess the insecticidal potential of a novel *Xenorhabdus* strain, overnight bacterial cultures were grown in Luria-Bertani (LB) broth. Following incubation, bacterial suspensions were harvested and their optical density (OD) at 590 nm (OD590) was measured using a Shimadzu spectrophotometer (Shimadzu, Kyoto, Japan). Cultures were standardized to an OD590 of 1.0, followed by serial dilutions to achieve final concentrations corresponding to OD590 values of 0.125, 0.25, 0.50, and 1.0. Subsequently, 0.01 mL aliquots of each bacterial suspension were injected into third-instar larvae of *G. mellonella*, with 20 larvae per dilution and strain (n=20). Larval mortality was evaluated every 24 hours for 3 days post-injection. Wax worms injected with LB medium and 0.85% NaCl-injected wax worms, as well as non-injected wax worms, served as negative controls. Repeated-measures ANOVA were applied to the data, with bacterial strain and time post-injection as independent variables. Assumptions of normality and homoscedasticity were confirmed via Shapiro–Wilk and Levene's tests, respectively. Multiple comparisons were performed using the Holm–Sidak post hoc method.

Virulence and efficacy of *Steinernema anantnagense* against *Helicoverpa armigera*

The pathogenic potential and bioefficacy of *Steinernema anantnagense* KP_CU were evaluated against the larvae of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). Bioassays were conducted using six-well plates, each measuring 3.5 cm in diameter, lined with double layers of Whatman No. 1 filter paper. Infective juveniles (IJs), aged one week, were employed as inoculants, following the protocol established by Bhat *et al.* (2019). Four graded concentrations of IJs—25, 50, 100, and 200 IJs—suspended in 450 µL of dH_2O were administered to the filter

paper, with untreated larvae serving as the negative control. Ten larvae, uniform in both size and weight, were exposed to each concentration, with one larva placed per well. The experimental setup was incubated at 28 ± 2 °C, and larval mortality was monitored at 12 h intervals until 100% mortality was achieved. To assess progeny production, larvae treated with different concentrations (25, 50, 100, and 200 IJs) were transferred after 7 days to a modified White trap (White, 1927) to determine infection persistence and monitor the emergence of IJs over an 18–20 day period. Mortality data were subjected to probit analysis to calculate the median lethal dose (LD50) with a 95% confidence interval. Statistical differences in mortality rates across the various IJ concentrations were analyzed using analysis of variance (ANOVA), and the outcomes were presented as mean percentages ± standard deviation (SD).

Statistical analysis

All statistical analyses including ANOVA, Shapiro–Wilk and Levene's tests, Multiple comparisons using the Holm–Sidak post hoc method and probit analysis were performed in GraphPad Prism 10.2.3 and IBM SPSS Statistics.

Results

Characterization of isolated nematode

The complete ITS rRNA sequences flanked with partial 18S and 28S rRNA sequences were submitted to NCBI under accession number PP882780. The ITS rRNA region of KP_CU. is 771 bp in length, with ITS1 comprising 274 bp, 5.8S comprising 157 bp, and ITS2 comprising 299 bp. BLASTn analysis showed that the isolate KP_CU exhibited 100% similarity with ITS rRNA sequences of *Steinernema anantnagense* (Bhat *et al*., 2023). Pairwise alignment of the ITS rRNA sequence of the isolate KP_CU revealed no nucleotide differences with the type population; hence they are considered conspecific. The percentage similarity and total character difference with type population and other closely related species of the "*feltiae* group" are shown in Table 1. It shows differences of 21-120 bp and sequence similarity values of 80-97%.

The construction of a phylogenetic tree based on the ITS rRNA gene sequences confirmed that the present nematode population is consspecific with *S. anantnagense,* and a formed a monophyletic clade with it. Together, they formed a sister clade with *S. kushidai* (Mamiya, 1988), *S. akhursti* (Qiu *et al*., 2005) and *S. populi* (Tian *et al*., 2022) (Rhabditida, Steinernematidae) (Fig. 1). The ITS rRNA genes sequence similarity between isolate KP_CU and type population *S. anantnagense* is 100%, indicating they are highly similar (Table 1). The phylogenetic tree also shows other sequences with lower scores when the sequence of validly published *Steinernema* species is compared with that of isolate KP_CU.

Phenotypic and biochemical characterization of bacteria

Bacterial colonies were grown on NBTA and MacConkey agar plates for phenotypic characterization. After 24 h of incubation, the bacterial colonies on NBTA plates exhibited colors ranging from green to brownish green. For neutral red adsorption analysis, bacterial cultures were incubated on MacConkey agar for 24 to 48 h. Phase I *Xenorhabdus* species formed reddish-brown colonies with significant neutral red adsorption, whereas Phase II colonies appeared pale yellow or off-white. Microscopic analysis revealed that the bacteria were rod-shaped, Gram-negative, and exhibited violet staining. No bioluminescence was observed under ultraviolet transillumination. The observed phenotypic traits and colony morphology suggest the bacterium is a member of the *Xenorhabdus* genus.

Additionally, the bacteria demonstrated growth across a temperature range of 20°C to 37°C, but cannot grow at 42°C. The strain also thrived in media containing 1%, 2%, and 3% NaCl and remained viable at pH levels 3, 5, 7, 8, and 9. Antibiotic susceptibility testing revealed sensitivity to tetracycline and gentamicin, but resistance to vancomycin.

The results of the biochemical tests indicate that the bacteria exhibit characteristics similar to *Xenorhabdus* entomopathogenic bacteria. These biochemical characteristics provide a comprehensive profile of the bacterial isolates, aligning them closely with the known properties of *X. anantnagensis* sp. XENO-2^{T} (Table 2). This detailed profiling is crucial for accurate identification and understanding of the bacterial species.

Table 1. Pairwise nucleotide similarities (%) of ITS rRNA gene sequences of *Steinernema* strains and *Steinernema anantnagense* KP_CU. Data for present species is in bold.

	ITS rRNA		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	***S. anantnagense***	1	**0**	**22**	**56**	**71**	**78**	**79**	**79**	**81**	**81**	**90**	**92**	**92**	**95**	**96**	**99**	**100**	**100**	**102**	**106**	**111**	**120**
2	*S. anantnagense*	100		22	56	71	78	79	79	81	81	90	92	92	95	96	99	100	100	102	106	111	120
3	*S. akhursti*	97	97		54	76	70	82	81	85	84	90	96	96	100	101	104	104	106	106	109	112	123
4	*\S kushidai*	93	93	93		95	99	95	102	98	96	112	108	111	112	111	117	114	116	116	121	129	129
5	*S. sangi*	89	89	88	85		103	69	72	61	57	71	69	75	73	71	82	71	81	75	85	75	109
6	*S. populi*	90	90	91	87	84		104	102	106	104	108	111	113	116	120	115	116	120	125	118	131	142
7	*S. xueshanense*	88	88	88	85	89	84		59	19	40	55	47	54	56	52	64	52	57	59	64	58	93
8	*S. jollieti*	88	88	87	84	89	83	91		48	57	62	55	45	45	43	56	63	65	44	60	47	79
9	*S. cholashanense*	89	89	89	87	91	86	97	93		32	40	31	46	49	47	52	39	45	56	54	52	92
10	*S. oregonense*	89	89	89	87	91	86	94	91	96		40	41	48	51	53	58	45	52	57	59	54	83
11	*S. tielingense*	87	87	87	83	89	84	92	91	94	94		36	60	69	61	63	40	51	68	64	61	94
12	*S. xinbinense*	88	88	87	86	89	85	93	92	96	95	95		49	58	54	58	30	36	59	58	51	91
13	*S. weiseri*	85	85	85	82	88	81	92	93	93	93	91	93		31	20	36	59	67	28	39	29	76
14	*S. ichnusae*	86	86	85	83	89	82	92	93	93	93	90	92	95		25	46	64	68	36	48	25	80
15	*S. africanum*	87	87	86	85	89	84	92	94	94	93	91	93	97	97		46	65	68	30	49	33	78
16	*S. citrae*	87	87	86	84	87	84	91	91	93	93	91	93	95	94	94		62	67	42	20	40	84
17	*S. kraussei*	87	87	86	85	89	84	93	90	95	94	94	96	91	91	92	92		35	70	64	62	95
18	*S. silvaticum*	87	87	86	84	87	84	92	90	94	94	93	96	90	90	91	91	96		73	68	68	100
19	*S. littorale*	86	86	86	84	88	83	91	93	93	93	90	92	96	95	96	95	91	91		45	35	72
20	*S. nguyeni*	86	86	85	83	86	84	91	91	93	92	91	93	94	93	94	98	92	91	94		41	81
21	*S. feltiae*	85	85	85	82	88	82	91	93	93	93	91	94	96	96	96	95	92	91	96	95		75
22	*S. hebeiense*	80	80	79	78	82	75	85	88	86	87	85	86	88	88	88	87	85	84	89	87	88	

Below diagonal: percentage similarity; above diagonal: total character differences.

Molecular and phylogenetic characterization of bacteria

Blastn analysis of the 16S rRNA sequence of *Xenorhabdus* sp. KP_CU showed 100% similarity with *X. anantnagensis* XENO-2^{T}, followed by 99.85% similarity with *X. japonica* strain BKP-4 and 99.03% with *X. vietnamensis* strain VN01, indicating they are closely related. The pairwise alignment confirmed that there was zero total character difference and 100% similarity in nucleotide sequences between the present strain *Xenorhabdus* sp. KP_CU and *X. anantnagensis* XENO-2 ^{T}, thus it is considered conspecific. When compared with other related *Xenorhabdus* species, it shows a difference of 12-62 bp and has sequence similarity values of 95-99% (Table 3). The absence of any nucleotide differences in the alignment confirmed that the present strain is genetically identical to *X. anantnagensis* XENO-2^{T}, leading to the conclusion that they are the same species or conspecific.

A phylogenetic tree based on 16S rRNA gene sequences revealed that *Xenorhabdus* sp. strain KP_CU forms a monophyletic clade with *Xenorhabdus anantnagensis* XENO-2^{T}, indicating similarity to the same species (Fig. 2). The phylogenetic tree also includes sequences with lower scores when compared to the sequence of each officially published *Xenorhabdus* species against *Xenorhabdus* sp. isolate KP_CU. These results were used to compare maximum identity and nucleotide differences with other *Xenorhabdus* species, highlighting variations in sequence length and base composition as distinguishing features. *Xenorhabdus* sp. strain KP_CU forms a sister clade with *X. japonica* and *X. vietnamensis*.

Ecological characterization

The present investigation elucidated the potent pathogenicity of the *Xenorhabdus* strain when introduced into waxworm larvae via hemocoel injection. A broad spectrum of bacterial concentrations was examined, revealing remarkable larvicidal efficacy. Notably, even at the minimal optical density (OD590 = 0.125), the strain induced over 80% larval mortality within merely 24 hours post-injection, emphasizing its elevated virulence (Fig. 3A), and achieved complete mortality (100%) across all concentrations evaluated within 72 h (Fig. 3C,D) This rapid onset of lethality underscores its extraordinary pathogenic capabilities, positioning it as a formidable candidate for biological control strategies.

Virulence and efficacy of *Steinernema anantnagense* against *Helicoverpa armigera*

The virulence of the *Steinernema anantnagense* isolate KP_CU against *Helicoverpa armigera* larvae is depicted in Fig. 4A. The results reveal that this isolate exhibit pronounced pathogenicity, inducing substantial larval mortality within a short period. Complete mortality of treated larvae was achieved within 60 h, irrespective of the inoculum size. Remarkably, larval mortality commenced as early as 36 h post-inoculation at a dose of 100 infective juveniles (IJs) per larva, while a higher dose of 200 IJs per larva resulted in larval death within 24 hours. In contrast, no mortality was recorded in the control groups, even after 72 h of observation.

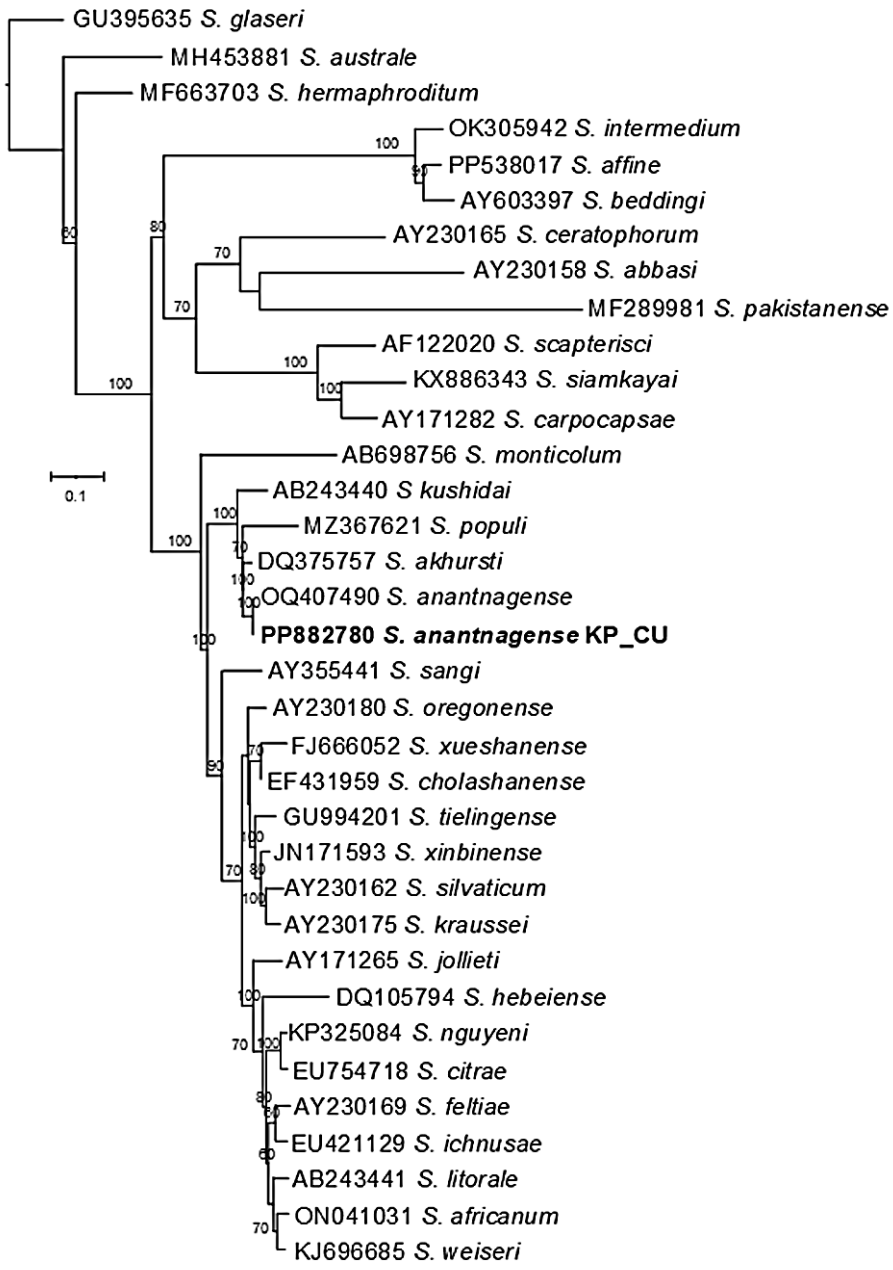

Fig. 1. Maximum-likelihood phylogenetic tree of *Steinernema* EPNs strains reconstructed from ITS rRNA gene sequences. The bootstrap value is represented by numbers at nodes and the bar represent average nucleotide substitutions per sequence position.

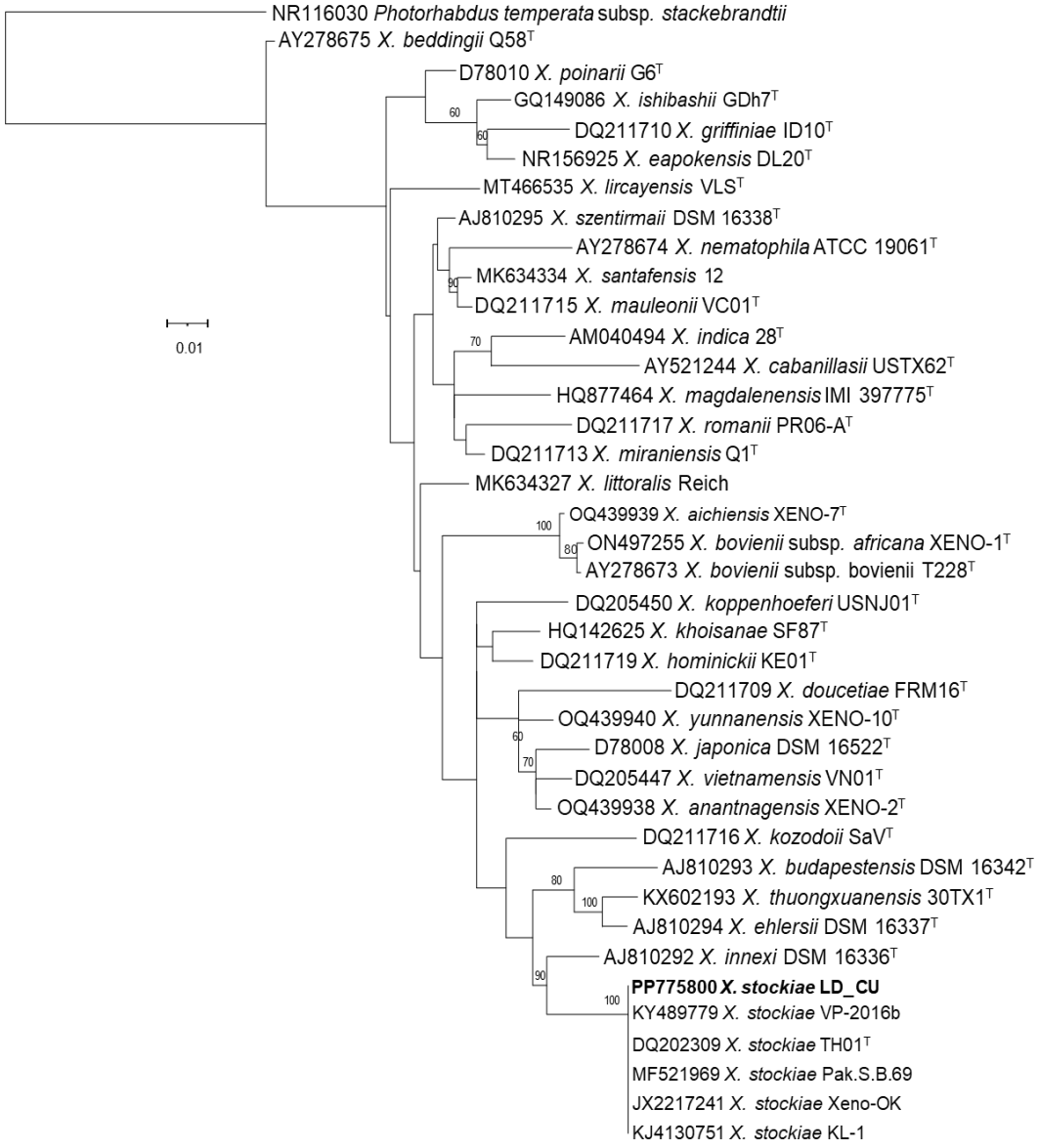

Fig. 2. Maximum-likelihood phylogenetic tree of *Xenorhabdus* bacterial strains reconstructed from 16S rRNA gene sequences. The bootstrap value is represented by numbers at nodes and the bar represent average nucleotide substitutions per sequence position.

Table 2. Biochemical characteristics of *Xenorhabdus stockiae* isolated from *Steinernema siamkayai*.

S. No.	Tests	Results	S. No.	Tests	Results
1.	β-Galactosidase	–	**13**	Gelatinase	+
2.	Lysine decarboxylase	+	**14**	Arabinose oxidation	–
3.	Citrate utilization	+	**15**	Glucose oxidation	+
4.	Urease	+	**16**	Mannitol oxidation	–
5.	Arginine dihydrolase	+	**17**	Sorbitol oxidation	–
6.	Ornithine decarboxylase	+	**18**	Amygdalin oxidation	–
7.	Tryptophan deaminase	–	**19**	Cytochrome oxidase	+
8.	Indole production	+	**20**	Melibiose oxidation	–
9.	Acetoin production	–	**21**	Inositol oxidation	–
10.	Sucrose oxidation	–	**22**	Rhamnose oxidation	–
11.	H_2S production	–	**23**	NO_2 production	–
12.	Catalase	–	**24**	NO_2 reduction to N_2 gas	–

+ = positive reaction, – = negative reaction.

Table 3. Pairwise nucleotide similarities (%) of 16S rRNA gene sequences of closely related *Xenorhabdus* strains with *Xenorhabdus* sp. KP_CU. Data for present species is in bold.

	16S rRNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	***Xenorhabdus* sp.**		**0**	**12**	**13**	**14**	**22**	**23**	**25**	**28**	**29**	**29**	**30**	**31**	**31**	**33**	**33**	**33**	**35**	**35**	**37**	**39**	**39**	**41**	**41**	**42**	**43**	**44**	**44**	**45**	**45**	**49**	**51**
2	*X. anantnagensis*	**100**		12	13	14	22	23	25	28	29	29	30	31	31	33	33	33	35	35	37	39	39	41	41	42	43	44	44	45	45	49	51
3	*X. vietnamensis*	**99**	99		18	20	25	29	28	31	29	35	38	35	37	36	34	37	39	40	39	38	45	39	46	45	47	45	43	51	41	49	55
4	*X. japonica*	**99**	99	99		21	27	32	30	35	33	34	35	40	34	36	35	38	41	45	40	43	41	41	44	43	45	46	42	49	42	54	48
5	*X. yunnanensis*	**99**	99	98	98		21	19	26	21	35	26	31	35	31	37	33	35	42	41	36	39	42	43	33	43	46	45	43	45	42	55	48
6	*X. doucetiae*	**98**	98	98	98	98		30	23	34	30	35	43	41	40	32	38	38	40	46	36	45	44	46	45	24	48	48	51	39	48	46	53
7	*X. khoisanae*	**98**	98	98	98	99	98		22	16	32	28	33	32	31	39	26	38	37	31	34	41	40	37	33	44	44	40	37	37	42	46	52
8	*X. thuongxuanensis*	**98**	98	98	98	98	98	98		25	12	34	42	30	37	40	29	39	28	33	24	43	43	40	46	42	39	36	39	26	42	36	44
9	*X. hominickii*	**98**	98	98	97	98	97	99	98		31	29	32	27	34	35	32	35	34	39	35	41	44	35	34	47	40	37	30	35	36	51	51
10	*X. ehlersii*	**98**	98	98	97	97	98	98	99	98		42	43	29	44	42	32	42	28	36	23	46	48	45	57	44	41	40	44	33	48	38	54
11	*X. koppenhoeferi*	**98**	98	97	97	98	97	98	97	98	97		36	40	31	39	41	40	44	45	40	46	48	43	39	51	43	45	45	51	42	59	47
12	*X. littoralis*	**98**	98	97	97	98	97	98	97	98	97	97		37	23	23	25	27	41	36	46	27	34	37	28	34	31	32	36	33	38	44	41
13	*X. stockiae*	**98**	98	97	97	97	97	98	98	98	98	97	97		33	34	33	28	24	35	33	40	46	46	49	44	33	37	45	36	49	50	48
14	*X. szentirmaii*	**98**	98	97	97	98	97	98	97	97	97	98	98	97		15	14	14	43	47	45	23	29	34	27	30	28	28	37	32	36	42	26
15	*X. mauleonii*	**97**	97	97	97	97	98	97	97	97	97	97	98	97	99		20	9	42	48	48	30	33	34	29	21	35	30	36	28	36	35	35
16	*X. miraniensis*	**97**	97	97	97	97	97	98	98	98	98	97	98	97	99	98		19	42	41	37	15	24	33	33	23	27	27	32	21	36	35	27
17	*X. santafensis*	**97**	97	97	97	97	97	97	97	97	97	97	98	98	99	99	99		44	46	48	27	35	38	31	28	33	28	39	26	39	37	34
18	*X. innexi*	**97**	97	97	97	97	97	97	98	97	98	97	97	98	97	97	97	97		45	34	52	48	49	58	51	44	46	50	45	54	53	49
19	*X. kozodoii*	**97**	97	97	97	97	96	98	97	97	97	97	97	97	96	96	97	96	97		46	48	48	50	46	51	43	41	48	45	53	43	63
20	*X. budapestensis*	**97**	97	97	97	97	97	97	98	97	98	97	96	97	97	96	97	96	97	96		47	52	43	56	46	41	48	47	45	47	51	49
21	*X. magdalenensis*	**97**	97	97	97	97	97	97	97	97	96	96	98	97	98	98	99	98	96	96	96		38	35	37	31	27	34	35	31	36	44	36
22	*X. beddingii*	**97**	97	97	97	97	97	97	97	97	96	96	97	96	98	97	98	97	96	96	96	97		43	36	32	38	27	46	33	48	44	39
23	*X. bovienii* subsp. *africana*	**97**	97	97	97	97	96	97	97	97	97	97	97	96	97	97	97	97	96	96	97	97	97		39	47	49	35	6	42	3	48	46
24	*X. lircayensis*	**97**	97	96	97	97	97	97	96	97	96	97	98	96	98	98	97	98	96	96	96	97	97	97		42	40	29	40	33	39	40	43
25	*X. romanii*	**97**	97	97	97	97	98	97	97	96	97	96	97	97	98	98	98	98	96	96	96	98	98	96	97		35	36	49	32	50	44	40
26	*X. indica*	**97**	97	96	97	96	96	97	97	97	97	97	98	97	98	97	98	97	97	97	97	98	97	96	97	97		33	47	32	51	44	31
27	*X. poinarii*	**97**	97	97	96	97	96	97	97	97	97	97	98	97	98	98	98	98	96	97	96	97	98	97	98	97	97		35	21	36	35	39
28	*X. aichiensis*	**97**	97	97	97	97	96	97	97	98	97	97	97	97	97	97	98	97	96	96	96	97	96	100	97	96	96	97		42	7	51	50
29	*X. eapokensis*	**97**	97	96	96	97	97	97	98	97	97	96	98	97	98	98	98	98	97	97	97	98	97	97	97	98	98	98	97		46	25	39
30	*X. bovienii* subsp. *bovienii*	**97**	97	97	97	97	96	97	97	97	96	97	97	96	97	97	97	97	96	96	96	97	96	100	97	96	96	97	99	96		53	48
31	*X. griffiniae*	**96**	96	96	96	96	96	96	97	96	97	95	97	96	97	97	97	97	96	97	96	97	97	96	97	97	97	97	96	98	96		54
32	*X. cabanillasii*	**96**	96	96	96	96	96	96	97	96	96	96	97	96	98	97	98	97	96	95	96	97	97	96	97	97	98	97	96	97	96	96	

Below diagonal: percentage similarity; above diagonal: total character differences.

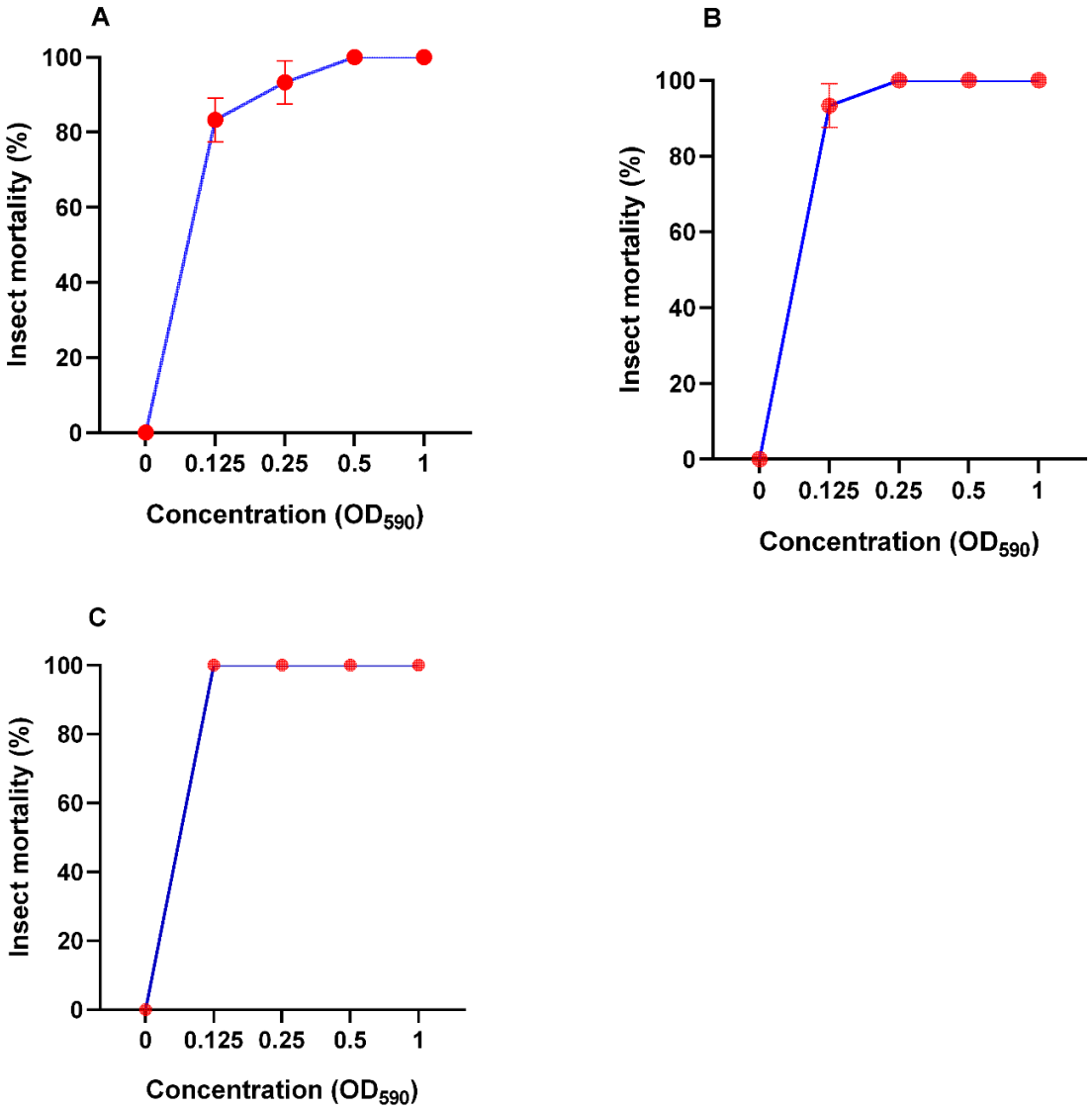

Fig. 3. Mortality rate (%) of waxworm larvae 24 h (A), 48 h (B) and 72 h (C) after injecting different *Xenorhabdus* strains at various cell concentrations (OD590 = 0.125, 0.25, 0.5 and 1).

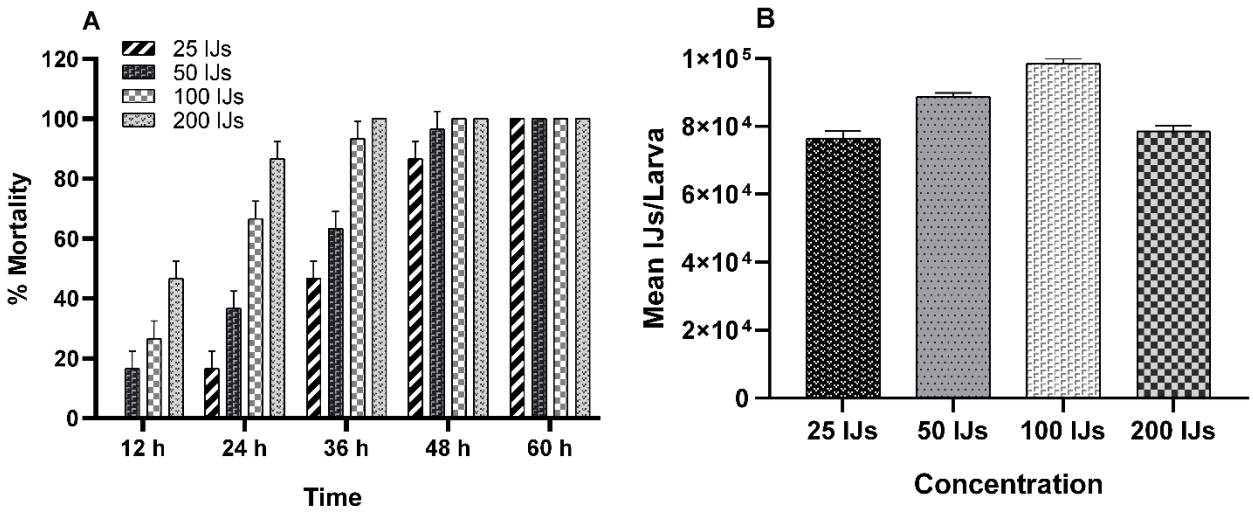

Fig. 4. (A) Percentage of mortality (mean and SD) of *Helicoverpa armigera* larvae with different doses of *Steinernema anantnagense* KP_CU. (B) Mean IJs production in *H. armigera* at 25, 50, 100 and 200 IJs/Larva.

The calculated LD50 values further substantiate the high virulence of the KP_CU isolate. At 24 h and 36 h post-infection, the LD50 was estimated to be 38 and 16 IJs per larva, respectively. Notably, this lethality was markedly intensified at 48 h post-infection, where the LD50 dropped to 9 IJs per larva, highlighting the rapid and potent lethality of this isolate.

Moreover, progeny production exhibited a clear dose-dependent pattern, with mean reproductive outputs of 76,500, 88,693, and 98,422 IJs/Larva recorded at inoculum levels of 25, 50, and 100 IJs per larva, respectively. Intriguingly, at the highest dose of 200 IJs per larva, a slight decline in reproductive output was observed, with progeny production reduced to 78,665 IJS/Larva, as shown in Fig. 4B. This reduction at elevated doses may suggest a potential trade-off between the virulence and reproductive success of the nematodes under high infection intensities.

Discussion

The results of this study demonstrate that the nematode KP_CU isolate, identified through ITS rRNA gene sequencing, exhibits complete sequence identity with *S. anantnagense*. This result corroborates previous findings by Bhat *et al.* (2023), who classified *S. anantnagense* as a novel species with distinctive genetic attributes. The phylogenetic tree construction reinforces this identification, as isolate KP_CU groups within a monophyletic clade with *S. anantnagense*, affirming their conspecific status. Additionally, KP_CU is positioned in a sister clade with *S. kushidai, S. akhursti*, and *S. populi*, members of the "*feltiae* group" of *Steinernema*. Similar clade arrangements have been reported in nematode studies where ITS rRNA molecular markers have effectively delineated species boundaries within the *Steinernema* genus (Mamiya, 1988; Qiu *et al.*, 2005; Tian *et al.*, 2022). Such molecular data are pivotal for elucidating evolutionary relationships within the genus, as emphasized in related phylogenetic analyses (Bhat *et al.*, 2021; Fukruksa *et al.*, 2017).

Regarding bacterial characterization, the phenotypic and biochemical attributes of the bacterial isolate from nematode KP_CU align with *X. anantnagensis*. XENO-2T, a symbiotic bacterium recently described by Machado *et al.* (2023). The distinctive blue-green coloration on NBTA and the reddish-brown colonies on MacConkey agar plates due to bromothymol blue and neutral red adsorption, respectively are characteristic features of Phase I *Xenorhabdus* species, consistent with previous findings (Bhat *et al.*, 2017; Fukruksa *et al.*, 2017). This distinct blue-green and neutral red hue aids in differentiating primary bacterial colonies (Phase I) from Phase II colonies, which develop a reddish color due to the reduction of tetrazolium chloride to formazan (Bhat *et al.*, 2017; Fukruksa *et al.*, 2017). These phenotypic characteristics, together with Gram-negative staining and rod-shaped morphology, correspond with earlier descriptions of *Xenorhabdus* species (Machado *et al.*, 2023). Furthermore, the bacterium's ability to grow across a range of temperatures and pH levels, coupled with its resistance profile, reinforces its identification as *X. anantnagensis* XENO-2^{T}, bolstering its classification within this group. The biochemical characteristics of the present strain show certain differences from other species such as *X. japonica* (Nishimura *et al.*, 1994), *X. budapestensis* (Lengyel *et al.*, 2005), *X. doucetiae* (Tailliez *et al.*, 2006), *X. hominickii* (Tailliez *et al.*, 2006), which are negative for citrate utilization. The present strain also shows no bioluminescence similar to other species of *Xenorhabdus* (Sajnaga & Kazimierczak, 2020).

Molecular analysis of the bacterial isolate KP_CU via 16S rRNA sequencing revealed 100% similarity with *X. anantnagensis* XENO-2^{T} and high similarity with *X. japonica* and *X. vietnamensis*. These findings are consistent with prior studies employing 16S rRNA as a molecular marker for bacterial identification, particularly within the *Xenorhabdus* genus (Fukruksa *et al.*, 2017; Kuwata *et al.*, 2017). The absence of nucleotide variations between KP_CU and *X. anantnagensis* XENO-2^{T} indicates genetic identity, a phenomenon similarly observed in other *Xenorhabdus* species where minimal 16S rRNA sequence variation confirms species identity (Bhat *et al.*, 2021; Sajnaga & Kazimierczak, 2020). Phylogenetic analysis further placed *X. anantnagensis* KP_CU in a monophyletic clade with *X. anantnagensis* XENO-2^{T}, substantiating their conspecificity. This outcome aligns with previous phylogenetic studies demonstrating the effectiveness of 16S rRNA gene sequencing in delineating phylogenetic relationships within *Xenorhabdus* (Tailliez *et al.*, 2006) and *Photorhabdus* (Bhat *et al.*, 2023). The formation of a sister clade with *X. japonica* and *X. vietnamensis* corresponds with other reports indicating close genetic relationships among these species (Lengyel *et al.*, 2005; Machado *et al.*, 2023; Nishimura *et al.*, 1994).

The integration of molecular, phenotypic, and biochemical data in this study aligns with existing research on *Steinernema* and *Xenorhabdus* species. The substantial genetic similarity to known species, supported by phenotypic characteristics, underpins the classification of both the nematode and bacterial isolates as *S. anantnagense* and *X. anantnagensis*, respectively. These findings corroborate the documented evolutionary and phylogenetic relationships, offering enhanced insight into the taxonomic and functional characterization of these organisms. The isolate *S. anantnagense* KP_CU exhibited virulence against *Helicoverpa armigera* across four concentration levels and killed all the larvae within 48 to 60 h. Comparative analyses revealed that *S. pakistanense* and *S. abbasi* achieved 100% mortality in *Spodoptera litura* Fabricius larvae within 48 to 192 hours at equivalent nematode doses, highlighting the superior efficacy of isolate KP_CU (Bhat *et al.,* 2019; Kalia *et al.*, 2014)). The variation in virulence across these insect hosts may be attributed to host-specific adaptations of the nematodes (Bhat *et al.,* 2017; Kalia *et al.*, 2014; Shapiro-Ilan *et al.,* 2003). Furthermore, factors such as nematode penetration efficiency, reproductive capacity, symbiotic bacteria, applied dosages, as well as biotic and abiotic environmental variables likely contribute to the observed disparities in virulence (Kamou *et al.*, 2024; Li *et al.*, 2020; Tarasco *et al.*, 2023).

Isolate KP_CU also exhibited remarkable reproductive capacity. In a related context, Susurluk *et al.* (2009) reported peak nematode reproduction at an optimal dose of 100 infective juveniles (IJs) per larva. This is consistent with the findings of Selvan *et al.* (1993), who demonstrated that *Heterorhabditis bacteriophora* produced the highest number of IJs at an initial dose of 100 IJs per larva, with reproductive success declining at higher doses due to intraspecific competition. These results underscore the significant potential of *Steinernema anantnagense* KP_CU as a potent biological control agent for *H. armigera* larvae in controlled laboratory settings. Future studies should thus focus on investigating its virulence and pathogenicity across a broader range of agricultural pests under field conditions and varying geographic regions. The incorporation of isolate KP_CU into biological control strategies holds the potential to significantly bolster the efficacy of integrated pest management (IPM) programs.

Conclusion

This study successfully isolated and identified *Xenorhabdus anantnagensis* from the nematode *Steinernema anantnagense*, collected from the Anantnag region of Jammu and Kashmir. Molecular and phylogenetic analyses, corroborated by ITS rRNA and 16S rRNA gene sequencing, verified the conspecific relationship between the isolated strain and *S. anantnagense* and *X. anantnagensis* XENO-2T. These findings provide significant insights into the geographical distribution of entomopathogenic nematodes (EPNs) and their symbiotic bacteria within India. Moreover, the phenotypic and biochemical characterizations further align the bacterial isolate with established *Xenorhabdus* species, consolidating its taxonomic classification. The present study highlights the potential of *X. anantnagensis* and its nematode partner in integrated pest management (IPM). The strain's adaptability to diverse environmental conditions, coupled with its susceptibility to commonly employed antibiotics such as tetracycline and gentamicin, underscores its applicability as an effective biocontrol agent. Utilizing biological agents such as this can substantially reduce the reliance on chemical pesticides, thereby mitigating risks to human health, preventing environmental contamination, and preserving soil fertility.

Further investigations are imperative to evaluate the pathogenicity and spectrum of efficacy of *X. anantnagensis* and *S. anantnagense* against a wider range of agricultural insect pests in field conditions. Research into the mass production and formulation of these biocontrol agents for field deployment should be prioritized to ensure their scalability and practical utility in sustainable agriculture. Furthermore, elucidating the interaction mechanisms between these organisms, insect pests, and host plants will be crucial for optimizing their application in IPM strategies. In light of the global shift towards sustainable agricultural practices, these findings align with key United Nations Sustainable Development Goals (UNSDGs), including SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production), and SDG 15 (Life on Land). The deployment of *X. anantnagensis* as a biocontrol agent has the potential to enhance food security by safeguarding crops from pest infestations, while also promoting responsible farming practices and conserving biodiversity through the reduced ecological impact of conventional pesticides. Additionally, future research should focus on assessing the long-term ecological consequences of utilizing *Xenorhabdus* species in pest control which will further bolster sustainable pest management initiatives in alignment with these global objectives.

Author's Contributions

Conceptualization: **Krishnapriya Okram** and **Aashaq Hussain Bhat**; Methodology: **Krishnapriya Okram**, **Aashaq Hussain Bhat**, **Ladoi Drema**; Formal analysis: **Krishnapriya Okram**, **Aashaq Hussain Bhat**; Investigation: **Krishnapriya Okram**, **Aashaq Hussain Bhat**, **Ladoi Drema**; Draft preparation: **Krishnapriya Okram**, **Aashaq Hussain Bhat**; Final review and editing: **Krishnapriya Okram**, **Aashaq Hussain Bhat**; Visualization: **Krishnapriya Okram**, **Aashaq Hussain Bhat**, **Ladoi Drema**; Validation: **Aashaq Hussain Bhat**; Supervision: **Aashaq Hussain Bhat**; Project administration: **Aashaq Hussain Bhat**. All the authors read and approved the manuscript.

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Funding

No funding was received

Data Availability Statement

All data supporting the findings of this study are available within the paper.

Acknowledgments

The authors express their deep gratitude to the Chandigarh University for laboratory assistance.

Ethics Approval

Insects, nematodes and bacteria were used in this study. All applicable international, national, and institutional guide lines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Citation: Okram, K., Bhat, A. H. & Drema, L. (2025) Biochemical, ecological and molecular characterization of *Xenorhabdus anantnagensis* associated with *Steinernema anantnagense* from India: Evaluating nematode efficacy against *Helicoverpa armigera*. *J. Entomol. Soc. Iran*, 45 (1), 121–138.

DOI: https://doi.org/10.61186/jesi.45.1.10

URL: https://jesi.areeo.ac.ir/article_131275.html

pISSN: 0259-9996 – eISSN: 2783-3968

Journal of Entomological Society of Iran

2025, 45 (1), 121–138

خصوصیات بیوشیمیایی، اکولوژیکی و مولکولی *Xenorhabdus anantnagensis* همزیست *Steinernema anantnagense* از هند: ارزیابی اثر نماتد روی *Helicoverpa armigera*

کریشناپریا اوکرام [۱]، عاشق حسین باهات [۲] و لادوی درما [۳]

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چکیده: این مطالعه، مشخصات مولکولی و فنوتیپی یک گونه نماتود بیمارگر حشرات و باکتری همزیستش که از خاک در هند جدا شده است را توصیف نموده و اطلاعات حشره‌کشی نماتود روی لارو *Helicoverpa armigera* نیز بررسی شده است. گونه نماتود به عنوان *Steinernema anantnagense* KP_CU معرفی می‌شود. توالی ناحیه ITS این نمونه، همسانی ۱۰۰٪ با جمعیت تیپ گونه *S. anantnagense* داشت. تجزیه و تحلیل فیلوژنتیک نیز این نمونه را با گونه‌های *S. kushidai*، *S. akhursti*، و *S. populi* در یک شاخه قرار داد. همزمان،توالی ناحیه 16S باکتری همزیست این نماتود با نام *Xenorhabdus* sp. KP_CU، 6S، شباهت ۱۰۰٪ با XENO- *Xenorhabdus anantnagensis* 2T نشان داد، که گویای همسانی است. خصوصیات فنوتیپی، نشان داد که باکتری قرابت بالایی با *X. anantnagensis* دارد و ویژگی‌های معمولی مانند سلول‌های میله‌ای شکل، گرم منفی و عدم وجود نور فلورسنت را برجسته کرد. آزمایش‌های بیوشیمیایی بیشتر از این شناسایی پشتیبانی کردند و KP_CU را از سایر گونه‌های *Xenorhabdus* متمایز کردند. تجزیه و تحلیل فیلوژنتیک بر اساس توالی 16S rRNA جدایه *Xenorhabdus* sp. KP_CU را با *X. anantnagensis*، در یک شاخه تک‌نیا قرار داد که گونه‌های *X. japonica* و *X. vietnamensis*، آرایه‌های خواهری این جدایه بودند. جدایه باکتری همچنین فعالیت لاروکشی روی *Galleria mellonella* نشان داد و حتی در کمترین چگالی نوری (OD590 = 0.125) تنها ۲۴ ساعت پس از تزریق بیش از ۸۰ درصد لاروها دچار مرگ و میر شدند که توان باکتری را نشان می‌داد. جدایه KP_CU قادر بود روی لارو گالریا با غلظت های ۹،۱۶ و ۳۸ لارو عفونت زا به ترتیب بعد از ۲۴، ۳۶ و ۴۸ ساعت مرگ و میر ایجاد نماید. جدایه نماتد KP_CU توان حشره‌کشی بالایی روی لارو *H. armigera* نشان داد، مرگ و میر در ۳۶ ساعت پس از تلقیح با تراکم لارو ۰ IJs / لارو شروع شد و در ۲۴ ساعت در ۲۰۰ IJs / لارو به دست آمد. مقادیر LD50 به طور قابل توجهی از ۳۸ IJ در ۲۴ ساعت به فقط ۹ IJ در ۴۸ ساعت کاهش یافت که نشان دهنده کشندگی قوی است. علاوه بر این، تولید نتاج افزایش وابسته به دوز را نشان داد، اگرچه در دوزهای بالاتر کمی کاهش یافت، که نشان دهنده‌ی یک ارتباط بین زهراگینی و تولیدمثل است. این نتایج نشان می‌دهد که *S. anantnagense* KP_CU پتانسیل آن را دارد تا به عنوان یک عامل کنترل زیستی روی *H. armigera* در مزارع در هندوستان بکار رود.

کلمات کلیدی: نماتودشناسی انتوموپاتوژنیک ، کنترل زیستی، آنالیز فیلوژنتیک، 16S rRNA

تاریخچه مقاله

دریافت: ۱۴۰۱/۰۴/۱۹

پذیرش: ۱۴۰۲/۰۶/۰۱

انتشار: ۱۴۰۳/۱۰/۱۲

دبیر تخصصی: جواد کریمی

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DOI: https://doi.org/10.61186/jesi.45.1.10