Toxicology International
DOI: 10.18311/ti/2026/v33i2/54312
INFORMATICS JOURNALS
eISSN: 0976-5131 | Vol 33(2), 2026 | pp. 355-370

Characterisation, Thermal Adaptability, and Growth Kinetics of *Photorhabdus luminescens*: An Isolate from the Hot Climates of Rajasthan, India

Parul Chauhan, Istkhar Rao*, Ayushi Chaudhary, and Kajol Yadav

Department of Bioscience and Biotechnology, Banasthali Vidyapith, Banasthali - 304022, Rajasthan, India; istkharrao@gmail.com

Abstract

The entomopathogenic bacterium *Photorhabdus luminescens*, a symbiont of the nematode genus *Heterorhabditis*, is a primary source of high-molecular-weight toxins and bioactive metabolites with potent biocontrol applications. The toxicological efficacy of these bacteria in tropical regions is often limited by the thermal instability of both the vegetative cells and their secreted toxins. This study focuses on the morphological, biochemical, and molecular characterisation, as well as the growth optimisation, of a new isolate, *Photorhabdus* sp. BSBT_XH, obtained from high-temperature agricultural soils in Tonk, Rajasthan, India. Molecular analysis using 16S rRNA gene sequencing confirmed the isolate's identity, showing 100% similarity to the reference species of *P. luminescens*, while maintaining high homology (98–99%) with other species in the complex. Growth kinetic analysis revealed that Tryptic Soy Broth optimised biomass production (OD600 of 1.040 ± 0.0010 at 36 hours), representing a 60% increase in yield compared to Nutrient Broth ($p < 0.05$), indicating a high potential for concentrated entomotoxin accumulation compared to standard media. Notably, the isolate exhibited exceptional temperature tolerance, with active proliferation observed from 15°C to 45°C and cellular viability (persistence) maintained up to 55°C. This thermal plasticity is toxicologically significant as it suggests the isolate can maintain pathogenic activity at temperatures that typically denature the toxic secretome of temperate species. Furthermore, the ability to thrive at 45°C necessitates a rigorous biosafety evaluation regarding potential non-target mammalian exposure.

Major Findings: The study concludes that *P. luminescens* isolate BSBT_XH is a highly resilient candidate for the development of heat-stable biopesticides, offering a specialised toxicological tool for pest management in extreme arid environments.

Keywords: Biocontrol, Entomopathogenic Bacteria, Thermotolerance, Tryptic Soy Broth, 16S rRNA Sequencing

Abbreviations: 16S rRNA - 16S ribosomal RNA, OD600 - Optical Density at 600 nm, EPN - Entomopathogenic Nematodes, IJ - Infective Juveniles, TRL - Temperature-Related Locus, NA - Nutrient Agar, NBTA - Nutrient Bromothymol Tetrazolium Agar, TSB - Tryptic Soy Broth, LB - Luria Bertani Broth, NB - Nutrient Broth, PBS - Phosphate Buffered Saline, BOD - Biochemical Oxygen Demand, DNA - Deoxyribonucleic Acid, PCR - Polymerase Chain Reaction, NCBI - National Center for Biotechnology Information, BLAST - Basic Local Alignment Search Tool, iTOL - Interactive Tree Of Life, CFUs/mL - Colony-Forming Units per millilitre, HSD - Honestly Significant Difference, ANOVA - Analysis of Variance, TNTC - Too Numerous To Count, SD - Standard Deviation, MLSA - Multilocus Sequence Analysis, WGS - Whole Genome Sequencing, NOAEL - No-Observed-Adverse-Effect Level, LD50 - Lethal Dose 50%

Article Received on: 08.01.2026 **Revised on:** 15.04.2026 **Accepted on:** 23.04.2026

1. Introduction

Entomopathogenic Nematodes (EPN) have been reported to cause insect pest mortality within a very short period, with their symbiotic bacterial partners playing a significant role[1,2]. The two main genera identified so far are *Steinernema* and *Heterorhabditis*, which are associated with bacteria *Xenorhabdus* and *Photorhabdus*, respectively[3,4]. The genus *Photorhabdus* is a Gram-negative bacterium within the Gammaproteobacteria class that

*Author for correspondence

lives in an obligate symbiosis with EPN of the genus *Heterorhabditis*[5]. Free-living Infective Juveniles (3rd stage IJ) of *Heterorhabditis* nematodes, found in soil, parasitise and kill insects after entry and reproduce rapidly. These IJ carry a secret weapon in their gut: The bioluminescent bacterium *P. luminescens*[6]. They are known for producing bioluminescence and a diverse array of secondary metabolites and insecticidal toxins that help them overcome insect hosts and inhibit competing microbes in the insect cadaver[7]. Usually, isolating *Photorhabdus* from nematodes or infected insect hemolymph involves using selective or indicator media and observing distinctive colony features, such as bioluminescence and pigment production[8]. Temperature is a key ecological and physiological factor for *Photorhabdus* and closely related entomopathogenic bacteria: Many insect-restricted species cannot grow at mammalian body temperatures (≥37 °C)[9], a trait linked to host range and infection biology. Recent studies have documented temperature-restricted phenotypes for *Photorhabdus* species and identified a rare isolate with broader temperature tolerance. Understanding temperature-dependent growth is therefore crucial for ecology, biosafety, and potential shifts in host range.

The arid and semi-arid regions of Rajasthan, India, with their unique soil compositions and changing environmental conditions, offer a compelling natural laboratory for studying the resilience and adaptability of biological control agents. The soil in Tonk, a district known for its specific agro-climatic zone, likely contains diverse populations of EPN, including *Heterorhabditis bacteriophora*[10]. These *Photorhabdus* spp. serve as key biological control agents, used to manage agricultural pests. They produce toxins and digestive enzymes that kill hosts, and also generate various bioactive substances, including antibiotics. Consequently, these organisms hold significant importance in biotechnology and medicine[6]. At the molecular level, temperature restriction in the genus *Photorhabdus* is governed by complex genetic architectures. Recent genomic insights have identified the Temperature-Related Locus (TRL) as a critical determinant of thermal niche expansion. While most entomopathogenic bacteria are mesophilic, constrained by the thermal instability of essential proteins and the absence of specific heat-tolerance genes, certain thermotolerant genotypes harbour genomic islands that enable metabolic maintenance at higher temperatures. For instance, studies on *P. luminescens* and *P. asymbiotica* have highlighted that the presence or absence of the TRL can determine whether a strain is restricted to an insect host or can adapt to the warmer environments of mammalian hosts or tropical soils. The lack of such literature for Indian isolates represents a gap that this study aims to bridge by characterising the thermal phenotype of *Photorhabdus* isolate BSBT_XH.

Unlocking the secrets of these locally adapted strains is essential for developing effective, region-specific biopesticides. This study focused on extracting and isolating *P. luminescens* from the *H. bacteriophora* nematodes collected from soils in Tonk, Rajasthan, India[10]. The primary objective was to isolate and characterise the thermal plasticity of *P. luminescens* isolate BSBT_XH. The study hypothesises that *P. luminescens* BSBT_XH, having evolved under localised high-temperature stress, exhibits a significantly shifted thermal niche that allows vegetative growth and biomass accumulation at temperatures (>37°C) typically inhibitory to temperate and clinical *Photorhabdus* strains. This study tests this hypothesis using comparative growth kinetics and thermal-resilience profiling. By establishing the toxicological baseline for this thermotolerant isolate, we address the instability of biological control agents in tropical climates, offering a robust alternative to chemical insecticides that often degrade rapidly at high temperatures.

2. Materials and Methods

2.1 Culture Media Preparation

The following culture media were purchased from Hi-Media, dissolved in distilled water, and subjected to a standard sterilisation procedure to evaluate the growth kinetics of *P. luminescens*. NA (nutrient agar); 5grams peptone, 5 grams sodium chloride, 1.5grams HM Peptone B, 1.5 grams yeast extract, 15grams agar, 1000mL distilled water, pH 7.4± 0.2. NBTA (Nutrient Bromothymol Tetrazolium Agar): 0.025grams bromothymol blue, 0.04grams 2,3,5-triphenyltetrazolium chloride, 20grams nutrient agar, 1000mL distilled water, pH 7.0. MacConkey Agar: 17grams peptone, 3grams proteose peptone, 10grams lactose, 1.50grams bile salts, 5grams sodium chloride, 0.03grams neutral red, 15grams agar, 1000mL distilled water, pH 7.0. Tryptic Soy Broth (TSB): 17grams tryptone, 3grams soya peptone, 5grams sodium chloride, 2.50 grams dipotassium hydrogen phosphate, 2.50 grams dextrose, 1000mL distilled water,

pH 7.3 ± 0.2. LB Broth (Luria Bertani Broth): 10 grams Casein enzymic hydrolysate, 10 grams sodium chloride, 5 grams yeast extract, 1000mL distilled water, pH 7.5 ± 0.2. NB Broth (Nutrient Broth): 10 grams peptone, 5 grams sodium chloride, 1.5 grams HM peptone B, 1.5 grams yeast extract, 1000mL distilled water, pH 7.4 ± 0.2.

2.2 Nematode Surface Sterilisation and Bacterial Extraction

To confirm that the recovered bacteria originated within the nematodes rather than from external soil contamination, IJ were surface-sterilised using a dilute cationic surfactant protocol adapted from Forst and Clarke[11]. About 200 IJ were rinsed in sterile distilled water, then treated with 0.1% sodium hypochlorite (NaOCl) solution for 2-3 minutes, and rinsed three times in distilled water[12]. Sterile IJ were then crushed in 50-100µL of PBS using a sterile micro-pestle to release the symbiotic bacteria. The resulting macerate was spread onto NBTA plates, which are designed explicitly for isolating *Photorhabdus*. All NBTA plates were incubated in a BOD incubator in the dark at 28°C for 24-48 hours to promote bacterial growth.

After incubation, colony development was evaluated on NBTA medium. Characteristic blue-pigmented colonies indicative of the phase I variant of *Photorhabdus* were observed, and the isolate was named BSBT_XH. Based on pigmentation profiles and colony morphology, isolates were purified by repeated streaking on fresh NBTA plates[13]. Axenic cultures were then prepared as glycerol stocks (20-30% v/v) and stored at -80°C for future studies.

2.3 Molecular Identification Using 16S rRNA

Following the initial isolation from Rajasthan soil, it was imperative to definitively establish the taxonomic identity of *Photorhabdus* isolate BSBT_XH using molecular markers before proceeding to physiological characterisation. Molecular identification and phylogenetic placement of the bacterial isolate involved detailed analysis of the 16S ribosomal RNA (rRNA) gene, a highly conserved genetic region essential for bacterial classification and evolutionary research. Genomic DNA was extracted from pure cultures of BSBT XH, initially grown on NBTA plates and then enriched in Luria-Bertani Broth (Miller, TM Media). The broth cultures were incubated at 35 ± 2 °C for 36 hours with continuous shaking at 180rpm on an orbital shaker to ensure optimal growth. After incubation, bacterial genomic DNA was isolated using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The 16S rRNA gene was then amplified via Polymerase Chain Reaction (PCR) in a 25µL reaction mixture containing 12. 5µL of green Taq PCR master mix (2X) (Thermo- Scientific), 7.5µL nuclease-free distilled water, 1µL of each universal bacterial primer (10 F: 5'- AGTTTGATCATGGCTCAGATTG- 3' and 1507 R: 5'- TACCTTGTTACGACTTCACCCCAG- 3')[14], and 3µL of the extracted DNA template. Thermocycling conditions included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 90 seconds. The process ended with a final extension at 72°C for 10 minutes, followed by a hold at 4°C. PCR products were confirmed by running them on a 1% agarose gel, then purified and sequenced using Sanger sequencing by Barcode Bioscience (Bangalore, India). The sequences obtained were analysed using the BioEdit Sequence Alignment Editor software (version 7.2.5)[15], and sequence similarity searches were conducted against the NCBI nucleotide database using BLAST[16]. For phylogenetic analysis, multiple sequence alignments were generated with CLUSTAL W, and phylogenetic trees were constructed with Molecular Evolutionary Genetics Analysis Version 11 (MEGA XI). The Neighbour-Joining method, with 1000 bootstrap replicates, was used to infer evolutionary relationships[17]. Tree visualisation and annotation were performed using the online tool Interactive Tree Of Life (iTOL). The 16S rRNA gene sequence of the *Photorhabdus* isolate BSBT_XH was deposited in GenBank under accession number PX446793.

2.4 Phenotypic and Biochemical Analysis

The phenotypic and biochemical properties of *Photorhabdus* isolate BSBT_XH were thoroughly evaluated to determine its key features. The phenotypic analysis began with the visual examination of colony appearance. *Photorhabdus* isolate BSBT_XH was grown on three different agar types: Nutrient Agar (NA), MacConkey Agar, and NBTA plates. After inoculation, the plates were incubated in darkness for 48 hours at 35°C, and then colony shape, size, and colour were carefully documented[18]. Next, microscopic identification involved Gram staining, using a Hi-Media K001-1KT kit. A thin smear of a 24-hour-old bacterial culture was prepared on a clean glass slide, air-dried, and heat-fixed. The smear was

stained sequentially with crystal violet (1 min), Gram's iodine (1 min), briefly decolourised with 95% ethanol, and then counterstained with safranin (30-60 sec). After a final rinse and air-drying, the stained bacteria were examined under oil immersion at 100X magnification to determine Gram reaction and cell shape[19]. For biochemical testing, a HiMedia Enterobacteriaceae Identification Kit (KB016 Hi24) was used to analyse the Gram-negative isolate. A single, pure colony from an NBTA plate was transferred into 5mL of Brain Heart Infusion Broth and incubated overnight at 35°C on a rotary shaker until the optical density reached 0.1 at 620nm. According to the manufacturer's instructions, 50µL of this standardised inoculum was carefully added to each of the 24 wells of the kit, which contained 11 carbohydrate utilisation tests and 13 other biochemical assays[20]. After incubation, the colour changes in the wells were interpreted to create a biochemical profile for *Photorhabdus* isolate BSBT_XH.

2.5 Growth Optimisation in Liquid Media

Having established its identity, we next evaluated the nutritional preferences of the *Photorhabdus* isolate BSBT_XH across three standard media to optimise biomass production for potential biocontrol applications. The successful cultivation of symbiotic bacteria associated with *Heterorhabditis* nematodes required evaluating various liquid media to optimise growth. The study compared three standard formulations: Tryptic Soy Broth (TSB), known for its rich amino acid and carbohydrate content (pH 7.3±0.2); Luria Bertani Broth (LB), a simpler, standard medium containing casein hydrolysate and yeast extract (pH 7.5±0.2); and Nutrient Broth (NB), which provides a moderate mix of peptones and yeast extract (pH 7.4±0.2). For the experiment, 50mL aliquots of each medium were inoculated with 100 µL of bacterial suspension in 250mL flasks. All cultures were incubated under standard conditions at 36°C ± 2°C with continuous orbital shaking at 180 rpm. Growth was monitored using a two-pronged approach over time: first, by spectrophotometric measurement of optical density at 600nm (OD600) every four hours to estimate total biomass[21]; and second, by performing spread plate techniques for standard plate counts, which directly measure live cells as Colony-Forming Units per millilitre (CFUs/mL)[22,23]. This comprehensive approach ensures accurate determination of both total growth and the viability of the *Photorhabdus* isolate BSBT_XH across different nutritional environments.

2.6 Cultivation Parameters and Thermal Sensitivity of Bacterial Isolate

A defining feature of the Rajasthan isolates is their exposure to extreme environmental heat; therefore, the thermal plasticity of *Photorhabdus* isolate BSBT_XH was characterised across a wide temperature gradient. The experiment began by reviving the isolate from a single blue-green colony on an NBTA plate. This colony was used to inoculate 50mL of TSB in a 250mL flask. The initial seed culture was incubated at 35°C with continuous orbital shaking at 180 rpm for 12 to 14 hours. To establish a uniform starting point for the thermal challenge, the culture was then standardised. Following the procedure described by Hall[24], the cell density was adjusted to achieve an initial Optical Density (OD600) of 0.1 at 600nm.

To evaluate the impact of temperature, the experiment was set up using 15mL sterile culture tubes, each containing 5mL of fresh TSB. Each tube was inoculated with 2mL of a standardised culture with an OD600 of 0.1. A wide range of nine incubation temperatures was tested: 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, and 55°C. All tubes were incubated in Biochemical Oxygen Demand (BOD) incubators with constant rotary agitation (180rpm). Triplicate samples were used for each temperature condition to ensure reliability. Growth was monitored over 60 hours, with measurements every 6 hours. Culture density was measured by recording the optical density at 600nm (OD600) using a UV-Vis double-beam spectrophotometer (Systronics, 2202TS). For each measurement, a 1mL aliquot was aseptically withdrawn, and sterile TSB served as the instrument blank. To differentiate between growth inhibition and cell death at extreme temperatures (50°C and 55°C), 100µL aliquots were sampled at 24 hours and plated onto TSB agar. The appearance of colonies after 48 hours of incubation at 30°C was used to confirm cell viability (Colony Forming Unit (CFU)-based confirmation) at these upper limits.

2.7 Data Analysis

The growth study data were carefully analysed using statistical methods to assess how different cultivation conditions quantitatively affected the *Photorhabdus* isolate BSBT_XH. To evaluate the impact of the three distinct liquid media (TSB, LB, and NB) on bacterial growth, the key growth kinetic parameters were examined. The maximum cell density, lag phase duration, and specific growth rate (µ) were calculated for the cultures. The

average and standard deviation of these metrics were calculated using XLSTAT. To visually illustrate the overall growth patterns, a detailed growth curve was created and plotted using GraphPad Prism (Version 8.0.2).

Data for growth optimisation and thermal kinetics were analysed using One-Way ANOVA to determine significant differences between treatment groups. Tukey's Honestly Significant Difference (HSD) test was used for post hoc pairwise comparisons ($p < 0.05$) in GraphPad Prism. All experiments were performed in triplicate to ensure reproducibility. For the temperature-dependent growth data, a detailed descriptive analysis was performed using IBM SPSS Statistics (Version 22). This analysis summarised the OD600 readings collected at various temperatures, with all results presented as the mean ± standard deviation. Following this statistical review, the entire growth pattern across the nine tested temperatures was visualised. Growth curve graphs were generated in Prism, plotting measured cell density (OD600) at 6-hour intervals to illustrate how temperature affects the isolate's growth trajectory.

3. Results

3.1 Molecular Identification and Phylogenetic Placement of *Photorhabdus* sp.

Molecular characterisation of the *Photorhabdus* isolate BSBT_XH involved sequencing the 16S rRNA gene, which yielded a sequence approximately 1424 base pairs long. An initial BLAST search of the NCBI GenBank database was conducted to identify the isolate's closest relatives. An initial BLAST search of the NCBI GenBank database revealed that the 16S rRNA gene sequence of *Photorhabdus* isolate BSBT_XH shared high homology with the genus, specifically exhibiting 100% identity with *P. luminescens* (Accession: KF780170) and 98%-99% similarity to several other well-known *Photorhabdus* species. To ensure precise taxonomic classification, the complete 16S rRNA sequence of *Photorhabdus* isolate BSBT_XH was submitted to GenBank (Accession number PX446793). The evolutionary position of the isolate was clearly established through phylogenetic analysis (Figure 1). A Neighbour-Joining tree was built in MEGA XI, with

Table 1. Pairwise distance matrix of 16S rRNA gene sequences for *Photorhabdus species*. Data for *Photorhabdus* isolate BSBT_XH is presented in bold.

S. No.	16S rRNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	**BSBT_XH**		0	22	21	14	36	59	53	15	13	42	17	50	35	26	93
2	*P. luminescens*	100		22	21	14	36	59	53	15	13	42	17	50	35	26	93
3	*P. aballayi*	98	98		21	21	30	45	46	18	20	37	18	51	17	19	66
4	*P. africana*	98	98	98		20	34	45	45	18	20	41	18	54	20	11	76
5	*P. akhurstii*	99	99	98	99		39	53	52	16	17	43	16	48	32	24	93
6	*P. antumapuensis*	97	97	98	97	97		53	57	28	31	35	32	48	39	40	85
7	*P. asymbiotica*	96	96	97	97	96	96		43	43	44	61	48	33	45	47	96
8	*P. australis*	96	96	97	97	96	96	97		45	40	49	44	36	51	50	92
9	*P. bodei*	99	99	99	99	99	98	97	97		18	36	16	54	15	19	70
10	*P. caribbeanensis*	99	99	99	99	99	98	97	97	99		41	20	43	31	22	72
11	*P. cinerea*	97	97	97	97	97	98	96	97	97	97		44	43	48	50	88
12	*P. hainanensis*	99	99	99	99	99	98	97	97	99	99	97		51	25	23	80
13	*P. heterorhabditis*	96	96	96	96	96	96	98	97	96	97	97	96		58	48	61
14	*P. kayaii*	98	98	99	99	98	97	97	96	99	98	97	98	96		23	97
15	*P. laumondii*	98	98	99	99	98	97	97	96	99	98	96	98	96	98		96
16	*X. japonica*	93	93	95	94	93	94	93	94	95	95	94	94	95	93	98	

Values below the diagonal represent the estimates of evolutionary divergence between sequences; values in above diagonal represent the percentage identity.

1000 bootstrap replicates to assess the robustness of the inferred branches. As seen in Figure 1, the *Photorhabdus* isolate BSBT_XH (highlighted in magenta, PX446793) consistently clustered within the main *Photorhabdus* genus clade. The isolate formed a distinct, well-supported branch, sister to *P. luminescens* (KF780170 and JX221723). This placement, supported by moderate to high bootstrap values (as indicated by the node size), confirms the genus-level identification and suggests a close evolutionary relationship to *P. luminescens*. The broader *P. luminescens* group was also closely related to *P. aegyptia* (MT355495) and *P. aballayi* (OP735347). *Xenorhabdus japonica* (D78008) was positioned as an outgroup, serving as a reference point for evolutionary divergence.

The genetic relationship between *the Photorhabdus* isolate BSBT_XH and closely related species was quantified using a pairwise distance matrix (Table 1) based on 16S rRNA gene sequences. The 16S rRNA gene sequence for the isolate BSBT_XH (Row 1) was found to be 100% similar to the reference strain of *P. luminescens* (Row 2), confirming its placement within the *P. luminescens* species complex.

The *Photorhabdus* isolate BSBT_XH also showed high sequence identity with several other *Photorhabdus* species, suggesting close evolutionary proximity. Specifically, it exhibited 99% sequence similarity to *P. bodei* (Row 9), *P. caribbensis* (Row 10), and *P. laumondii* (Row 15). A 98% similarity was observed with *P. aballayi* (Row 3), *P. africana* (Row 4), *P. akhurstii* (Row 5), *P. australis* (Row 8), and *P. kayaii* (Row 14). The lowest similarities within the genus were 96% to *P. asymbiotica* (Row 7) and *P. heterorhabditis* (Row 13). The outgroup, *Xenorhabdus japonica* (Row 16), showed lower overall similarity (93%), further supporting the isolate's taxonomic assignment to the genus *Photorhabdus*. These high similarity values (ranging from 96% to 100%) confirm the close genetic relatedness among the *Photorhabdus* species, consistent with the clustering observed in the phylogenetic tree (Figure 1).

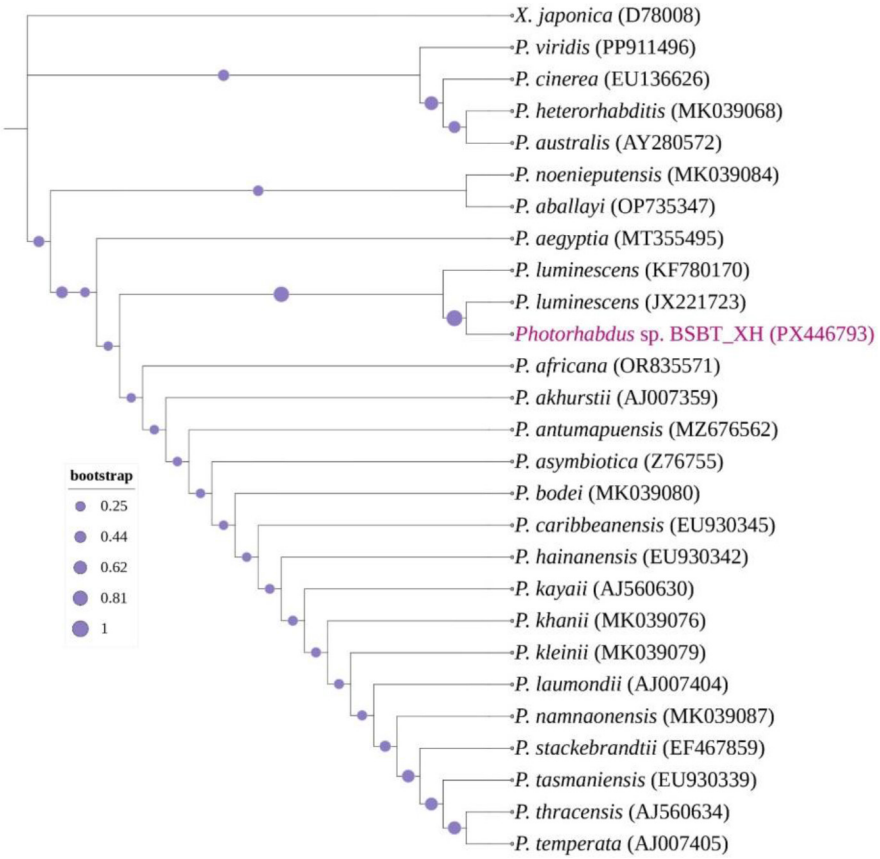


Figure 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, illustrating the phylogenetic position of *Photorhabdus* isolate BSBT_XH. The tree was constructed using the Neighbour-Joining method (MEGA XI) with the Maximum Composite Likelihood model. Node support was assessed with 1000 bootstrap replicates (shown by the size of the purple circles). *X. japonica* served as the outgroup. All accession numbers for reference sequences are provided in parentheses.

3.2 Morphological and Biochemical Characterisation

The *Photorhabdus* isolate BSBT_XH underwent detailed morphological examination and a series of standard biochemical tests to confirm its identity and metabolic profile, with results summarised in Table 2. Motility testing yielded a positive outcome. Growth characteristics were observed on three differential media: (A) NBTA; colonies were circular, slightly larger (2-4 mm), and brick red, with a slightly convex surface. (B) MacConkey Agar; colonies appeared circular and small (2-3mm), light pink, with a slightly convex surface. The light pink hue confirms its status as a non-lactose fermenter, consistent with *Photorhabus* species. (C) Nutrient Agar; colonies were circular with a convex surface, typically measuring 2-3mm in diameter (Figure 2). Pigmentation was white, and the colonies had a smooth texture. Microscopic analysis confirmed that the isolate is a Gram-negative, rod-shaped bacterium, as illustrated in Figure 2(D).

The biochemical profile revealed several key metabolic capabilities that are essential for distinguishing between genera and species within the Enterobacteriaceae. The isolate tested positive for Urease activity and α-Galactosidase. In contrast, the isolate showed negative results for indicating no β-galactosidase activity (ONPG), Lysine and Ornithine utilisation, Phenylalanine deaminase, Voges-Proskauer, Methyl red, Indole production, and Pyrrolidonyl Arylamidase (PYR). Regarding carbohydrate utilisation, the *Photorhabdus* isolate BSBT_XH fermented Trehalose and Mannose and hydrolysed α-Galactosidase.

3.3 Enumeration and Standardisation of *Photorhabdus* Isolate BSBT_XH Inoculum

To accurately determine the viable cell concentration and establish a standardised starting inoculum for subsequent growth kinetics studies, a ten-fold serial dilution series

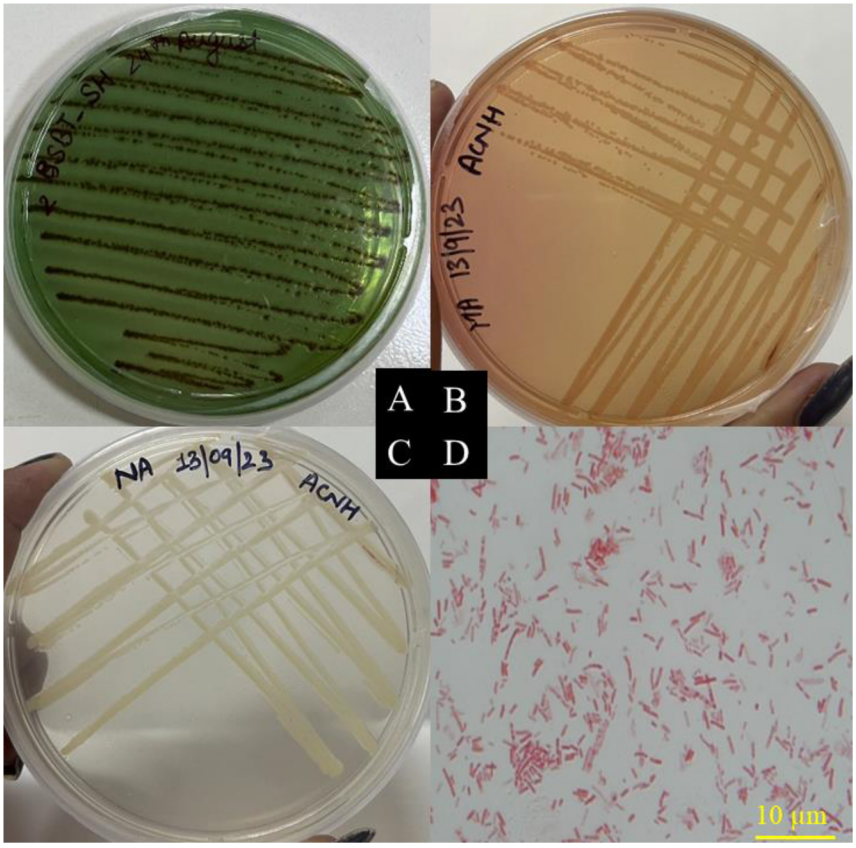


Figure 2. Morphological and microscopic characteristics of *Photorhabdus* isolate BSBT_XH, **(A)** Colonies on NBTA plates, **(B)** Colonies on MacConkey Agar, **(C)** Colonies on Nutrient Agar (NA), **(D)** Gram stain micrograph showing the characteristic pink (Gram-negative), rod-shaped cells of the isolate, observed at ×1000 magnification.

Table 2. Morphological and biochemical characteristics of isolate *Photorhabdus* isolate BSBT_XH

	Test	BSBT_XH			Peel *et al.*[31]
		NA	**NBTA**	**MacConkey**	
Morphological Characters	Shape	Circular	Circular	Circular	
	Size	2-3mm	2-4mm	2-3mm	
	Surface	Convex	Slightly Convex	Slightly Convex	
	Pigmentation	White	Brick Red	Light-Pink	
Gram staining	Reaction	-			-
	Cell Shape	Rod Shape			Rod Shape
Conventional Biochemical Tests	ONPG	-			-
	Lysine Utilisation	-			-
	Ornithine Utilisation	-			-
	Urease	+			+
	Phenylalanine Deamination	-			-
	Voges Proskauer's	-			-
	Methyl Red	-			-
	Indole	-			-
	PYR	-			NA
	β- Glucuronidase	-			NA
	α- Galactosidase	+			NA
	β- Xylosidase	-			NA
	Esculin Hydrolysis	-			-
	Sucrose	+			+
	Sorbitol	+			+
	Trehalose	+			+
	Glucose	+			+
	Cellobiose	-			NA
	Melibiose	-			NA
	Salicin	-			NA
	Mannose	+			NA
	Maltose	-			NA
	Raffinose	-			NA
	Lactose	-			NA
Motility		Positive			Positive

Biochemical tests were conducted using the standardized KB016 kit; media-specific observations (NA, NBTA, MacConkey) are restricted to morphological characteristics.

of the *Photorhabdus* isolate BSBT_XH culture was prepared. 0.1mL from each dilution was plated onto agar medium using the spread plate technique and incubated at 28 ± 2 °C for 24 to 48 hours. Following the incubation period, colonies were counted from the plates falling within the statistically reliable range, and the viable cell concentration was calculated as Colony-Forming Units per millilitre (CFUs/mL) (Table 3).

The calculated cell concentration spanned a wide range, from 8.12×10^5 CFUs/mL at the 10^{-3} dilution factor to a maximum of 6.5×10^{10} CFUs/mL at the 10^{-9} dilution. Given the high concentration of the original

culture, the lower dilutions (10^{-1} and 10^{-2}) yielded plates with Too Numerous To Count (TNTC). For consistency and precision in the subsequent growth experiments, a single, isolated colony from the most dilute plate (10^{-9} dilution factor) was aseptically selected to prepare the final working inoculum.

The fermentation kinetics of *Photorhabdus* isolate BSBT_XH were monitored over 60 hours in three distinct liquid media: LB, NB, and TSB. The growth curves (Figure 3) demonstrated a typical bacterial growth pattern across all media, encompassing lag, exponential (log), stationary, and death phases. The composition of the medium significantly affected both growth rate and final biomass yield (OD600). A standardised starting dose of 6.5×10^{10} CFUs/mL (10^{-9} dilution factor) was used for all treatments to ensure consistent exposure. The initial OD600 at hour 0 was similar across all media (LB: 0.1980 ± 0.0017; NB: 0.1730 ± 0.0017; TSB: 0.1070 ± 0.0017). The isolate exhibited a short lag phase of approximately 8 hours in TSB and LB, but a slightly longer lag phase of up to 12 hours in NB (Figure 3). The fastest growth rate was observed in TSB, followed by LB, and then NB. By 12 hours, TSB had already reached an OD600 of 0.9850 ± 0.0058, significantly higher than LB (0.8550 ± 0.0058) and NB (0.6140 ± 0.0023). TSB promoted the earliest and most rapid entry into the stationary phase. The TSB medium yielded the highest and most sustained cell density. The culture reached its maximum OD600 of 1.040 ± 0.0010 at 36 hours and maintained a high level (e.g., 1.030 ± 0.0020 at 40 hours) until 48 hours. LB reached its peak density of 0.9120 ± 0.0008 at 36 hours and remained stable until 44 hours. NB provided the lowest overall biomass, peaking at 1.020 ± 0.0058 at 36 hours before a gradual decline. Cell viability decreased rapidly after 48 hours across all media.

Table 3. Viable cell count (CFUs/mL) of *Photorhabdus* isolate BSBT_XH at different dilution factors

S. No	Dilution Factor	CFU Counted	CFUs/mL of Samples
1	10^{-1}	TNTC	TNTC
2	10^{-2}	TNTC	TNTC
3	10^{-3}	812	$812 \times 10^3 = 8.12 \times 10^5$ CFUs/mL
4	10^{-4}	668	$668 \times 10^4 = 6.68 \times 10^6$ CFUs/mL
5	10^{-5}	423	$423 \times 10^5 = 4.23 \times 10^7$ CFUs/mL
6	10^{-6}	305	$305 \times 10^6 = 3.05 \times 10^8$ CFUs/mL
7	10^{-7}	198	$198 \times 10^7 = 1.98 \times 10^9$ CFUs/mL
8	10^{-8}	106	$106 \times 10^8 = 1.06 \times 10^{10}$ CFUs/mL
9	10^{-9}	65	$65 \times 10^9 = 6.5 \times 10^{10}$ CFUs/mL

CFUs: Colony-Forming Units; TNTC: Too Numerous to Count

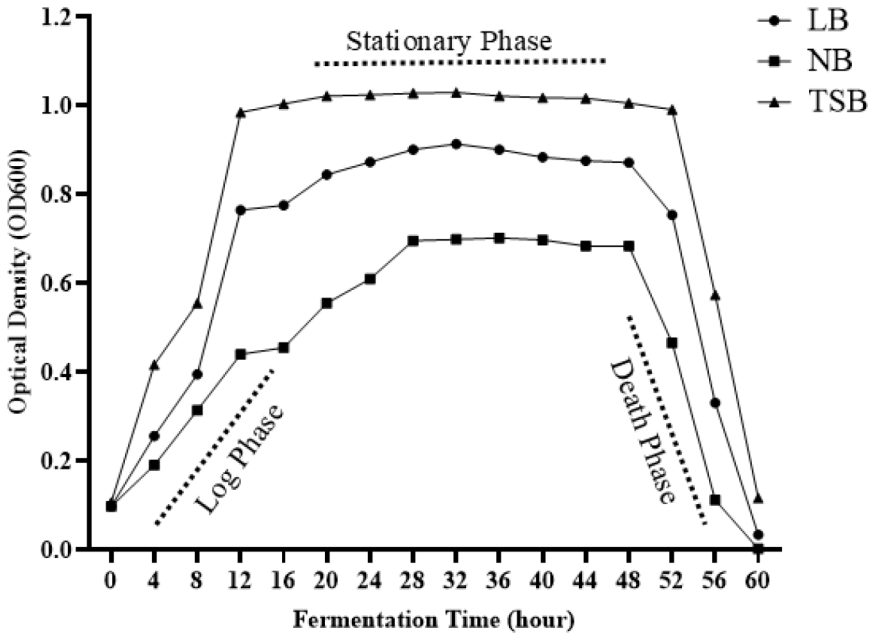


Figure 3. Analytical growth kinetics of *Photorhabdus* isolate BSBT_XH over a 60-hour exposure period. Significant differences in biomass accumulation (OD600) between TSB and NB indicate media-dependent metabolic flux. Data represent the mean ± SD of triplicate assays.

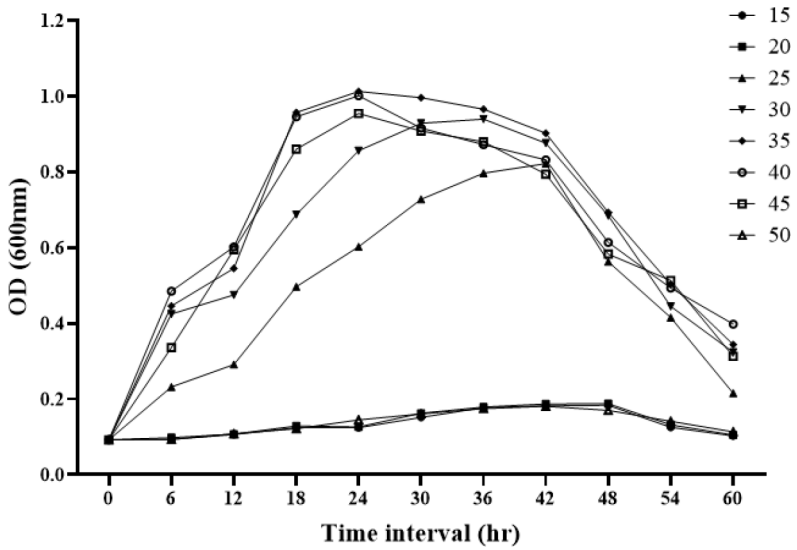


Figure 4. Growth curves of *Photorhabdus* isolate BSBT_XH at nine different temperatures (15°C to 55°C). Growth was monitored over 60 hours by measuring the Optical Density at 600 nm (OD600).

By the final measurement at 60 hours, the OD600 values were 0.1040 ± 0.0020 (LB), 0.1020 ± 0.0020 (NB), and 0.1363 ± 0.0020 (TSB).

3.4 Temperature-Dependent Growth of *Photorhabdus* Isolate BSBT_XH

The growth of the *Photorhabdus* isolate BSBT_XH was assessed over a wide temperature range (15°C to 55°C), and the resulting growth curves are shown in Figure 4. The isolate demonstrated the ability to grow over a broad temperature range, from 15°C to 55°C, although growth efficiency varied significantly.

The optimal temperature range, defined as the conditions that promote the fastest growth rate and highest final biomass (OD600), was determined to be 35°C to 40°C. A temperature of 35°C yielded the highest maximum cell density (1.013 ± 0.0016) at 24 hours. The growth rate was rapid, with the culture reaching an OD600 of 0.957 ± 0.0012 at 18 hours. 40°C temperature also supported rapid growth, peaking at 1.002 ± 0.0022 at 24 hours. The growth kinetics closely mirrored those at 35°C during the exponential phase. Growth was clearly inhibited at the extreme ends of the tested range. Specifically, at low temperatures (15°C and 20°C), the isolate exhibited minimal growth. The maximum OD600 achieved at 15°C was only 0.184 ± 0.0012 at 48 hours, and at 20°C it was 0.187 ± 0.0005 at 42 hours. This suggests that while the isolate survives at these temperatures, metabolic activity and division are severely limited. While 50°C initially showed slight growth (peaking at 0.407 ± 0.0031 at 54 hours), 55°C was highly inhibitory, while exponential growth was recorded between 15°C and 45°C; the OD600 at 55°C remained near baseline levels. However, subsequent sub-culturing on TSB agar confirmed the presence of viable CFUs at 55°C, indicating that while active binary fission is inhibited at this extreme, the cells remain viable (thermal persistence) for at least 24 hours. Growth was robust but less rapid at intermediate temperatures (25°C to 45°C): 25°C, the culture peaked at 0.797 ± 0.0012 at 36 hours. 30°C, growth was strong, peaking at 0.997 ± 0.0026 at 30 hours, but the decline was steeper than at 35°C and 40°C. Statistical analysis confirmed that the biomass yield at 40°C did not significantly differ from the yield at 30°C ($p > 0.05$), highlighting a unique thermal plateau. However, growth at 50°C showed a significant decline compared to 45°C ($p < 0.01$), marking the upper threshold of metabolic division. 45°C, growth was significantly reduced compared to the optimum, peaking at 0.955 ± 0.0021 at 24 hours, but showing an earlier onset of the death phase.

4. Discussion

The overall aim of this study was to characterise the *Photorhabdus* isolate BSBT_XH and define its optimal cultivation parameters, with particular focus on its thermal characteristics, given its origin in the hot climate of Rajasthan, India. The results provide essential data

for the mass production and potential field application of this entomopathogenic bacterium. The isolate was identified primarily through a combination of phenotypic traits and 16S rRNA gene sequencing. Subsequent 16S rRNA gene sequencing confirmed a 100% sequence identity with the reference strain of *P. luminescens*. The 16S rRNA gene is widely regarded as the gold standard for bacterial phylogeny and identification due to its highly conserved nature and extensive coverage within public databases[25]. Given the 100% sequence match to a known species and the high bootstrap support within the *P. luminescens* clade in the phylogenetic tree, the strain described from *Heterorhabditis indica* nematode[26], while 16S rRNA provides a reliable primary identification, future Multilocus Sequence Analysis (MLSA) and Whole-Genome Sequencing (WGS) will be essential to precisely define the subspecies or potential novel status of this Rajasthan ecotype. The *Photorhabdus* isolate BSBT_XH displayed significant sequence similarity to a range of recognised *Photorhabdus* species. A 99% identity was observed with *P. bodei*, *P. caribbensis*, and *P. laumondii*[27]. Furthermore, 98% similarity was detected with *P. aballayi*, *P. africana*, *P. akhurstii*, and *P. kayaii*. It is noteworthy that these latter taxa, which were initially considered subspecies, have since been formally characterised as distinct species by Machado[27,28]. These findings collectively underscore the close phylogenetic relationships and shared evolutionary history within the *Photorhabdus* genus[29]. Such high genetic similarity among multiple species implies a recent common ancestry and limited genomic differentiation within the genus. This conservation complicates the identification of finer-scale ecotype distinctions when using this particular gene.

The morphological and biochemical profile confirms the isolate's status as a typical *Photorhabdus* strain. The characteristic blue-green pigmentation on NBTA and the Gram-negative, rod-shaped morphology (Figure 2) immediately identified the isolate as belonging to the expected genus as described by Castaneda-Alvarez[30]. The detailed biochemical characterisation of the *Photorhabdus* isolate BSBT_XH provides a metabolic fingerprint that is crucial for differentiating it within the Enterobacteriaceae and for understanding its capacity to exploit nutrients within the insect host. When compared to the reference *Photorhabdus* strain described by Peel[31], the conventional biochemical tests performed on *Photorhabdus* isolate BSBT_XH revealed a relatively unreactive profile, consistent with specific *Photorhabdus* species. As shown in Table 1, the majority of tests yielded negative results, indicating a limited range of metabolic capabilities under the tested conditions. Specifically, only three tests were observed to be positive: urease, α-galactosidase, and nitrogen reduction, as well as the utilisation of Sucrose, Sorbitol, Trehalose, Glucose, and Mannose, which also supports the description[20] for the *P. akhurstii* isolate.

Comparative growth analysis demonstrated a significant impact of the liquid medium on biomass production. While NB has been the traditional standard for culturing entomopathogenic bacteria, recent optimisation trials suggest that highly nutritious media like TSB provide superior carbon-to-nitrogen ratios for certain species, yielding the highest maximum cell density (1.040 ± 0.0010 at 36 hours) and the fastest exponential phase[23]. TSB produced more biomass, which is the vehicle for toxin delivery. The high cell density achieved in TSB (6.5×10^{10} CFUs/mL) is toxicologically significant, as it represents the peak accumulation of primary-phase secondary metabolites. In entomopathogenic bacteria, pathogenicity is a density-dependent phenomenon where a toxic threshold must be reached to overcome host immunity. TSB is recognised for its high concentration of enzymatic digests of casein and soybean meal, which provide a broad spectrum of amino acids, peptides, vitamins, and other growth factors[32]. In contrast, NB and LB, while also providing essential nutrients, are generally considered less nutritionally complex, particularly in terms of the variety of peptide and amino acid sources. The preference for TSB by *Photorhabdus* isolate BSBT_XH aligns with the general understanding that many fastidious or environmentally specialised bacteria, including some entomopathogens, often benefit from a comprehensive nutrient supply for optimal proliferation. This finding is consistent with optimisation studies in other bacteria, in which complex media usually promote superior growth metrics due to reduced biosynthetic burden[21]. The significant biomass increase observed in our isolate when grown in TSB confirms that *Photorhabdus* isolate BSBT_XH requires high protein availability to maximise its metabolic flux. This suggests that for commercial-scale toxin production, traditional low-nutrient media may be a limiting factor.

A critical physiological characteristic with significant implications for the ecological niche and potential application of the *Photorhabdus* isolate BSBT_XH is its remarkable thermal tolerance. The investigation revealed

that *Photorhabdus* isolate BSBT_XH exhibits a wide temperature growth range, from 15°C to an impressive 55°C, with an optimal growth range between 35°C and 40°C. The transition of *Photorhabdus* species from soil-dwelling insect symbionts to potential environmental hazards depends largely on their thermal limits. While *P. luminescens* is primarily recognised for its efficacy against agricultural pests (animal/*in vitro* relevance), the thermal profile of *Photorhabdus* isolate BSBT_XH (optimal growth at 35°C to 40°C) warrants discussion of its human health relevance. The ability of *Photorhabdus* isolate BSBT_XH to maintain metabolic activity at 37°C–40°C is a critical toxicological finding. While most biocontrol *Photorhabdus* strains are restricted by temperate climates, this isolate's thermal plasticity allows it to remain active at human physiological temperatures. This necessitates further investigation into its No-Observed-Adverse-Effect Level (NOAEL) for non-target mammalian systems before large-scale environmental release. This profile represents a significant departure from what is typically observed for many *Photorhabdus* species. Foundational studies established 28°C as the optimal temperature for most temperate *Photorhabdus* strains. Most *Photorhabdus* species are generally classified as mesophiles, with optimal growth temperatures often reported to be closer to 25°C to 30°C. Most entomopathogenic bacteria are considered safe for humans because they cannot survive at mammalian body temperature (37°C). However, the high-temperature adaptation of the Rajasthan isolate brings it into the range of human physiological conditions. In clinical toxicology, *P. asymbiotica* is known to cause human infections, whereas *P. luminescens* is generally excluded from clinical cases due to its thermal sensitivity. For instance, *P. luminescens* subsp. *luminescens* has been described to grow optimally around 28°C–30°C [33,34]. Similarly, *P. temperata* isolates are often reported with optimal growth temperatures not exceeding 40°C [35]. The high cell density achieved by *Photorhabdus* isolate BSBT_XH in TSB (6.5 × 10^{10} CFU/mL) exceeds the typical 10^8 to 10^9 CFU/mL threshold reported in literature as necessary for consistent oral toxicity (Table 4), suggesting this isolate has high potential for commercial formulation development. While some studies indicate survival at slightly higher temperatures, an upper growth limit of 40°C–42°C is frequently cited for many characterised *Photorhabdus* species. In contrast, the isolate *Photorhabdus* isolate BSBT_XH demonstrates robust growth at temperatures up to 50°C, a particularly noteworthy characteristic. The ability of *Photorhabdus* isolate BSBT_XH to thrive at these elevated temperatures, achieving a peak optical density of 1.002 ± 0.0022 at 24 hours when incubated at 40°C, underscores its distinct thermal adaptation. This trend suggests a strong correlation between regional agro-climatic conditions and the evolution of thermal plasticity. The findings for *Photorhabdus* isolate BSBT_XH (optimal growth at 35°C–45°C) push these known biological boundaries even further, indicating that Rajasthan isolates have evolved a unique metabolic resilience to extreme tropical heat.

While this study provides a foundational physiological profile of *Photorhabdus* sp. BSBT_XH, certain limitations remain. Identification was primarily based on 16S rRNA sequencing; therefore, future MLSA is required for deeper taxonomic resolution. Additionally, although the thermal stability of the vegetative cells was established, the direct insecticidal efficacy, as assessed by LD50 bioassays, and the quantification of specific Tc were outside the current scope and remain subjects for subsequent investigation.

Table 4. Toxicological parameters of *P. luminescens* toxins from representative literature

Toxin Type	Model System	Route of Admin.	Dose/Conc. (LD50 or LC50)	Exposure Duration	Outcome	Reference
Tc Toxins	*Manduca sexta*	Oral (Bioassay)	1.5 - 3.2 ng/cm²	48 - 72 h	Midgut paralysis	Blackburn *et al.*[36]
Mcf1 Toxin	*G. mellonella*	Hemocoelic Injection	10^3 cells/larva	24 h	Apoptosis/ Death	Kushwah and Somvanshi[37]
Photox	*In vitro*	Cellular Assay	100 µg/mL	12 - 24 h	Cell lysate	Visschedyk *et al.*[38]
Whole Culture	*Drosophila suzukii*	Oral (Spray)	10^7–10^9 CFU/ mL	7 days	85-100% Mortality	Mastore *et al.*[39]

5. Critical Summary

While 16S rRNA gene sequencing remains the standard for generic assignment, it is important to note that this locus often lacks the resolution to distinguish between closely related subspecies within the *P. luminescens* complex. The 100% similarity observed in the *Photorhabdus* isolate BSBT_XH is a strong indicator of identity, but relying solely on a single conserved marker is considered preliminary. Future studies utilising MLSA or whole-genome sequencing would provide a more robust confirmation of the unique genomic traits that support this isolate's specific adaptation to the Rajasthan climate. The clear superiority of TSB for biomass production (OD600 1.040) suggests that protein-rich environments are essential for this isolate. However, a limitation of this observation is that high biomass does not always correlate linearly with high toxin pathogenicity. Some studies suggest that Nutrient Broth (NB), while supporting lower growth, may trigger different stress-response pathways that lead to higher specific toxin yields per cell. Therefore, while TSB is optimal for mass production, the quality of the toxicological profile in different media remains an area requiring further comparative bioassays. The robust growth observed at 35°C and 40°C directly contradicts the widely accepted thermal limits for *Photorhabdus* species (typically 25°C–34°C). This evidence supports the existence of high-temperature ecotypes in arid regions. The evidence for thermal adaptability provided here is strong for vegetative growth, but it remains preliminary regarding the thermal stability of the secreted toxins themselves, which may denature at lower temperatures than the bacteria can survive.

6. Conclusion

This study successfully characterised a bacterial isolate, designated *Photorhabdus* isolate BSBT_XH, recovered from high-temperature agricultural soil in Tonk, Rajasthan, India. Molecular analysis (16S rRNA gene sequencing) confirmed its identity as *P. luminescens*, a finding supported by typical morphological (Gram-negative rods, characteristic colony pigmentation) and biochemical profiles. Furthermore, TSB was identified as the optimal medium for the efficient, high-yield cultivation of the strain. The most significant finding is the isolate's exceptional thermal adaptability and thermotolerance. Originating from a hot climate, *P. luminescens* BSBT_XH demonstrated viable growth across an extensive range from 15°C and robust activity up to 50°C. This is a considerable increase over the typical 25°C -30°C optimum for most mesophilic *Photorhabdus* isolates. Traditional biopesticides often lose efficacy due to sensitivity to high field temperatures in tropical and subtropical regions. The inherent thermotolerance of *P. luminescens* BSBT_XH, which thrives up to 50°C, directly addresses this major limitation. While this study focuses on the physiological limits, the results establish the necessary parameters for future investigations into the specific signalling pathways triggered by this thermotolerant isolate during host-pathogen interactions. Significant research gaps remain, particularly regarding the biochemical stability of the secreted toxin complexes (Tc and Mcf) under extreme heat. It is currently unknown whether the high-temperature growth observed translates to sustained toxicological efficacy or if the proteomic profile shifts under thermal stress. Therefore, while *P. luminescens* BSBT_XH is a promising candidate for heat-resilient biopesticides; its deployment is contingent upon rigorous biosafety screening, including mammalian cell line cytotoxicity and acute inhalation toxicity assays. Future studies should focus on the genomic basis of this thermotolerance and the signalling pathways (e.g., Nrf2 and MAPK) involved in its host-interaction at elevated temperatures. Therefore, this resilient isolate represents an up-and-coming candidate for developing robust, environmentally friendly biopesticides that can maintain effectiveness and survival even under the extreme heat stress typical of regions like Rajasthan, offering a sustainable alternative to chemical pest control.

7. Acknowledgements

The authors express their gratitude to the Vice Chancellor of Banasthali Vidyapith and the Head of the Department of Bioscience and Biotechnology for providing the necessary facilities to conduct this research. The support provided by DST-FIST to the Vidyapith is also highly acknowledged.

8. Funding

The work of I.R. was supported by the Indian Council of Medical Research (ICMR), New Delhi, India (Grant No. IIRP-2023-0918).

9. Availability of Data and Material

Sequence data supporting the study (accession number PX446793) have been deposited in the National Centre for Biotechnology Information nucleotide database. All other data generated or analysed during this study are included in this published article.

10. References

1. Istkhar, Chaubey AK, Garg AP. Entomopathogenic nematodes in the biological control of insect pests with reference to insect immunity. In Plant Biotic Interactions: State of the Art. 2019; pp. 181-209. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-26657-8_11 PMid:28118817.
2. Istkhar, Chaubey AK. Changes in protein profile and encapsulation avoiding responses of entomopathogenic nematode in the American bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Egypt J Biol Pest Control. 2019; 29:69. https://doi.org/10.1186/s41938-019-0173-1
3. Chaudhary A, Chauhan P, Rao I. Biosystematics of entomopathogenic nematodes: Integrating morphological, molecular, and ecological Approaches. In Studies on Entomopathogenic Nematodes and their behavior. 2024; pp. 17-36. Nova Science, USA.
4. Chauhan P, Istkhar, Chaudhary A. Entomopathogenic nematodes and their mutualistic bacteria: Enemy of the enemies. In Parasitology: Risks and challenges for health and sustainability. 2023; pp. 115-159. Nova Science, USA.
5. Sajnaga E, Kazimierczak W. Evolution and taxonomy of nematode-associated entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus*: An overview. Symbiosis. 2020; 80:13. https://doi.org/10.1007/s13199-019-00660-0
6. Clarke DJ. *Photorhabdus*: A tale of contrasting interactions. Microbiology. 2020; 166:335-348. https://doi.org/10.1099/mic.0.000907.
7. Gangwar P, Trivedi M, Tiwari RK. Entomopathogenic bacteria. In Microbial approaches for insect pest management. 2022; pp. 59-79. Singapore: Springer Singapore. https://doi.org/10.1007/978-981-16-3595-3_2 PMCid: PMC11541078.
8. Muller A. Glowing belowground: Investigating the evolution and biological functions of "*Photorhabdus*" bioluminescence 2024; (Doctoral dissertation, University of Bern).
9. Carter EL, Waterfield NR, Constantinidou C, Alam MT. A temperature-induced metabolic shift in the emerging human pathogen *Photorhabdus asymbiotica*. Msystems. 2024; 9:23. https://doi.org/10.1128/msystems.00970-23 PMid:39445821 PMCid:PMC11575385.
10. Chaudhary A, Sharma R, Chauhan P, Ohri P, Rao I. Antagonistic effects of *Steinernema siamkayai*, *S. pakistanense*, and *Heterorhabditis bacteriophora* on gall hatching and infective juvenile stages of *Meloidogyne incognita*. Russ J Nematol. 2025; 33:19-29. https://doi.org/10.24412/0869-6918-2025-1-19-29
11. Forst S, Clarke D. Bacteria-nematode symbiosis. In Entomopathogenic nematology 2002; pp. 57-77. Wallingford, UK: CABI Publishing. https://doi.org/10.1079/9780851995670.0057
12. Akhurst RJ. Morphological and functional dimorphism in *Xenorhabdus spp*., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. Microbiology. 1980; 121:303-309. https://doi.org/10.1099/00221287-121-2-303
13. Aliyu HU, Tahir F, Agbo EB, Kela SL. Isolation and characterization of *Photorhabdus* spp.(Enterobacteriaceae) isolated from *Heterorhabditis bacteriophora* in Kachere, Nigeria. J Appl Sci Environ Manag. 2021; 25:1345-1348. https://doi.org/10.4314/jasem.v25i8.9
14. Sandström JP, Russell JA, White JP, Moran NA. Independent origins and horizontal transfer of bacterial symbionts of aphids. Mol Ecol. 2001; 10:217-228. https://doi.org/10.1046/j.1365-294X.2001.01189.x PMid:11251800.
15. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In Nucleic acids symposium series. 1999; 41(41):95-98.
16. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res 1997; 25:3389-3402. https://doi.org/10.1093/nar/25.17.3389 PMid:9254694 PMCid:PMC146917.
17. Horiike T. An introduction to molecular phylogenetic analysis. Rev Agric Sci. 2016; 4:36-45.
18. Machado RA, Somvanshi VS, Muller A, Kushwah J, Bhat CG. *Photorhabdus hindustanensis* sp. nov., *Photorhabdus akhursti*i subsp. *akhurstii* subsp. nov., and *Photorhabdus akhurstii* subsp. *bharatensis* subsp. nov., isolated from *Heterorhabditis* entomopathogenic nematodes. Int J Syst Evol Microbiol. 2021; 71:004998. https://doi.org/10.1099/ijsem.0.004998 PMid:34524954.
19. Kumar KV, Vendan KT, Nagaraj SB. Isolation and characterization of entomopathogenic symbiotic bacterium, *Photorhabdus luminescens* of *Heterorhabditis indica* from soils of five agro-climatic zones of Karnataka. Biosci Biotech Res Asia. 2014; 11:129-139.
20. Kajol, Bhat AH, Chaubey AK. Biochemical and molecular characterization of *Photorhabdus akhurstii* associated with

Heterorhabditis indica from Meerut, India. Pak J Nematol. 2020; 38:10. https://doi.org/10.18681/pjn.v38.i01.p15-24

21. Begot C, Desnier I, Daudin JD, Labadie JC, Lebert A. Recommendations for calculating growth parameters by optical density measurements. J Microbiol Methods. 1996; 25:225-232. https://doi.org/10.1016/0167-7012(95)00090-9
22. Olsen RA, Bakken LR. Viability of soil bacteria: Optimization of plate-counting technique and comparison between total counts and plate counts within different size groups. Microb Ecol. 1987; 13:59-74. https://doi.org/10.1007/BF02014963 PMid:24213103
23. Chen G, Maxwell P, Dunyhy GB, Webster JM. Culture conditions for *Xenorhabdus* and *Photorhabdus* symbionts of entomopathogenic nematodes. Nematologica. 1996; 42:124-130. https://doi.org/10.1163/187529296X00139
24. Hall BG, Acar H, Nandipati A, Barlow M. Growth rates made easy. Mol Biol Evol. 2014; 31:232-238. https://doi.org/10.1093/molbev/mst187 PMid:24170494 PMCid:PMC5760743.
25. Brunel B, Givaudan A, Lanois A, Akhurst RJ, Boemare N. Fast and accurate identification of *Xenorhabdus* and *Photorhabdus* species by restriction analysis of PCR-amplified 16S rRNA genes. Appl Environ Microbiol. 1997; 63:574-580. https://doi.org/10.1128/aem.63.2.574-580.1997 PMid:9023937 PMCid:PMC168349.
26. Vishrutkumar KS, Tamilvendan K, Mahadevaswamy M, Prabhuraj A. Biochemical diversity of *Photorhabdus luminescens* isolated from *Heterorhabditis indica* collected from different agroclimatic regions of Karnataka. 2013; 596-602.
27. Machado RA, Wüthrich D, Kuhnert P, Arce CC, Thönen L, Ruiz C, Erb M. Whole-genome-based revisit of *Photorhabdus* phylogeny: proposal for the elevation of most *Photorhabdus* subspecies to the species level and description of one novel species, *Photorhabdus bodei* sp. nov., and one novel subspecies *Photorhabdus laumondii* subsp. *clarkei* subsp. nov. Int J Syst Evol Microbiol. 2018; 68:2664-2681. https://doi.org/10.1099/ijsem.0.002820 PMid:29877789 PMCid:PMC6267812.
28. Machado RA, Bhat AH, Castañeda-Álvarez C, Půža V, San-Blas E. *Photorhabdus aballayi* sp. nov. and *Photorhabdus luminescens* subsp. *venezuelensis* subsp. nov., isolated from *Heterorhabditis amazonensis* entomopathogenic nematodes. Int J Syst Evol Microbiol. 2023; 73:005872. https://doi.org/10.1099/ijsem.0.005872 PMid:37171451
29. Goodrich-Blair H, Clarke DJ. Mutualism and pathogenesis in *Xenorhabdus* and *Photorhabdus*: Two roads to the same destination. Mol Microbiol. 2007; 64:260-268. https://doi.org/10.1111/j.1365-2958.2007.05671.x PMid:17493120.
30. Castaneda-Alvarez C, Machado RA, Morales-Montero P, Boss A, Muller A, Prodan S, Aballay E. *Photorhabdus antumapuensis* sp. nov., a novel symbiotic bacterial species associated with *Heterorhabditis atacamensis* entomopathogenic nematodes. Int J Syst Evol Microbiol. 2022; 72:005525 https://doi.org/10.1099/ijsem.0.005525 PMid:36223180
31. Peel MM, Alfredson DA, Gerrard JG, Davis JM, Robson JM, McDougall RJ, Akhurst RJ. Isolation, identification, and molecular characterization of strains of *Photorhabdus luminescens* from infected humans in Australia. J Clin Microbiol. 1999; 37:3647-3653. https://doi.org/10.1128/JCM.37.11.3647-3653.1999 PMid:10523568 PMCid:PMC85716.
32. Ferreira T, Addison MF, Malan AP. *In vitro* liquid culture of a South African isolate of *Heterorhabditis zealandica* for the control of insect pests. Afr Entomol. 2014; 22:80-92. https://hdl.handle.net/10520/EJC150972
33. Forst S, Nealson K. Molecular biology of the symbiotic-pathogenic bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. Microbiol Rev. 1996; 60:21-43. https://doi.org/10.1128/mr.60.1.21-43.1996 PMid:8852894 PMCid:PMC239416.
34. Grewal PS, Abrol DP, Shankar U. Entomopathogenic nematodes as tools in integrated pest management. Integrated pest management: Principles and practice, 2012; 162-236. https://doi.org/10.1079/9781845938086.0162
35. Tailliez P, Laroui C, Ginibre N, Paule A, Pagès S, Boemare N. Phylogeny of *Photorhabdus* and *Xenorhabdus* based on universally conserved protein-coding sequences and implications for the taxonomy of these two genera. Proposal of new taxa: *X. vietnamensis* sp. nov., *P. luminescens* subsp. *caribbeanensis* subsp. nov., *P. luminescens* subsp. *Hainanensis* subsp. nov., *P. temperata* subsp. *Khanii* subsp. nov., *P. temperata* subsp. *tasmaniensis* subsp. nov., and the reclassification of *P. luminescens* subsp. *thracensis* as *P. temperata* subsp. *thracensis* comb. nov. Int J Syst Evol Microbiol. 2010; 60:1921-1937. https://doi.org/10.1099/ijs.0.014308-0 PMid:19783607.
36. Blackburn M, Golubeva E, Bowen D, Ffrench-Constant RH. A novel insecticidal toxin from *Photorhabdus luminescens*, toxin complex a (Tca), and its histopathological effects on the midgut of *Manduca sexta*. Appl Environ Microbiol. 1998; 64:3036-3041. https://doi.org/10.1128/AEM.64.8.3036-3041.1998 PMid:9687470 PMCid:PMC106812.
37. Kushwah J, Somvanshi VS. Photorhabdus: A microbial factory of insect-killing toxins. Microbial Factories: Biodiversity, Biopolymers, Bioactive Molecules. 2015; 2:235-240. New Delhi: Springer India. https://doi.org/10.1007/978-81-322-2595-9_15
38. Visschedyk DD, Perieteanu AA, Turgeon ZJ, Fieldhouse RJ, Dawson JF, Merrill AR. Photox, a novel actin-targeting

mono-ADP-ribosyltransferase from *Photorhabdus luminescens*. J Biol Chem. 2010; 285:13525-13534. https://doi.org/10.1074/jbc.M109.077339 PMid:20181945 PMCid:PMC2859513.

39. Mastore M, Caramella S, Quadroni S, Brivio MF. *Drosophila suzukii* susceptibility to the oral administration of *Bacillus thuringiensis*, *Xenorhabdus nematophila* and its secondary metabolites. Insects. 2021; 12:635. https://doi.org/10.3390/insects12070635 PMid:34357295 PMCid:PMC8305655.

Authors Contributions

Conceptualisation: I.R., P.C.; Data curation: P.C., A.C., I.R.; Formal analysis: P.C., I.R., A.C., K.Y.; Funding acquisition: I.R.; Investigation: I.R., P.C.; Methodology: I.R., P.C.; Project administration: I.R.; Resources: I.R.; Supervision: I.R.; Validation: I.R., P.C., K.Y.; Visualisation: P.C., I.K.; Writing original draft– P.C., I.R.; Writing review & editing: I.R. All the authors read and approved the final version of the manuscript.

Access this journal online	
Website: https://informaticsjournals.com/journals/toxi/home	**Quick Response Code**