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Research Article

Thermal tolerance and characterisation of *Xenorhabdus* isolates from *Steinernema pakistanense* in semi-arid regions of Rajasthan, India

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Abstract: *Xenorhabdus* bacteria, symbionts of entomopathogenic nematodes of the genus *Steinernema*, influence the effectiveness of these nematodes in biological pest control and exhibit different temperature tolerances across species and strains. Certain strains can endure a wide range of temperatures, whereas others are more sensitive. This study investigated heat-tolerant isolates of *Xenorhabdus* species, the symbiotic bacteria associated with the entomopathogenic nematode *Steinernema pakistanense*, from semi-arid regions of Rajasthan, India. Bacteria were obtained from their nematode host, *S. pakistanense*, isolated from agricultural fields in Tonk district, Rajasthan, India. Their biochemical characterisation showed positive results for catalase, oxidase, and protease activities, and they metabolise various carbohydrates, including glucose, sucrose, and maltose. The isolates demonstrated remarkable thermotolerance, maintaining viability and metabolic activity up to 45°C temperature. Growth kinetics analysis revealed optimal growth between 25°C and 45°C for all isolates, with significant metabolic activity persisting at elevated temperatures. A comparative study with temperate *Xenorhabdus* species showed that the Rajasthan isolates exhibited significantly greater thermotolerance. This adaptation to high temperatures appears crucial for the bacterium's survival in arid agricultural soils where summer temperatures frequently exceed 40°C. Our research lays the foundation for developing thermotolerant formulations of heat-resistant *Xenorhabdus*, thereby improving its effectiveness and sustainability in pest control across diverse environmental conditions.

Keywords: Biological control, entomopathogenic nematode, thermotolerance, viability, *Xenorhabdus*

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INTRODUCTION

Agricultural pests pose a significant threat to global food security, and sustainable pest management is a priority. Although chemical pesticides are effective, their use raises concerns about their effects on environmental conditions and human health, as well as the development of pest resistance (Zhou *et al*., 2024). Therefore, there is a need to introduce microbial-based biocontrol agents, including Entomopathogenic Nematodes (EPNs) and their symbiotic bacteria (Singh *et al*., 2022). The genus *Xenorhabdus* represents a unique group of facultatively anaerobic, gram-negative bacteria within the order Enterobacterales, in a symbiotic relationship with EPNs exclusively associated with the genus *Steinernema*. First described by Poinar in 1990, they were initially classified as a new genus due to their unique physiological traits, distinct morphological characteristics, and symbiotic behaviour. These bacteria play a significant role in symbiotic relationships, which help the nematode utilise the insect host by causing infection and killing it (Forst & Clarke, 2002). The nematode partner acts as a vector for bacteria. It helps enter the hemocoel cavity of the host insect, where the bacteria proliferate and release several bioactive compounds, which eventually kill the insect by depleting nutrients and suppressing the insect's immune response inside the insect's body (Istkhar *et al*., 2019; Vashisth *et al*., 2013; Hazir *et al*., 2003). *Xenorhabdus* has attracted attention for its potential applications in agriculture, as it produces a range of bioactive compounds, including insecticidal toxins, antibiotics, antifungals, and nematicidal agents. Despite its significance, EPN diversity remains largely unexplored in India.

The nematode *S. pakistanense* has recently been reported to be effective in targeting insect pests (Bhat *et al*., 2019). This nematode has been reported from the semi-arid region of Rajasthan, and its pathogenicity has been established against lepidopteran and coleopteran insect pests (Chaudhary *et al.*, 2025). To optimise its potential for Integrated Pest Management (IPM) strategies, it is essential to understand the symbiotic bacterium's physiology and environmental tolerance, especially across various agroclimatic conditions. The potential of *Xenorhabdus* species under different climatic conditions, especially at high temperatures, is a crucial factor that determines their effectiveness and viability for biocontrol applications, particularly in regions with high temperature

variability. One of the most critical abiotic factors for microorganisms is temperature, which affects their survival, growth, and pathogenicity. For field applications of biocontrol agents like *Xenorhabdus,* they need to be heat-tolerant to ensure their feasibility. In tropical or subtropical areas, climatic conditions vary significantly, with temperatures often exceeding 40°C. Therefore, for successful biocontrol programs, microbial agents must exhibit heat adaptability. Although *Xenorhabdus* has significant economic and ecological importance, little is known about its growth potential at high temperatures. Therefore, understanding its physiology at high temperatures can provide insights into selecting resilient strains suitable for a variety of agricultural zones.

The present study focuses on isolating and identifying symbiotic bacteria *Xenorhabdus* from *S. pakistanense*, isolated from agricultural soils in Tonk district, Rajasthan (Chaudhary *et al.*, 2025), and on determining their growth potential at high temperatures. The morphological and biochemical characterisation of the bacterial symbiont, *Xenorhabdus,* was also performed. The bacterial symbiont's potential to colonise its nematode host, exhibit insecticidal activities, and produce secondary metabolites is adversely affected by high temperatures. Therefore, the study also contributes to the heat tolerance of *Xenorhabdus*, thereby enhancing the efficacy of biological control programs in areas with high temperatures. Moreover, *Xenorhabdus's* heat tolerance enables its use in practical applications.

ABBREVIATIONS

EPNs - Entomopathogenic Nematodes; IPM - Integrated Pest Management; IJs - Infective Juveniles; NBTA - Nutrient Agar supplemented with Bromothymol Blue and Triphenyltetrazolium Chloride; CFUs - Colony-Forming Units; TSB - Tryptic Soy Broth; OD - Optical Density; BOD - Biochemical Oxygen Demand; NA - Nutrient Agar; LB - Luria-Bertani Broth; NB - Nutrient Broth

MATERIALS AND METHODS

Isolation of symbiotic bacteria

Soil sampling was carried out in agricultural fields at a surface depth of 15-20 cm, at temperatures of 28°C-33°C, in the Tonk district of Rajasthan. Ten positive samples with *S. pakistanense* were identified (Chaudhary *et al*., 2025). The bacteria associated with EPN *S. pakistanense* were isolated directly from Infective Juveniles (IJs). Approximately 150 IJs were stored in double-distilled water, surface sterilised with 0.1% sodium hypochlorite solution for 2-3 minutes, and then washed three times in distilled water (Forst & Clarke, 2002). To release bacteria from the IJs, they were transferred to a sterile microcentrifuge tube containing 100-150 µL nutrient broth. Then, the nematodes were crushed and macerated under laminar airflow using a sterile micropestle (Akhurst, 1980). Samples of macerate were spread onto NBTA plates (0.025gm/l bromothymol blue, 0.04 gm/l 2,3,5-triphenyltetrazolium chloride, 28 gm/l nutrient agar, pH 7) for the isolation of *Xenorhabdus*. All the NBTA plates were incubated in a BOD incubator (REMI CI-6 PLUS LED) for 24-48 h at 28°C in the dark to support bacterial growth. The bacterial colonies were then observed on NBTA plates. Characteristic blue colony plates were designated as BSBT_X5, BSBT_X6, BSBT_X7, BSBT_X8, BSBT_X9, BSBT_X10, BSBT_X11, BSBT_X12, BSBT_X13, and BSBT_X14, which were found to be *Xenorhabdus* species. Based on pigmentation and specific morphology of *Xenorhabdus* species, colonies were subcultured onto fresh NBTA plates (Boemare *et al*., 1993). For long-term storage, a pure culture of bacteria is transferred to 20-30% glycerol stocks at -80°C.

Phenotypic and biochemical characterisation of bacteria

Phenotypic characterisation of bacteria were observed like colony shape, size and color on different agar plates; Nutrient Agar (peptone: 5 g, beef extract: 3 g, sodium chloride: 5 g, agar: 15 g, pH 7.0 in 1000 ml distilled water), MacConkey Agar (peptone: 17 g, proteose peptone: 3 g, lactose: 10 g, bile salts: 1.50 g, sodium chloride: 5 g, neutral red: 0.03 g, agar: 15 g, pH 7.0 in 1000 ml of distilled water) and NBTA plates, after 48 h of incubation period at 35°C in dark (Aly & Mona 2009).

The biochemical interpretation was performed using the Enterobacteriaceae Identification Kit KB016 Hi24TM from Hi-media (Mumbai, India), which identifies Gram-negative Enterobacteriaceae. This identification kit is based on a standardised colorimetric system comprising 11 assays for carbohydrate utilisation and 13 for standard biochemical assays. For biochemical interpretation, only a pure culture is used. The bacterial colonies are cultured on MacConkey Agar, a differential agar medium. A single colony of bacteria is inoculated into 5 ml Brain Heart Infusion Broth (Hi-Media). The inoculated broth was then incubated overnight at 35°C in a BOD incubator on an orbital shaker until the inoculum reached 0.1 OD at 620 nm. Under aseptic conditions, using the surface inoculation method, 50 µl of inoculum was transferred onto all 24 wells of the kit and incubated according to the manufacturer's guidelines. Changes in the well colour were observed (Bhat *et al*., 2019; Okram *et al*., 2025).

Microscopic identification of bacteria was performed using the Gram Staining Kit (Hi-Media K001-1KT) (Okram *et al*., 2025). A sterile loopful of 24-hour-old bacterial culture was placed on a clean slide, and a thin smear was prepared. It was heat fixed and stained with Gram's stain. The air-dried slide

was observed and photographed under a microscope (Nikon Eclipse Ni-U, Japan) at 100× oil-immersion magnification.

Standardisation of liquid media kinetics for bacterial growth

To understand the growth conditions required for *Xenorhabdus*, fermentation characteristics were defined using three different liquid media (HiMedia) with varying component compositions: Soyabean Casein Digest Medium (Tryptic Soy Broth) (tryptone: 17 g, soya peptone: 3 g, sodium chloride: 5 g, dipotassium hydrogen phosphate: 2.50 g, dextrose: 2.50 g, pH 7.3± 0.2 in 1000 ml distilled water), Luria Bertani Broth Miller (Casein enzymic hydrolysate: 10 g, sodium chloride: 10 g, yeast extract: 5 g, pH 7.5± 0.2 in 1000 ml distilled water), and Nutrient Broth (Peptone: 5 gram; sodium chloride: 5 g, HM peptone B: 1.5 g, yeast extract: 1.5 g, pH 7.4± 0.2 in 1000 ml distilled water). The 100 µL bacterial suspension was added to 50 ml of liquid media in a 250 ml Erlenmeyer flask, and the flask was incubated at a standard bacterial growth temperature (36±2°C) with orbital shaking at 180 rpm. Optical Density (OD600) was measured at 4-h intervals, and viable cell counts were performed using the standard plate count method by the spreading plate method described by Roszak *et al*. (1987), which is recorded as Colony-Forming Units (CFUs) per millilitres (CFUs/ml) (Thomas *et al*., 2012). Growth characteristics were tracked for maximum cell density, lag phase duration, and specific growth rate, and mean and standard deviation values were determined using XLSTAT. The growth curve was plotted using GraphPad Prism (Version 8.0, GraphPad Software Inc., San Diego, CA, United States).

Determining the optimum growth temperature

Xenorhabdus isolates were revived on NBTA media plates. A single blue-green colony was picked and inoculated into 50 ml of TSB broth in a sterile 250 ml Erlenmeyer flask. The inoculated broth culture was incubated in an orbital shaking BOD incubator for 12-14 h at 180 rpm and 35°C until the turbidity reached the mid-log phase. In 15 ml sterile culture tubes, 5 ml aliquots of TSB broth were prepared. The inoculated bacterial culture was diluted to a 0.1 OD at 600nm to ensure the uniform starting inoculum by following the method described by Hall *et al*. (2014). 2 ml of diluted culture was added to each tube with TSB broth and incubated at temperatures 15ºC, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, and 50°C in BOD incubators with 180 rpm orbital shaking. To ensure isolate reproducibility, each temperature condition was examined in triplicate. Bacterial growth was monitored at 6-h intervals over 60 h by measuring OD600 with a UV-Vis Double Bean Spectrophotometer (Systronics, 2202TS). For each reading, 1 ml of culture was aseptically withdrawn from culture tubes, and the OD at 600 nm was recorded against a blank of sterile TSB broth. Descriptive analysis of the data was performed using IBM SPSS Statistics (Version 22). The results are expressed as mean ± standard deviation. The growth curves were plotted against OD 600 nm and a 6 h time interval using GraphPad Prism.

RESULTS AND DISCUSSION

Phenotypic and biochemical characterisation

A total of 10 bacteria isolated from *S. pakistanense* (BSBT X5-X14) were cultured on NBTA plates, generating both Phase I and II variants, which were identified by their dye-absorbance profiles. The morphological variations across different media plates demonstrate the versatile growth characteristics of *Xenorhabdus*. Consistent colony morphology suggests stable physiological properties across nutrient environments (Figure 1). When streaked onto (a) Nutrient Agar (NA) plates, circular colonies with white to cream colour pigmentation with a diameter of 2-3 mm appeared. As described by Boemare (2002), the Phase I variant of the genus *Xenorhabdus* can adsorb dyes, such as bromothymol blue and neutral red. On (b) NBTA plates, it appeared to be blue-green, and on (c) MacConkey agar plates, it formed red colonies. Interpretations of results are shown in Table 1.

The results of overnight inoculated culture in the Enterobacteriaceae Identification Kit KB016 Hi24™ from Hi-media were interpreted using the standard identification index provided with the kit, which mainly showed adverse reactions

Table 1. Phenotypic and morphological characteristics of bacteria

		Differential Media		
	Colony properties	**Nutrient agar**	**MacConkey agar**	**NBTA**
Morphology	Colony shape	Circular	Circular	Circular
	Margin	Smooth edge	Smooth edge	Smooth edge
	Elevation	Slightly convex	Slightly convex	Slightly convex
	Colony size	1 to 3 mm	2 to 4 mm	2 to 4 mm
	Surface	Smooth	Smooth and shiny	Smooth and shiny
	Texture/ Consistency	Butyrous	Butyrous	Butyrous
	Pigmentation	Cream-yellow	Reddish pink	Greenish-blue
	Optical properties	Opaque	Opaque	Opaque
Microscopy	Gram-staining	Gram-negative, pink colour, rod-shaped cells		

Table 2. Biochemical identification of *Xenorhabdus* isolates from the Enterobacteriaceae identification kit KB016 Hi24™

S. No.	Conventional biochemical tests	Results									
		BSBT_ X5	BSBT_ X6	BSBT_ X7	BSBT_ X8	BSBT_ X9	BSBT_ X10	BSBT_ X11	BSBT_ X12	BSBT_ X13	BSBT_ X14
1	ONPG	-	-	-	-	-	+	-	-	-	-
2	Lysine utilisation	-	-	-	-	-	-	-	-	-	-
3	Ornithine utilisation	-	-	-	-	-	-	-	-	-	+
4	Urease	-	-	-	-	-	-	-	-	-	-
5	Phenylalanine Deamination	-	-	-	-	-	-	-	-	-	-
6	Voges Proskauer's	-	-	-	-	-	-	-	-	-	-
7	Methyl red	-	-	+	-	-	-	-	-	-	-
8	Indole	-	-	-	-	-	-	-	-	-	-
9	PYR	-	-	-	-	-	-	-	-	-	-
10	β- Glucuronidase	-	-	-	-	-	-	-	-	-	-
11	α- Galactosidase	-	+	-	-	-	-	-	+	-	-
12	β- Xylosidase	-	-	-	+	-	-	-	+	-	-
13	Esculin hydrolysis	+	-	-	-	-	+	-	-	-	-
14	Sucrose	-	-	-	-	+	-	-	-	+	-
15	Sorbitol	-	-	-	-	-	-	-	-	-	-
16	Trehalose	-	-	-	-	-	-	-	-	-	+
17	Glucose	-	-	-	+	-	-	+	-	-	-
18	Cellobiose	-	-	-	-	-	-	+	-	-	-
19	Melibiose	-	+	-	-	-	-	-	-	-	-
20	Salicin	-	-	-	-	-	-	-	-	-	-
21	Mannose	-	-	-	-	-	-	-	-	-	-
22	Maltose	-	-	-	-	-	-	-	-	-	-
23	Raffinose	-	-	-	-	-	-	-	-	-	-
24	Lactose	-	-	-	-	-	-	+	-	-	-

*Based on %, more than 95% isolates showed '-' results, '-', negative, '+', positive, ONPG, ortho-nitrophenyl-β-D-galactopyranoside, PYR, PyrrolidonylArylamidase

(Table 2). The biochemical characterisation of the isolates using the Enterobacteriaceae Identification Kit confirmed their placement within the Enterobacteriaceae family as oxidase-negative and nitrate-positive rods, but also revealed significant deviations when compared to the reference species *Xenorhabdus indica* (Bhat *et al*., 2019). While the isolates were consistently negative for many tests, such as ONPG, lysine utilisation, and indole, aligning with the profile of *X. indica*, notable adverse reactions were observed in carbohydrate utilisation and other biochemical markers. For example, all BSBT isolates tested negative for sorbitol, and most were negative for glucose, both of which are positive for *X. indica* (Bhat *et al*., 2019). This biochemical variability is not unusual within the genus, as different species and even strains of the same species can exhibit distinct metabolic profiles (Dreyer *et al*., 2018; Abd-Elgawad, 2022). Studies have shown that variations in the ability to utilise different carbohydrates and other biochemical traits are common among *Xenorhabdus* isolates (Akhurst, 1983). This inherent diversity makes species identification based solely on traditional biochemical tests challenging. Therefore, the unique pattern of adverse reactions observed in the BSBT isolates, when contrasted with the published data for *X. indica*, strongly suggests potential strain-level or even species-level diversity that warrants further molecular investigation.

The microscopic examination of Gram-staining slides reveals the properties of Gram-negative bacteria, supporting the taxonomic classification of *Xenorhabdus* sp. The results of Gram staining at 100X magnification show pink/red colouration and rod-shaped morphology of bacteria (Figure 1).

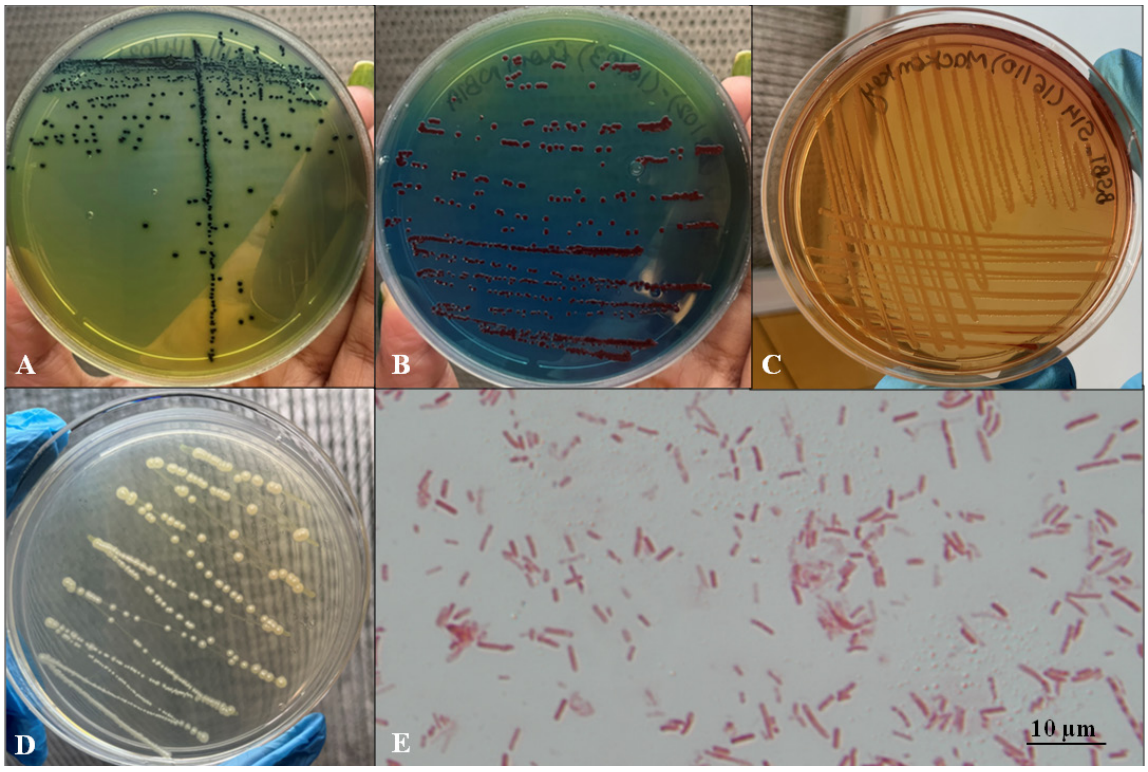


Figure 1. Phenotypic characteristics of bacterial isolates. **(A, B)** Streaking on NBTA plates, Phase I and Phase II colonies, **(C)** Streaking on MacConkey agar plate, **(D)** Streaking on NA plate, **(E)** Gram staining slide.

Bacterial growth characteristics in different liquid media

To obtain plates with countable colonies, a series of tenfold dilutions of the *Xenorhabdus* isolate was prepared in different test tubes labelled from 10^{-1} to 10^{-9}. 0.1 ml of each dilution was spread on the agar medium. Plates were incubated at the optimal growth temperature for *Xenorhabdus,* that is, 28°C±2°C, for 24 to 48 h. After incubation, single colonies appearing on plates with different dilution factors of the *Xenorhabdus* isolate were counted. Colonies in the 30-300 CFU range were counted (Thomas *et al*., 2012), as fewer or more colonies may lead to inaccurate results. CFUs/ml was calculated (Table 3) as a result of which the dilution factor of bacteria ranges from 8.65×10^5 CFUs/ml of 10^{-3} dilution factor to 7.8×10^{11} CFUs/ml of 10^{-9} dilution factor. Therefore, to further assess the growth of *Xenorhabdus* isolates under different media compositions, a single colony from an agar plate was diluted 10^{-9} as the inoculum.

Fermentation was conducted in three different liquid media, and bacterial growth was monitored over 60 h. The growth pattern in all three media is consistent with an intense exponential growth phase within 12-16 h, followed by a stationary phase up to 48 h, and then reaching the death phase, as depicted in Figure 2. According to the growth curve, the maximum growth of bacteria was observed in TSB media within 16 h, with a bacterial cell density of OD 600 nm 1.004±0.004, followed by LB and NB. The maximum

Table 3. CFUs at different dilution factors

S. No.	Dilution Factor	CFUs Counted	CFUs/ml of sample
1	10^{-1}	TNTC	TNCT
2	10^{-2}	TNTC	TNTC
3	10^{-3}	865	$865 \times 10^3 = 8.65 \times 10^5$ CFUs/ml
4	10^{-4}	735	$735 \times 10^4 = 7.35 \times 10^6$ CFUs/ml
5	10^{-5}	428	$428 \times 10^5 = 4.28 \times 10^7$ CFUs/ml
6	10^{-6}	302	$302 \times 10^6 = 3.02 \times 10^8$ CFUs/ml
7	10^{-7}	225	$225 \times 10^7 = 2.25 \times 10^9$ CFUs/ml
8	10^{-8}	154	$154 \times 10^8 = 1.54 \times 10^{10}$ CFUs/ml
9	10^{-9}	78	$78 \times 10^9 = 7.8 \times 10^{11}$ CFUs/ml

*CFUs, Colony Forming Units; TNTC, Too Numerous To Count

cell density of bacteria in TSB media was determined to be 1.028±0.001 at an OD600 of 28 h. In contrast, in LB media, the maximum cell density was observed at 32 h (1.013±0.002), and the lowest cell density was observed in NB media at 36 h (1.002±0.001). Based on the growth curves shown in Figure 2, the lag phase duration for *Xenorhabdus* isolates appears to be approximately 4 h across the tested media. The specific growth rate, calculated as the maximum slope of the exponential growth phase that is, 0.16 h^{-1} the highest specific growth rate in TSB media, indicates it is most favorable for *Xenorhabdus* isolates' proliferation in

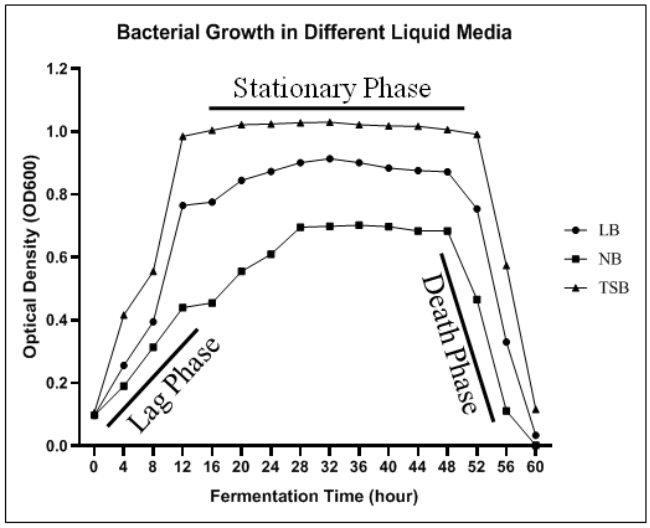


Figure 2. Growth curve of bacteria in different liquid media.

comparison with other two medium (LB 0.12 h^{-1} and NB 0.09 h^{-1}), where the 'h^{-1}' indicates the inverse of an hour; the rate at which the bacterial population is increasing in size over time during the exponential growth phase.

Since *Xenorhabdus* bacteria are symbionts of EPNs, they have complex metabolic requirements for their development. The TSB medium, rich in soy peptone and other growth-promoting factors such as amino acids, peptides, and vitamins, provides a nutrient-rich environment that supports better bacterial fermentation compared to NB and LB. In a study by Ferreira *et al*. (2014), Xenorhabdus sp. can grow in any medium, but specifically, it requires a protein-rich environment. Moreover, Han *et al*. (2021) suggest that, for *X. nematophila*, TSB media is a better nutrient source, with maltose and proteose peptone identified as the best carbon and nitrogen sources, respectively, affecting the production of its secondary metabolites.

Optimum growth temperatures

Optimum growth temperatures for *Xenorhabdus* isolates (BSBT X5-X14) were determined at 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, and 50°C (Figure 3). The findings revealed that there was no clear growth of bacteria at 15ºC, whereas the OD600 range began to increase at 20ºC, with a maximum of 0.196 in isolate BSBT_X11 at 46 h. At 25°C, isolate BSBT_X11 shows a maximum OD600 of 0.900 at 30 h. At 30°C, isolates BSBT_X10, BSBT_X11, BSBT_X12, BSBT_13, and BSBT_X14 have the highest OD600 of 0.995, with moderate growth, reaching the exponential phase within 18-24 h. As the bacterial growth rate was moderate up to 30°C, the maximum growth of isolates was observed above 35°C, extending to 45°C, where the maximum OD600 reached 1.02 in isolates BSBT_X9 and BSBT_X14. Additional data is provided in Table 4. No significant growth was observed at 50°C, indicating the threshold temperature for survival of the isolates.

The study demonstrates that all symbiont isolates from *S. pakistanense* can survive at high temperatures, with the majority showing significant growth at 35°C, 40°C, and 45 °C. There is a knowledge gap regarding how *Xenorhabdus* bacterial species in India respond to high temperatures. The study conducted by Somvanshi *et al.* (2006) shows that the nematode host of *X. indica*, *S. thermophilum,* can survive at temperatures up to 40°C. In contrast, the growth of bacteria was found to be maximum at 41°C in TSB, whereas at 36°C in NB media. Moreover, in a study by Shahina *et al.* (2004), bacteria isolated from *S. pakistanense* were identified as *X. nematophila*, which showed maximum growth at 38°C. One of the key findings of this research highlights *Xenorhabdus* isolates exceptional heat tolerance when found in *S. pakistanense*. This

Table 4. OD600 at different temperatures of isolates

Temperatures	20°C		25°C		30°C		35°C		40°C		45°C		50°C	
Isolates	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**
BSBT_X5	0.181	0.096	0.811	0.212	0.931	0.333	1.011	0.399	0.996	0.352	0.856	0.343	0.183	0.096
BSBT_X6	0.175	0.094	0.812	0.233	0.931	0.333	1.014	0.352	1	0.399	0.956	0.322	0.183	0.096
BSBT_X7	0.195	0.098	0.899	0.231	0.945	0.341	1.04	0.405	1.007	0.396	0.908	0.308	0.196	0.097
BSBT_X8	0.184	0.098	0.894	0.209	0.933	0.355	1.014	0.39	1.01	0.352	0.914	0.296	0.183	0.097
BSBT_X9	0.103	0.184	0.894	0.224	0.945	0.395	1.021	0.404	1.014	0.394	0.975	0.376	0.186	0.1
BSBT_X10	0.186	0.099	0.885	0.307	0.965	0.397	1.018	0.456	1.005	0.405	0.974	0.389	0.196	0.039
BSBT_X11	0.196	0.101	0.9	0.31	0.913	0.315	1.014	0.398	1.006	0.324	0.989	0.342	0.185	0.105
BSBT_X12	0.184	0.105	0.813	0.21	0.993	0.304	1.014	0.421	1.003	0.414	0.975	0.345	0.186	0.997
BSBT_X13	0.185	0.098	0.812	0.243	0.995	0.304	1.014	0.347	1.007	0.316	0.977	0.316	0.196	0.102
BSBT_X14	0.187	0.102	0.895	0.274	0.963	0.385	1.022	0.456	1.004	0.406	0.995	0.396	0.189	0.098

*Max, OD600 Maximum; Min, OD600 Minimum

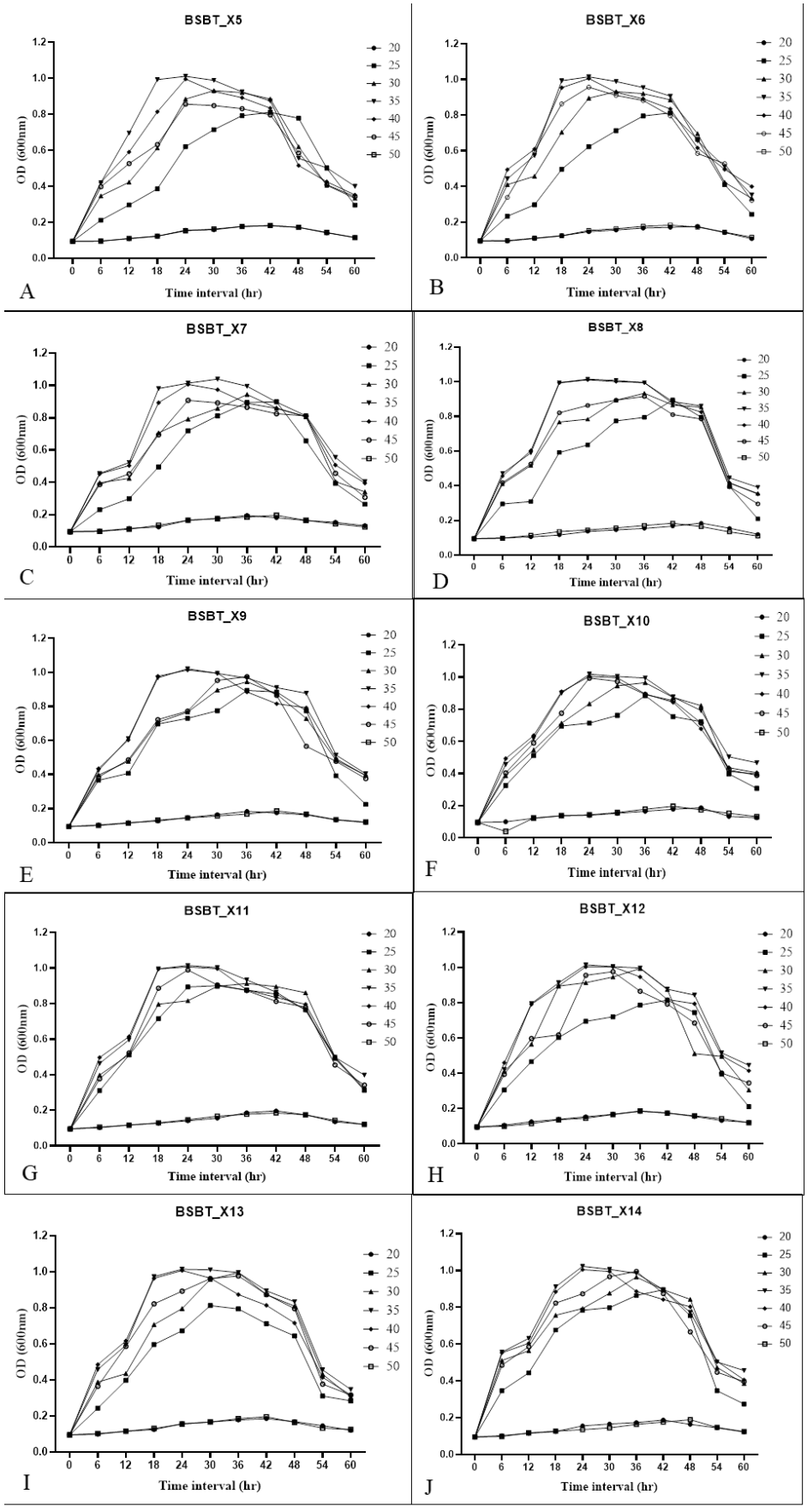


Figure 3. Growth curve of bacterial isolates at optimum temperatures. **(A)** BSBT_X5, **(B)** BSBT_X6, **(C)** BSBT_X7, **(D)** BSBT_X8, **(E)** BSBT_X9, **(F)** BSBT_X10, **(G)** BSBT_X11, **(H)** BSBT_X12, **(I)** BSBT_X13, **(J)** BSBT_X14.

adaptation is particularly significant in South Asian regions, as documented by Shahina *et al*. (2004). The bacteria's heat tolerance represents an evolutionary adaptation to the challenging climate of South Asia, where high temperatures, arid conditions, and semi-arid environments are typical. Research has revealed that *X. indica* demonstrates superior heat tolerance when it is a symbiont of *S. thermophilum*. Compared to other *Xenorhabdus* species, particularly *X. nematophila* and *X. bovienii*, which are commonly found in cooler regions. Henceforth, the *S. pakistanense* isolates from the Rajasthan region may represent evolutionary adaptations to heat tolerance, as summer temperatures there can exceed 40 °C. Their symbiont bacteria possess traits that enable them to survive in arid, semi-arid, and high-temperature conditions, as all isolates survived at 45°C.

CONCLUSIONS

The research examining thermal tolerance in *Xenorhabdus* isolates isolated from *S. pakistanense* in Rajasthan's semi-arid regions reveals essential aspects of how entomopathogenic bacteria adapt to challenging environments. These *Xenorhabdus* isolates exhibited exceptional thermal resilience, surviving and functioning across a wide range of temperatures typical of semi-arid climates. This adaptation is particularly significant for their role as biocontrol agents, as it ensures their effectiveness despite temperature fluctuations. The bacteria's ability to maintain activity under thermal stress makes them especially valuable for pest management strategies in regions experiencing significant temperature variations. This thermal adaptability represents a crucial evolutionary trait that enhances their potential applications in agricultural pest control, especially in areas where temperature extremes could limit the effectiveness of other biological control agents.

Additional research is needed to understand the genetic mechanisms underlying these bacteria's heat tolerance, including identifying the key genes and metabolic pathways that enable this adaptation. Understanding these molecular mechanisms could allow scientists to develop more resilient bacterial strains through targeted genetic modification or selective breeding, ultimately creating more effective biocontrol agents for field applications. Decoding the genetic basis of thermal tolerance would not only advance our understanding of bacterial adaptation but also provide practical tools for enhancing the performance of these organisms in agricultural settings where temperature stress is a significant challenge.

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