



Thermal sensitivity of *Xenorhabdus bovienii* (Enterobacterales: Morganellaceae) isolated from *Steinernema feltiae* (Rhabditida: Steinernematidae) originating from different habitats

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Received: 26 September 2021 / Accepted: 29 June 2022 / Published online: 21 July 2022
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Abstract

The soil-dwelling nematode *Steinernema feltiae* is found across a wide range of environmental conditions. We asked if its only bacterial symbiont, *Xenorhabdus bovienii*, shows intraspecific variability in its thermal range, which may affect effectiveness of *S. feltiae* against host insects. We isolated *X. bovienii* from *S. feltiae* from six different natural locations with different mean annual temperatures and two laboratory cultures. We estimated *X. bovienii* thermal range and determined the specific growth rate based on optical density measurements and mathematical modeling using the Ratkowsky model. The minimal temperature (T_{\min}) of *X. bovienii* growth ranged from 0.9 ± 2.2 °C to 7.1 ± 1.4 °C. The optimal temperature (T_{opt}) varied between 25.1 ± 0.2 °C and 30.5 ± 0.2 °C. The model showed that *X. bovienii* stops multiplying at around 36 °C. The calculated specific *X. bovienii* growth rate ranged from 2.0 ± 0.3 [h⁻¹] to 3.6 ± 0.5 [h⁻¹]. No differences in T_{\min} , T_{opt} , and T_{\max} between the isolated bacteria were found. Additionally, *X. bovienii* T_{opt} did not correlate with the mean annual temperature of *S. feltiae* origin. However, the obtained growth curves suggested that the analyzed *X. bovienii* may show some variability when comparing the growth curves characteristics.

Keywords Entomopathogenic nematodes · Growth curve modeling · Optimal temperature · Symbiotic bacteria · Thermal range

Introduction

Soil-dwelling nematodes of *Steinernema* species, the obligate parasites of insects, are symbiotically associated with the Gram-negative bacteria *Xenorhabdus* species residing in their digestive tract. These nematodes are very successful insect larvae killers (Arthurs et al. 2004; Grewal et al. 2005) and therefore used in agriculture as biological control agents against insect pests (Ehlers 2001; Ciche et al. 2006; Lewis et al. 2006). During their life cycle, they either live free in the soil where at some points their infective juveniles search for insect prey and infect it, or live within their host prey and complete their life cycle (Boemare 2002). The juvenile nematodes leaving the host's cadaver carry bacteria inside of

an intestine vesicle (Boemare 2002), protecting them from the soil environment and eventually transferring them to the host prey. Nematodes symbiotic bacteria cannot spread on their own and need vectors (Ehlers 2001). The bacterium's main service for their symbiotic partner is killing the prey by inducing septicemia, providing nutrients for the nematodes by digesting prey's tissues, and protecting prey's cadaver from saprophytes (Boemare 2002; Dillman et al. 2012).

Steinernema feltiae Filipjev, 1934 carrying the symbiotic bacteria *Xenorhabdus bovienii* Akhurst, 1983 is widely spread in temperate regions (Tailliez et al. 2006). It is suspected to be highly thermally plastic as it is found on all continents except for Africa and Greenland (Campos-Herrera et al. 2012). It is known that *S. feltiae* infects its prey hosts at temperatures between 8 and 30 °C and reproduces between 10 and 25 °C (Richardson and Grewal 1993). Hazir et al. (2001), in their work on *S. feltiae* isolated from different climate zones, showed that the temperature directly affects the time of death, penetration rate, emergence time, and the number of emerging infective juveniles. They suggested that

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the geographic isolation of *S. feltiae* resulted in its adaptation to the given region (Hazir et al. 2001).

A relatively great amount of data are known about the thermal response of entomopathogenic nematodes (Kaya 1977; Grewal et al. 1994; Sulsuruk 2008; Ulu and Sulsuruk 2014; Evans et al. 2015). However, fewer data have been provided for associated bacteria, although bacterial enzymatic activity and growth rate depend strongly on temperature (Tailliez et al. 2006; Hapeshi et al. 2020). According to Tailliez et al. (2006), *Xenorhabdus* species differ in the ability to grow at high temperatures. They found that some *Xenorhabdus* species can grow at 35–42 °C, while the others grow only below 35 °C. They suggested that some *Xenorhabdus* species may be adapted to tropical or temperate regions (Tailliez et al. 2006). This fact is not surprising as *X. bovienii* is associated with at least nine *Steinernema* spp. (Tailliez et al. 2006; Lee and Stock 2010; Campos-Herrera et al. 2012; Murfin et al. 2015; Bisch et al. 2016).

However, *S. feltiae* is associated only with *X. bovienii* (Stock and Blair 2008; Murfin et al. 2015), making this symbiotic relationship a good research model in studies on intraspecific variability of symbiotic partners. We know that symbiotic bacteria are key elements for the nematode's reproductive success. Some studies showed that temperature directly affects the reproductive success of *S. feltiae* (Hazir et al. 2001) and that the infectivity rate and soil survival time depend on the nematode's origin: with wild strains showing higher infectivity rate but shorter survival time, and the laboratory-bred strains showing longer survival time but lower infectivity rate (Chapuis et al. 2011; Grewal et al. 1999; Wang and Grewal 2002). Thus, we asked if *X. bovienii* bacteria isolated from the same nematode species living in different thermal conditions and originating from different environments, natural vs. laboratory, show differences in thermal sensitivity. The analysis of the literature showed a lack of such information. The answer to this question could help to estimate the partake of bacteria in the nematodes thermal sensitivity. It would also allow us to experiment with the nematodes' reproductive success by creating new symbiotic connections. Using *X. bovienii* isolates with different thermal ranges, we could check *S. feltiae* reproductive success and infectivity rate, which could translate into its possible future applications in agriculture. However, since the question about *X. bovienii* thermal range remained unanswered, we focused on this basic science issue first.

The presented study aimed to evaluate if intraspecific variation in temperature sensitivity occurs in *X. bovienii* isolates and depends on local climatic conditions, measured as the mean annual temperature, of the place of *S. feltiae* origin. To do this, we examined the bacterial growth rate in a temperature gradient for eight *X. bovienii* isolates from *S. feltiae*: six collected at different geographical latitudes with different mean annual temperatures and two isolates cultured in

constant laboratory conditions. We choose the mean annual temperature as a factor showing climatic differences between locations. Since air temperature strongly correlates with soil temperature on ground level (Dwyer et al. 1990; Xu et al. 2011; Wojkowski and Skowera 2017), it can be used as an indicator of habitat conditions of nematodes living in the soil. We suspected that *X. bovienii* isolated from *S. feltiae* originating from different habitats might differ in thermal range (minimum, optimal, and maximal growth temperatures), which would explain the nematodes' ability to spread over a wide range of temperatures. We also suspected that the thermal range of isolates bred in laboratory conditions over a longer time is different from the thermal range of isolates from *S. feltiae* collected from natural habitats.

Materials and methods

Study material and its origin

The experiment was conducted on *X. bovienii*, entomopathogenic bacteria isolated from the nematode *S. feltiae*. The nematodes came from the collection of the Institute of Environmental Sciences of the Jagiellonian University (Kraków, Poland). They were identified as *S. feltiae* using standard 16S RNA sequencing and the BLAST method (Tailliez et al. 2006).

Originally, the nematodes were collected from natural habitats by scientists across Europe and Asian part of Turkey (six samples) or were bred in a controlled laboratory environment (two samples). Table 1 shows the exact places of origin of the nematodes used for the experiment. Samples FRA45 and FRA44, as well as WG01 and WG02, were collected from the proximal locations in the same country of origin, France and Poland, respectively. All nematodes originating from the natural habitats were collected shortly before the experiment and stored in a storage solution (NaCl, $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, MgSO_4 , ascorbic acid, 0.25% formaldehyde) by two to three generations in 4 °C. Sample Commercial, purchased from Koppert Biological Systems (the Netherlands) and OBS III, collected from the natural habitat in 1980 (Noordoostpolder, the Netherlands) and stored under laboratory conditions since then (Scheepmaker et al. 1998), represent nematodes bred in a controlled environment for a long time.

Isolation of *Xenorhabdus bovienii*

Bacteria have been isolated from the nematodes using the *Galleria mellonella* (Lepidoptera: Pyralidae) method (Akhurst 1980). Final-stage larvae of the greater wax

Table 1 Location and local climatic conditions data (Copernicus Data Store 2021) of the place of origin of *Steinernema feltiae* used for isolation of *Xenorhabdus bovienii* used in the experiment

<i>S. feltiae</i> sample name	Place of origin	Longitude (°E)	Latitude (°N)	Mean annual temp. (°C)	Daily maximum temperature of the warmest month (°C)	Daily minimum temperature of the coldest month (°C)
09–38	Turkey, Aydni	27.5	37.5	17.8	32.4	14.2
FRA45	France, Aigues-Mortes	4.2	43.5	14.1	27.6	12.5
FRA44	France, St. Gely	3.8	43.7	13.6	29.8	9.3
Prosenice	Czech Republic, Prosenice ⁻	17.5	49.5	8.6	24.5	4.7
WG-01	Poland, Winna Góra ⁻	17.6	51.3	8.3	24.9	5.0
WG-02	Poland, Winna Góra ⁻	17.6	51.3	8.3	24.9	5.0
OBSIII ^L	The Netherlands, Noordoostpolder	5.7	52.7	25*	25*	25*
Commercial ^L	Koppert Biological Systems, The Netherlands	–	–	> 10.0**		

L – bred in laboratory conditions for a longer time

**Steinernema feltiae* kept in laboratory conditions since 1980 (Scheepmaker et al. 1998)

**According to data provided by the producer, the best working conditions for *S. feltiae* are between 10 and 30 °C

moth were infected with *S. feltiae* larvae on moist sand. After 24 h of incubation at 25 °C in the dark, the larvae of *G. mellonella* were dissected in sterile conditions, and samples of infected hemolymph were collected with an inoculation loop, streaked on a culture plate and incubated for 3–5 days at 25 °C in the dark. The culture plates with NTBA agar (Akhurst 1980) containing nutrient agar (Merck, Germany) and two dyes: bromothymol blue (Avantor Performance Materials Poland S.A., Poland) and triphenylotetrazolium chloride (Merck, Germany) that were used to identify the symbiotic bacteria. Bacteria of *Xenorhabdus* spp. have two forms: I and II. Form I enters the symbiotic relationship with nematodes, produces antibiotics and other substances that allow nematodes to propagate, and forms green–blue colonies on the NTBA medium. Form II, mostly found only in laboratory conditions (Adams et al. 2006), presents low metabolic activity and produces red colonies on the NTBA medium (Akhurst 1980; Thanwisai et al. 2012). Only bacteria forming green–blue colonies were used for further experiments. Additionally, the bacteria were identified as *X. bovienii* using 16SP1 and 16SP2 primers as described by Taillez et al. (2006).

After the incubation on the NTBA medium, the single green–blue colonies were propagated in LB Agar (Difco™, USA) for 48 h at 25 °C. Then, bacteria were frozen with 15% glycerol (1:1) and stored at – 80 °C until needed for the experiment. After defrosting, bacteria were first streaked on the NTBA medium, incubated for 3–5 days h at 25 °C in the dark, and then used in further experimental steps.

Experimental design

The thermal sensitivity of *X. bovienii* isolated from *S. feltiae*, originating from natural habitats located at different geographical latitudes, differing in annual temperatures and bred in artificial conditions for a long time, was estimated using the three-step method, designed for this study. The first step aimed to determine the minimal and maximal temperature at which the isolated *X. bovienii* grows. The second step aimed to measure the bacterial growth rate at several temperatures between the minimal and maximal temperatures determined in the first step. The third step aimed to estimate minimal, maximal, and optimal growth temperatures for *X. bovienii* isolated with high precision. The modeling of the bacterial growth curves performed at this step used data obtained at the first two steps of the experiment.

Minimal and maximal temperature of *Xenorhabdus bovienii* growth

Bacteria were propagated in the dark, at 25 °C for 48 h in the LB medium on an orbital shaker (200 rpm) (ELMI, DOS-10 M, Estonia). Samples of liquid culture (10 µl) were diluted tenfold and streaked on the LB agar medium and then incubated in the dark at temperatures ranging from 2 to 36 °C. Bacterial growth was observed daily for two weeks. Each day, growth was classified as “–” no growth, “(–)” very weak growth, “(+)” moderate growth, or “+” strong growth – colony highly visible. The experiment was repeated for 6 individual colonies obtained from one *X. bovienii* isolate.

Bacteria originating from one colony were tested in triplicates in the whole temperature range.

Xenorhabdus bovienii specific growth rate

The optical density (OD) in the exponential phase of growth for each *X. bovienii* isolate at several temperatures was measured to estimate the bacterial specific growth rate (Sugar et al. 2012). Temperatures ranging between the minimal and maximal temperature of growth of each isolate were chosen based on the results of the previous step. If the bacterial growth at a given temperature was too slow and it was difficult to measure it in a short time, the bacteria were propagated in the LB medium at 25 °C for 48 h. Then they were serially diluted (1:10) and incubated at the chosen temperature in a 96-well plate on an orbital shaker (200 rpm). The OD measurements were taken at 600 nm every hour until the stationary phase was established (microplate reader Infinite® 200, TECAN, Austria). The OD measurements were taken for six individual colonies obtained from one *X. bovienii* isolate. Bacteria originating from one colony were tested in triplicates in the whole temperature range at least twice. The mean value of OD was calculated and then used for bacterial growth rate calculations and modeling.

Xenorhabdus bovienii growth modeling

Basic mathematical models for bacterial growth across temperatures were described by Zwietering et al. (1991). The growth curve is defined as the logarithm of the relative population size [$y = \ln(N/NO)$] as a function of time (t). For bacteria, the growth rate shows a lag phase that is followed by an exponential phase and, finally, a stationary phase when the growth rate decreases to zero and the number of bacterial cells reaches a maximum. A growth model with three parameters can describe this growth curve: the maximum specific growth rate μ_m , which is defined as the tangent of the inflection point; the lag time X , which is defined as the t -axis intercept of this tangent; and the asymptote A , which is the maximal value reached (Zwietering et al. 1991). In our research, we used the extended Ratkowsky model (Ratkowsky et al. 1983), which allowed us to model thermal reaction norms across a wide range of temperatures. The bacterial growth rate across the thermal range was modeled using the square root model (Ratkowsky et al. 1983):

$$\mu = [b(T - T_{\min})]^2 \{1 - \exp[-c(T - T_{\max})]\},$$

where μ is the bacterial specific growth rate, T_{\min} and T_{\max} is the theoretical maximum and minimum temperatures at which the growth rate is zero, b and c are the coefficients calculated during the modeling process.

The specific growth rate (μ) was calculated based on the OD measurements. To estimate T_{opt} (the temperature of the highest μ), we calculated the maximum of the described function for each bacterial isolate. Growth curves were calculated for every single repetition of the experiment for all isolates of *X. bovienii*, then the mean value for each estimated point— T_{\min} , T_{\max} , T_{opt} was calculated.

Data analysis

All statistical analyses were performed using the R environment (R Core Team 2017).

Bacterial growth rate at selected temperatures was estimated using a linear regression model (lm). Regression coefficients were tested using a t test with the confidence interval was set at 95%. The calculated growth rate values were then used for data modeling. Data were fit to model using a non-linear regression model (nls). All calculated parameters: T_{\min} , T_{\max} , b , and c coefficients were also tested using Student's t test with the confidence interval set at 95%. T_{opt} was calculated based on the calculated bacterial growth curves as the function's maximum. The errors were estimated using the bootstrap function. The one-way ANOVA was calculated using aov function and used to test variance between *X. bovienii* isolates of *S. feltiae*. Pearson's correlation coefficients were calculated using the cor function and tested using a t test with the confidence interval set at 95%.

Results

Xenorhabdus bovienii thermal range

The first step of determining the thermal sensitivity of *X. bovienii* isolated from *S. feltiae* aimed to find the minimal and maximal temperature at which the isolated bacteria grow. We observed no growth for any isolated *X. bovienii* at 2 °C (Table 2). At 8 and 10 °C, we observed no growth for the FRA44 sample, poor growth for *X. bovienii* isolated from laboratory-bred *S. feltiae* and those originating from Poland and the Czech Republic, and good growth for bacteria isolated from FRA45 and 09–38 samples. Between 16 and 30 °C, the bacterial growth was strong, and the colonies were large and visible for most of the samples. At temperatures above 30 °C, the bacterial growth was hardly observed, with only *X. bovienii* isolated from the commercially available *S. feltiae* forming visible colonies at 34 °C (Table 2).

Xenorhabdus bovienii specific growth rate and growth curves

Based on the results obtained in the first step of determining the thermal sensitivity of *X. bovienii* isolated from *S. feltiae*,

Table 2 The results of the thermal range test of *Xenorhabdus bovienii* isolated from *Steinernema feltiae* of different origin

<i>Xenorhabdus bovienii</i> isolate	Temperature (°C)									
	2	8	10	16	20	23	25	30	34	36
09–38	–	+	+	+	+	+	+	–	–	–
FRA45	–	+	+	+	+	+	+	+	–	–
FRA44	–	–	–	(+)	+	+	+	+	–	–
Prosenice	–	(–)	(+)	+	+	+	+	+	–	–
WG-01	–	(+)	(+)	+	+	+	+	+	–	–
WG-02	–	(+)	(+)	+	+	+	+	(+)	–	–
OBSIII ^L	–	(+)	(+)	+	+	+	+	+	–	–
Commercial ^L	–	(+)	(+)	+	+	+	+	+	+	–

The results are presented as a mean of six individual colonies of each *X. bovienii* isolate and marked as: “–” no growth, “(–)” very weak growth, “(+)” – moderate growth “+” strong growth observed over the two weeks incubation period

L – bred in laboratory conditions for a longer time. The place of *S. feltiae* origin was as follows: 09–38 – Turkey, FRA45, FRA44 – France, Prosenice – Czech Republic, WG–01, WG–02 – Poland, OBSIII – The Netherlands, Commercial – The Netherlands

Table 3 Minimal (T_{min}), optimal (T_{opt}), and maximal (T_{max}) temperature of *Xenorhabdus bovienii* estimated based on the growth curves obtained using the Ratkowsky model for specific bacterial growth

<i>Xenorhabdus bovienii</i> isolate	T_{min} (°C)	T_{opt} (°C)	T_{max} (°C)
09–38	3.1 ± 1.9	29.9 ± 0.2	36.0 ± 0.1
FRA45	6.5 ± 0.9	26.8 ± 0.1	36.1 ± 0.2
FRA44	7.1 ± 1.4	29.7 ± 0.2	36.0 ± 0.2
Prosenice	6.9 ± 1.0	25.1 ± 0.2	36.0 ± 0.2
WG-01	5.6 ± 1.9	26.5 ± 0.2	36.2 ± 0.4
WG-02	0.9 ± 2.2	30.5 ± 0.2	36.0 ± 0.1
OBSIII ^L	6.9 ± 1.1	25.8 ± 0.2	36.1 ± 0.3
Commercial ^L	4.2 ± 1.9	29.4 ± 0.2	36.0 ± 0.2

Results are presented as a mean ± standard deviation ($n=241$)

L – bred in laboratory conditions for a longer time, T_{max} – maximal temperature, T_{min} – minimal temperature, T_{opt} – optimal temperature. The place of *S. feltiae* origin was as follows: 09–38 – Turkey, FRA45, FRA44 – France, Prosenice – Czech Republic, WG–01, WG–02 – Poland, OBSIII – The Netherlands, Commercial – The Netherlands

we choose temperatures between 12 and 35 °C to determine bacterial specific growth rate based on the OD measurements and modeling.

The Ratkowsky model showed that the minimal temperature of *X. bovienii* growth varies between 0.9 ± 2.2 °C for *X. bovienii* isolated from *S. feltiae* WG02 sample and 7.1 ± 1.4 °C for *X. bovienii* isolated from *S. feltiae* FRA44 sample (Table 3). It is worth noting that 95% coefficient intervals for minimal growth temperature are much wider than optimal and maximal temperatures of growth. The minimal temperatures of growth estimated for *X. bovienii* isolated from *S. feltiae* FRA45, FRA44, OBSIII, and Prosenice samples were highly precise ($p < 0.001$), for WG01 and Commercial samples were moderately precise ($p 0.001–0.1$),

and for 09–38 and WG02, samples were not strongly recognized ($p > 0.1$). The model also showed that *X. bovienii* stops to multiply at around 36 °C, and the maximal temperature of *X. bovienii* growth was estimated for all samples with a very narrow 95% confidence interval ($p < 0.001$).

The optimal temperature for *X. bovienii* growth calculated with the Ratkowsky model varied between 25.1 ± 0.2 °C for the Prosenice sample and 30.5 ± 0.2 °C for the WG-02 sample (Table 3).

The calculated optimal temperature of *X. bovienii* growth did not correlate with the mean annual temperature (T_{ann}) of *S. feltiae* place of origin ($R = -0.009$, $p = 0.986$, $n = 8$). For this correlation, we assumed that the T_{ann} for *X. bovienii* originating from *S. feltiae* bred in laboratory conditions (OBSIII) or for commercial purposes (Commercial) was 25 °C. ANOVA analysis showed that *X. bovienii* isolates show no difference between the isolates for the calculated T_{min} ($F = 2.371$, $p = 0.138$), T_{opt} ($F = 0.057$, $p = 0.813$) and T_{max} ($F = 0.002$, $p = 966$). Also, the analysis showed no differences in T_{min} ($F = 0.165$, $p = 0.688$), T_{opt} ($F = 2.412$, $p = 0.135$), and T_{max} ($F = 0.108$, $p = 0.746$) between *X. bovienii* isolated from *S. feltiae* originating from a natural environment and isolates from *S. feltiae* kept in laboratory conditions.

The calculated specific *X. bovienii* growth rate (Table 4) ranged between 2.0 ± 0.3 [h⁻¹] for *X. bovienii* isolated from *S. feltiae* from Prosenice sample and 3.6 ± 0.5 [h⁻¹] for *X. bovienii* isolated from *S. feltiae* from Commercial sample (Table 4). The modeled growth curves, based on the OD measurements, for *X. bovienii* isolated from *S. feltiae* of different origin, are shown in Fig. 1.

ANOVA analysis showed no differences in the specific growth rate at T_{opt} between the examined *X. bovienii* isolates ($F = 0.001$, $p = 0.977$) and no differences between the isolates cultured from *S. feltiae* originating from a

Table 4 *Xenorhabdus bovienii* specific growth rate for optimal temperature (T_{opt}) calculated based on OD measurements ($n=241$) using the Ratkowsky model, and *X. bovienii* specific growth rate for mean annual temperature (T_{ann}) extrapolated from the growth curves

<i>Xenorhabdus bovienii</i> isolate	Specific growth rate (h^{-1}) at T_{opt} ($n=241$)	Specific growth rate (h^{-1}) at T_{ann}
09–38	3.1 ± 0.4	1.87
FRA45	2.7 ± 0.3	1.30
FRA44	3.1 ± 0.4	1.01
Prosenice	2.0 ± 0.3	0.42
WG-01	2.9 ± 0.5	0.50
WG-02	3.3 ± 0.4	0.89
OBSIII ^L	2.9 ± 0.4	2.93
Commercial ^L	3.6 ± 0.5	3.22

L – bred in laboratory conditions for a longer time, T_{ann} – mean annual temperature, T_{opt} – optimal temperature. The place of *S. feltiae* origin was as follows: 09–38 – Turkey, FRA45, FRA44 – France, Prosenice – Czech Republic, WG–01, WG–02 – Poland, OBSIII – The Netherlands, Commercial – The Netherlands

natural habitat and *S. feltiae* kept in laboratory conditions ($F=0.844$, $p=0.394$).

The calculated specific *X. bovienii* growth rate at T_{opt} did not correlate with the T_{opt} calculated for the *X. bovienii* isolates ($R=0.046$, $p=0.914$).

When looking at the growth curves, we noticed that the curves form two groups (Fig. 1). The first one is characterized by a lower maximal growth rate (below $3.0 h^{-1}$), lower T_{opt} ($< 27 ^\circ C$), and also a lower difference between T_{min} and T_{opt} (between 18.2 and $20.9 ^\circ C$) and higher difference between T_{opt} and T_{max} (between 9.3 and $10.9 ^\circ C$). The second one is characterized by a higher maximal growth rate (above $3.0 h^{-1}$), higher T_{opt} ($> 29 ^\circ C$), and also a higher difference between T_{min} and T_{opt} (between 22.6 and $29.6 ^\circ C$) and a smaller difference between T_{opt} and T_{max} (between 5.5 and $6.6 ^\circ C$) (Tables 3 and 4). The first subgroup is represented by *X. bovienii* isolated from *S. feltiae* of FRA45, Prosenice, WG-01, and OBSIII samples. The second subgroup is represented by *X. bovienii* isolated from *S. feltiae* of 09–38, FRA44, WG-02, and Commercial samples. The division into the two identified subgroups has been confirmed by pairwise comparison of T_{opt} of *X. bovienii* growth (Table 5). However, the pairwise comparison of the *X. bovienii* specific growth rate showed that the difference exists only between *X. bovienii* isolated from *S. feltiae* from Prosenice sample (with the lowest specific growth rate) and *X. bovienii* isolated from *S. feltiae* of WG-02 and Commercial samples (with the highest specific growth rates) ($p < 0.05$ for both comparisons).

Fig. 1 Growth curves of *Xenorhabdus bovienii* isolated from *Steinernema feltiae* originating from different environments. The growth curves were obtained using the Ratkowsky model. The place of *S. feltiae* origin was as follows: 09–38 – Turkey, FRA45, FRA44 – France, Prosenice – Czech Republic, WG-01, WG-02 – Poland, OBSIII – The Netherlands, Commercial – The Netherlands. OBSIII and Commercial were bred in laboratory conditions for a longer time

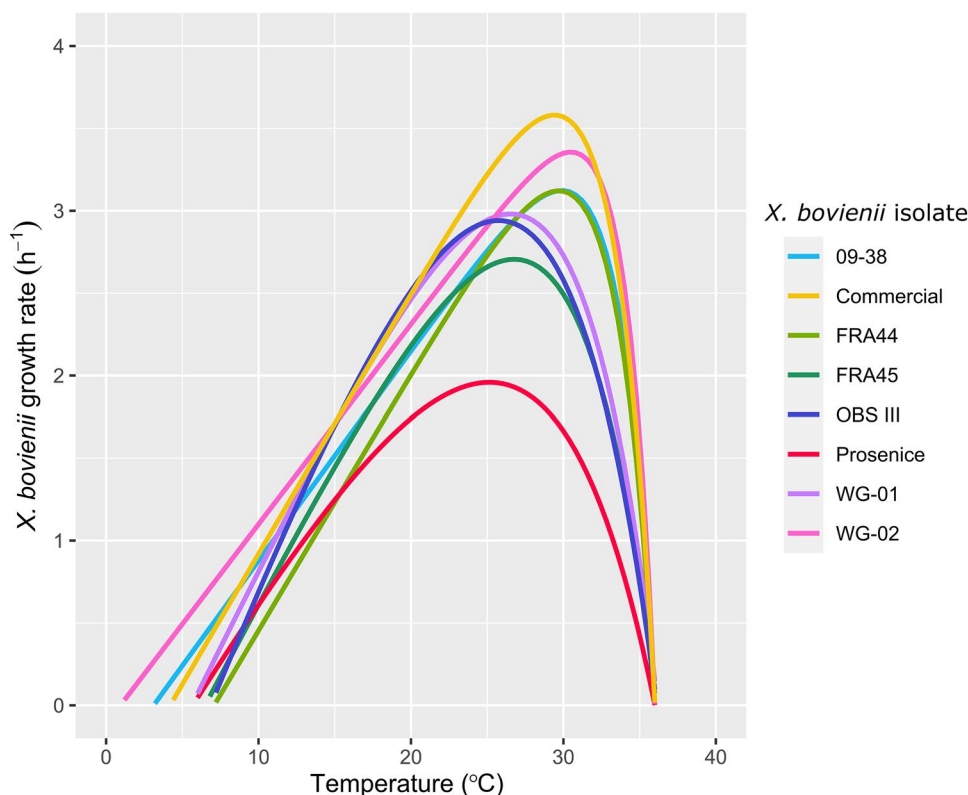


Table 5 Results of pairwise comparison of T_{opt} and the specific bacterial growth rate of *Xenorhabdus bovienii* isolated from *Steinernema feltiae* originating from different environments using Student's *t* test

<i>Xenorhabdus bovienii</i> isolates	09–38	FRA45	FRA44	Prosenice	WG-01	WG-02	OBSIII	Commercial
09–38	–							
FRA45	0.00572	–						
FRA44	1	0.0096	–					
Prosenice	0.00016	0.64701	0.00026	–				
WG-01	0.00327	1	0.00548	1	–			
WG-02	1	0.00124	1	0.000038	0.00072	–		
OBSIII ^L	0.00046	1	0.00072	1	1	0.00011	–	
Commercial ^L	1	0.01931	1	0.00051	0.01039	1	0.00136	–

Significance was set at $p < 0.05$

Legend: L – bred in laboratory conditions for a longer time. The place of *S. feltiae* origin was as follows: 09–38 – Turkey, FRA45, FRA44 – France, Prosenice – Czech Republic, WG–01, WG–02 – Poland, OBSIII – The Netherlands, Commercial – The Netherlands

Discussion

The presented study aimed to check if *X. bovienii* isolated from *S. feltiae* originating from different environmental conditions, have different thermal sensitivity. We also aimed to check if the thermal sensitivity of *X. bovienii* correlates with the mean annual temperature of the place of *S. feltiae* origin. The minimal and maximal temperatures of *X. bovienii* growth are, respectively, much above and below the thermal range of *S. feltiae* reproduction (Richardson and Grewal 1993). We decided to discuss the optimal temperature of bacterial growth because it can impact the host's infection and reproduction rate. We also decided to choose the mean annual temperature as the only factor showing differences between locations.

The lack of significant differences in the optimal, minimal, and maximal temperature of *X. bovienii* isolates growth, and the lack of correlation between the optimal temperature of *X. bovienii* isolates growth and the mean annual temperature of *S. feltiae* place of origin may have various reasons. Calculated optimal temperature for *X. bovienii* growth did not correlate with the mean annual temperature of *S. feltiae* place of origin. When looking at the reproductive cycle of the nematode and its symbiotic bacteria, it seems that *X. bovienii* we isolated are not necessarily exposed to extreme temperatures. In our experiment, we isolated *X. bovienii* from the nematode's infective juvenile form. During this stage, the bacteria are carried in a specialized intestine vesicle, mainly in the quiescent state, when no significant bacterial propagation occurs (Adams et al. 2006).

It might be possible that the bacteria also are less prone to selection by other external factors, like temperature to which the nematodes are exposed during the infective juvenile stage. Therefore, we conclude that since *X. bovienii* are protected by the vesicle during the infective juvenile stage, the lack of correlation between *X. bovienii* thermal range and the

mean annual temperatures of *S. feltiae* place of origin proves that they were not subjected to selection that would force them to adapt to growth at different temperature conditions.

The presence of *S. feltiae* with symbiotic *X. bovienii* in sites characterized by different climatic conditions may result from two mechanisms: both nematodes and bacteria are thermally plastic, or local adaptations occur. Our results show that *X. bovienii* are more thermally plastic than their host. The studies of others showed that *S. feltiae* can adapt to local conditions (Hazir et al. 2001). However, our results also indicate that some degree of adaptation may emerge between *X. bovienii* isolated from *S. feltiae*. We found that the examined *X. bovienii* isolates form two subgroups with different T_{opt} . Each subgroup is represented by one of the isolates originating from proximal locations (FRA45 and FRA44, WG-01 and WG-02) or kept in laboratory conditions (OBSIII and Commercial), indicating that *X. bovienii* can show some variance. The observed segregation of *X. bovienii* isolated from proximally sourced *S. feltiae* into physiologically distinct subgroups might indicate that the seasonal and local variations in the temperature have had forced the changes between isolates. In the case of *X. bovienii* isolated from laboratory kept nematodes, the difference between the isolates is more pronounced. *X. bovienii* from the OBSIII sample was isolated from *S. feltiae* collected from a natural habitat in 1981 in the Netherlands (Ehlers et al. 1997) and kept in the laboratory conditions since then (Scheepmaker et al. 1998). It shows a lower specific growth rate and lower T_{opt} than *X. bovienii* isolated from commercially available *S. feltiae*. In the case of commercially bred *S. feltiae*, the nematode is mass-produced in bioreactors, optimized for maximal efficiency to be sold worldwide as a biocontrol agent that can be applied under different climatic conditions in fields to protect the plants from insect pests (Gaugler and Kaya 2018). This isolate showed the highest growth rate and the widest thermal range (31.8 °C), when

omitting 09–48 and WG-02 samples with not strongly recognized T_{\min} . It seems that the two laboratory-derived isolates, OBSIII and Commercial, are distinct from each other. The noted differences between *X. bovienii* isolated from *S. feltiae* originating from the laboratory conditions suggest the influence of different laboratory storage and culture conditions, focused on achieving different goals.

Conclusion

Our study showed that *X. bovienii* have a wider thermal range than their nematode host, *S. feltiae* and *X. bovienii* carried by *S. feltiae* live below their thermal optimum. In a broader perspective, this bacteria's trait can impact *S. feltiae* survival. Hypothetically, the bacteria would be able to kill the prey faster in higher temperatures, but the nematodes would fail to reproduce in such conditions. The lack of differences in the thermal range of *X. bovienii* isolates, observed in our study, reflects no phenotypical differences in this fundamental and highly conserved bacterial trait.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by PEK, JPM, and AR. The first draft of the manuscript was written by JPM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the Polish National Centre of Science [grant Preludium V DEC-2013/09/N/NZ8/03220] and Jagiellonian University [grant number DS/WBINOZ/INOS/756].

Availability of data and code Both datasets generated and analyzed during the current study and the code are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical approval Not applicable.

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