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Temperature Effects on Korean Entomopathogenic Nematodes, *Steinernema glaseri* and *S. longicaudum*, and their Symbiotic Bacteria

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Abstract We investigated the temperature effects on the virulence, development, reproduction, and motility of two Korean isolates of entomopathogenic nematodes, *Steinernema glaseri* Dongrae strain and *S. longicaudum* Nonsan strain. In addition, we studied the growth and virulence of their respective symbiotic bacterium, *Xenorhabdus poinarii* for *S. glaseri* and *Xenorhabdus* sp. for *S. longicaudum*, in an insect host at different temperatures. Insects infected with the nematode-bacterium complex or the symbiotic bacterium was placed at 13°C, 18°C, 24°C, 30°C, or 35°C in the dark and the various parameters were monitored. Both nematode species caused mortality at all temperatures tested, with higher mortalities occurring at temperatures between 24°C and 30°C. However, *S. longicaudum* was better adapted to cold temperatures and caused higher mortality at 18°C than *S. glaseri*. Both nematode species developed to adult at all temperatures, but no progeny production occurred at 13°C or 35°C. For *S. glaseri*, nematode progeny production was best at inocula levels above 20 infective juveniles/host at 24°C and 30°C, but for *S. longicaudum*, progeny production was generally better at 24°C. *Steinernema glaseri* showed the greatest motility at 30°C, whereas *S. longicaudum* showed good motility at 24°C and 30°C. Both bacterial species grew at all tested temperatures, but *Xenorhabdus* sp. was more virulent at low temperatures (13°C and 18°C) than *X. poinarii*.

Keywords: *Steinernema*, mutualistic bacterium, *Xenorhabdus*, entomopathogenic nematode

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In recent years, biological control of pests with microorganisms has received attention both in plant diseases [7, 22] and insect pests [8, 18]. Entomopathogenic nematodes in the genus *Steinernema* are mutualistically associated with symbiotic bacteria in the genus *Xenorhabdus*, and some species of this nematode-bacterium complex are being used as biological control agents against a variety of soil insect pests. Each *Steinernema* species is closely associated with a *Xenorhabdus* species, but a *Xenorhabdus* species may be associated with more than one nematode species [2, 3]. For example, *X. nematophila* is associated only with *S. carpocapsae*, *X. poinarii* is associated with *S. glaseri* [30] and *S. cubanum*, and *X. beddingii* with *S. longicaudum* and two undescribed nematode species from Australia [2].

The life cycle of steinernematids demonstrates the mutualistic association between the nematode and the symbiotic bacterium. The infective 3rd-stage juvenile, the only free-living stage, harbors the bacterium in its intestine. The infective juvenile searches for an insect host, enters the insect through natural openings such as the mouth, anus, and spiracles, and then penetrates into the hemocoel. Once the infective juvenile penetrates into the insect's hemocoel, it releases the symbiotic bacterium. The bacterium multiplies, produces toxins to kill the insect within 48 h of infection, and also produces antibiotics that prevent the growth of most other microorganisms. Because nearly all steinernematids are amphimictic, they require a male and female nematode to enter a host for reproduction. These nematodes feed on insect tissues and bacterial cells, develop to adults, and produce through 1–3 generations.

As resources are depleted, the nematodes produce the 3rd-stage infective juveniles that sequester the *Xenorhabdus* bacterial cells in their intestine and emerge from the cadaver to search for new hosts [2, 16].

Because entomopathogenic nematodes are poikilothermic, temperature plays an important role in their motility, infectivity, development, and survival [13, 24, 26]. Temperature ranges for survival, infectivity, and development vary with nematode species and strains [10, 12]. Each species has its own upper and lower thresholds as well as optimal temperatures for survival, infectivity, and development [19, 20]. In some cases, the infective juveniles develop to adults and produce progeny at upper and lower threshold limits, but progenies may remain inside the cadaver and eventually die [4].

Interestingly, the entomopathogenic nematodes have different temperature thresholds for the symbiotic bacteria. For example, the upper threshold for the development of *S. feltiae* is 25°C and the lower threshold is 8°C [12], whereas for its symbiotic bacterium, *X. bovienii*, these are 32°C and 5°C, respectively [2]. Temperature is one of the important factors affecting both entomopathogenic nematodes and their symbiotic bacteria, but temperature studies have generally been on the nematodes.

We report herein the performance of two Korean steinernematid isolates and their symbiotic bacteria at different temperature regimes. These two Korean isolates, *S. glaseri* Dongrae strain and *S. longicaudum* Nonsan strain, are both large nematodes with the infective juvenile averaging 1,130 μm and 1,063 μm in length, respectively. However, they have different symbiotic bacterium, *S. glaseri* associated with *X. poinarii* [30] and possibly *S. longicaudum* associated with *X. beddingii* [2]. Both nematode-bacterium complexes show promise as biological control agents of certain Korean pest insects.

MATERIALS AND METHODS

Nematode and Bacterial Cultures

Steinernema glaseri Dongrae was isolated from larval cadavers of the oriental beetle *Exomala orientalis*, at the Dongrae Benest Golf Club in Busan [5]. *S. longicaudum* Nonsan was isolated from soil samples collected from a forest at Nonsan in Chungnam Province using the insect baiting method [27]. The nematodes were reared in the last instar of the greater wax moth larvae *Galleria mellonella*, using the method of Dutky *et al.* [9]. Infective juveniles were harvested from a White trap and stored in sterilized distilled water at 10°C for no more than 21 days before they were used [17].

Symbiotic bacteria were isolated from nematode-killed insects and cultured on MacConkey agar (Difco, Sparks, MD, U.S.A.) using a modified method of Akhurst [1]. Bacterium isolated from each species was identified using the method described by Kaya and Stock [17] and Boemare [2]. Phase I bacterium was selected based on the colony pigmentation and morphology. On MacConkey agar, phase I colonies are smaller, convex, opaque, and more pigmented than those of phase II [17].

Nematode Virulence

A concentration-response assay against the last instar of *G. mellonella* larvae was performed individually with both nematode species to evaluate their virulence. A *G. mellonella* larva (180–200 mg) was placed in a 5-cm petri dish with two filter papers that had received infective juveniles of 0, 5, 10, 20, 40, 80, or 160 infective juveniles/0.5 ml. There were four replicates with 10 insects/concentration, temperature, and species. The petri dishes were grouped in a completely randomized block design and wrapped with polyethylene film that was punctured with 4 small holes. The grouped petri dishes were placed in a plastic case (37×30×22 cm) and covered with 5 wet paper towels to minimize evaporation. These plastic cases were at 13°C, 18°C, 24°C, 30°C, or 35°C in the dark, and mortality was recorded daily. The experiment was terminated after 15 days of inoculation. This experiment and all subsequent experiments described below were conducted a total of three different times in an incubator at the temperatures indicated above.

Nematode Development

Cadavers of *G. mellonella* from the above experiments ("Nematode virulence") were removed from the incubator, placed in a new petri dish, and returned to the same incubator. The cadavers were dissected to check on nematode development at different time intervals. At 24, 30, and 35°C, nematode development was checked 2 days after death, whereas at 13 and 18°C, nematode development was checked 4 or 5 days after death. A subsample of 5 to 10 cadavers was dissected from each temperature regime and the number of nematode adults within each cadaver was recorded.

Nematode Reproduction

Using the same experimental protocol described for the "Nematode Virulence," a new set of larvae was exposed to the nematode species. At each concentration and temperature, up to 10 cadavers were placed individually on a White trap 7 days after mortality was recorded and kept at the same temperature regime. The first day of infective juvenile emergence was recorded. The infective juveniles were harvested daily until no infective juveniles emerged. The infective juveniles that were produced daily were counted and averaged. The number of infective juveniles per day and their total numbers were analyzed based only on those cadavers that produced progeny.

Nematode Motility

Soil (20% compost, 30% sand, and 50% clay) was thoroughly mixed in a container. Based on preliminary tests, soil moisture of 32% was suitable to test nematode motility. Thus, 16 g of soil was placed in a 5-cm petri dish, which provided a 5-mm gap between the soil surface and the lid. Five-hundred infective juveniles/0.5 ml of *S. glaseri* or *S. longicaudum* was placed on the soil surface, and each petri dish was covered with the lid and placed in an incubator at the temperature regimes described previously. The infective juveniles were washed from the lid daily and counted. There were four replicate petri dishes for each temperature and nematode species.

Bacterial Growth on MacConkey Agar

Phase I colonies of *X. poinarii* or *Xenorhabdus* sp. were selected and transferred to a test tube containing sterilized distilled water. The tube was vortexed for 10 sec and 0.1 ml of the bacterial suspension was streaked on MacConkey agar. As needed, serial dilutions were made to obtain 30–300 colony forming units (CFU)/dish [29]. The dishes were incubated at the indicated temperature regimes and checked daily for bacterial colonies. There were 10 replicates for each temperature regime and bacterial isolate.

Virulence of Bacterium

Bacterial colonies of phase I of *X. poinarii* or *Xenorhabdus* sp. selected from cultures on MacConkey agar were inoculated directly into YS broth at 24°C. The bacterial cells that were in the exponential growth period were used for injection into the last larval instar of *G. mellonella*. The number of bacterial cells had been previously determined through correlation by optical density and CFU, and 20 µl containing ca. 100 cells of *Xenorhabdus* sp. or 10,000 cells of *X. poinarii* in sterilized Ringer's solution (9 g NaCl, 0.4 g KCl, 0.4 g $CaCl_2$, 0.2 g NaH_2CO_3 in 1 l of sterilized distilled water) was injected into each larva. Each larva that had been surface sterilized with 70% ethanol at the point of injection (*i.e.*, at the base of the abdomen) was inoculated using a 27-gauge needle mounted on a 1-ml syringe and placed in a 90-mm petri dish containing a piece of moistened filter paper. A group of 10 larvae/dish was kept in an incubator at the temperature regimes described above. Each control larva was injected with an equivalent volume of sterilized Ringer's solution and held similarly as the treated larva. There were four replicates at each temperature regime with 10 larvae/replicate. Mortality was checked every 12 h.

Data Analysis

Mortality data were arcsine square root transformed before analysis using ANOVA, and means separation was made with the Student-Newman-Keul's test [25]. The nematode concentration was correlated with numbers of developed adults at the given temperature with SAS PROC CORR [25]. Nematode development and progeny production were analyzed by analysis of variance, and means at given temperatures were separated by Student-Newman-Keul's test (PROC GLM) [25]. Data are expressed as means ± standard error (SE).

RESULTS

Nematode Virulence

Temperature and concentration significantly influenced virulence of *S. glaseri* (Sg) ($F=24.56$; df=29, 90; $P<0.0001$) and *S. longicaudum* (Sl) ($F=12.96$; df=29, 90, $P<0.0001$) to *G. mellonella* larvae. *S. glaseri* showed different virulence at low temperature and concentration; that is, virulence was significantly different at 13°C and 18°C at <40 infective juveniles compared with 24°C, 30°C, and 35°C. Nematode virulence increased with increasing

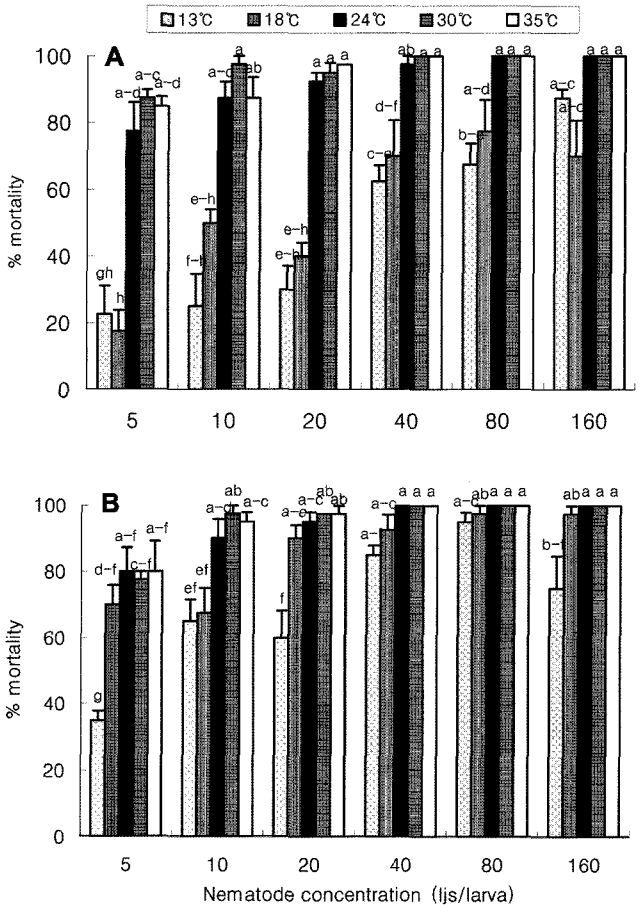

Fig. 1. Effect of nematode concentration on the mortality of (**A**) *Steinernema glaseri* Dongrae and (**B**) *S. longicaudum* Nonsan against *Galleria mellonella* larvae.
Vertical bars represent standard error of the means. The same lower case letters above the bars indicates no significant difference among means ($P<0.05$). Ijs, Infective juveniles of nematodes.

concentrations at <40 infective juveniles at 13°C and 18°C, but there were no differences in virulence for >10 infective juveniles at 24°C, 30°C, and 35°C. Virulence of *S. longicaudum* was similar to that of *S. glaseri*, but significant difference occurred at 5 infective juveniles. *Steinernema glaseri* caused higher mortality than *S. longicaudum* at 35°C, whereas *S. longicaudum* caused greater host mortality than *S. glaseri* at 18°C. Virulence of *S. glaseri* and *S. longicaudum* was not significantly different at all tested temperatures. However, virulence of *S. longicaudum* was significantly different at <18°C at <40 infective juveniles and that of *S. glaseri* at 18°C only at <10 infective juveniles (Fig. 1). Mortality by both species was reduced at 13°C and the percentage mortality was 22.5–87.5% by *S. glaseri* and 35–95% by *S. longicaudum* (Fig. 1). No control mortality occurred.

Nematode Development

Nematode development was significantly influenced by temperature and concentration. The number of developed *S. glaseri* adults was increased with increasing concentrations at all tested temperatures. The highest number was obtained at 30°C at 160 infective juveniles representing 47.2. However, the number of adults was significantly low at 13°C representing 9.2 (F=58.21, df=29, 256, $P<0.0001$) (Fig. 2A). The number of developed *S. longicaudum* adults showed the same tendency as in *S. glaseri*. The highest number was 42.3 at 30°C at 160 infective juveniles, followed by 24°C and 18°C representing 32.9 and 25.9, respectively. The lowest number was recorded at 35°C representing 15.3 (F=36.92; df=29, 259; $P<0.0001$) (Fig. 2B).

Nematode Reproduction

Temperature and concentration significantly influenced nematode production. Although both nematode species

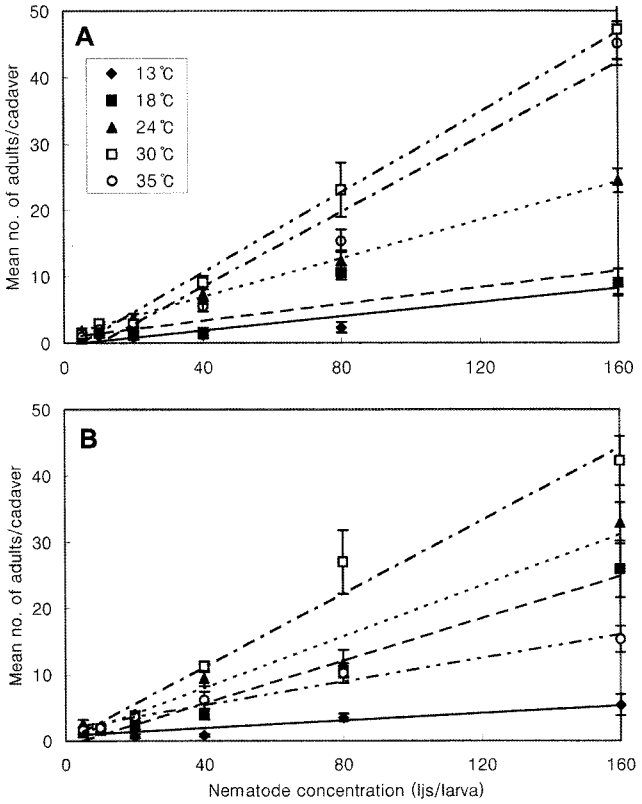

Fig. 2. Effect of nematode concentration on the development of (**A**) *Steinernema glaseri* Dongrae and (**B**) *S. longicaudum* Nonsan in *Galleria mellonella* larvae.
Vertical bars represent standard error of the means.

developed to adults at 13°C and 35°C, *S. glaseri* and *S. longicaudum* produced no infective juveniles at those temperatures (Sg: F=7.71; df=11, 98; $P<0.0001$; Sl: F=10.95; df=11, 98; $P<0.0001$) (Table 1). Generally, low numbers of infective juveniles were produced at 18°C and

Table 1. Infective juvenile production of *Steinernema glaseri* Dongrae and *S. longicaudum* Nonsan in *Galleria mellonella* larvae at different temperatures and nematode concentration.

Nematode	Concentration (Ijs/larva)	Number of IJs/cadaver±SE				
		13°C	18°C	24°C	30°C	35°C
Steinernema glaseri	5	0	0 a	27,999±5,500 b	33,255±15,807 b	0
	10	0	2,706±2,401 a	41,083±6,339 ab	26,774±5,817 b	0
	20	0	1,593±523 a	60,766±4,670 a	51,411±4,063 ab	0
	40	0	2,894±1,766 a	45,634±4,699 ab	55,499±4,386 ab	0
	80	0	7,550±3,356 a	59,418±1,0740 ab	82,238±6,070 a	0
	160	0	6,183±1,313 a	64,545±1,5611 a	99,209±33,070 a	0
Steinernema longicaudum	5	0	1,691±256 a	19,959±3,229 f	29,952±9,233 def	0
	10	0	2,909±841 a	47,094±5,453 b–e	33,903±4,848 ef	0
	20	0	2,566±42 a	36,764±8,185 ef	28,537±4,617 ef	0
	40	0	8,726±2,609 a	58,898±5,609 a–d	31,126±2,034 ef	0
	80	0	6,772±925 a	69,286±8,052 ab	42,071±2,802 c–f	0
	160	0	10,902±5,628 a	71,111±6,020 a	60,611±3,935 abc	0

The same lowercase letters for the same nematode species within a column are not significantly different (Student-Newman-Keul's test, $P<0.05$).

higher numbers were produced at 24°C and 30°C (Table 1). The highest numbers of *S. glaseri* infective juveniles were produced at 30°C at the inocula between 20 and 160 infective juveniles/larva. However, the number of *S. glaseri* infective juveniles produced overlapped at 24°C and 30°C at the 80 and 160 inocula levels. At 18°C, the number of *S. glaseri* infective juveniles produced was lower than the number of *S. longicaudum* infective juveniles. In contrast, *S. longicaudum* produced more progeny at 24°C at the inocula between 40 and 160 infective juveniles/larva. At 30°C, *S. longicaudum* infective juvenile production overlapped at 160 infective juveniles/larva.

Temperature and concentration affected the first progeny emergence of *S. glaseri* (F=8.34; df=11, 100; $P<0.0001$) and *S. longicaudum* (F=8.04; df=11, 100; $P<0.0001$) from cadavers. Progeny emergence took longer at both low temperatures and low concentrations. The earliest emergence of *S. glaseri* infective juveniles was at 24°C at 8.3 days post infection at 160 infective juveniles/larva followed by 30°C at 8.4 days post infection. By day 14, 80% of the infective juveniles that would emerge from the cadaver had emerged at 24°C and 30°C. No significant difference was obtained for *S. longicaudum* as infective juveniles began emerging at 8.1 days post infection at 30°C at 20 infective juveniles/larva, followed by the same temperature at 8.2 days post infection at 80 and 160 infective juveniles/larva. Peak emergence occurred at 11 days for *S. glaseri* at 30°C at 160 infective juveniles/larva and 12 days for *S. longicaudum* at 24°C at 80 infective juveniles/larva.

Nematode Motility

Time and temperature influenced *S. glaseri* motility (F=36.6; df=4, 15; $P<0.0001$), but only temperature influenced *S. longicaudum* motility (F=228.4; df=4, 15; $P<0.0001$). Based on cumulative numbers, *S. glaseri* was most active at 30°C and *S. longicaudum* at 24°C and 30°C (Fig. 3). The total number of *S. glaseri* infective juveniles recovered was twice that of *S. longicaudum*, with the most infective juveniles recovered the first 2 days after placing the dish in the incubator (Fig. 3).

Bacterial Growth on MacConkey Agar

The symbiotic bacteria, *X. poinarii* (Xp) and *Xenorhabdus* sp. (Xs), grew at all tested temperatures ranging from 13°C

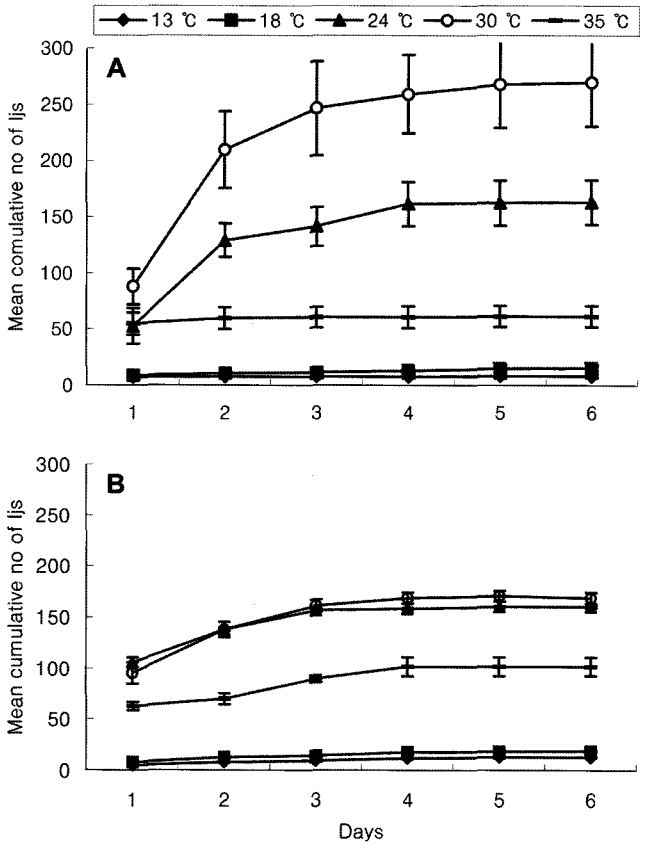

Fig. 3. Changes of the motility of nematodes with time at different temperatures from 13°C to 35°C.
The motility of nematodes was measured based on the cumulative number of infective juveniles (Ijs) on the petri dish lids. (**A**) *Steinernema glaseri* Dongrae and (**B**) *S. longicaudum* Nonsan. Vertical bars represent standard error of the means.

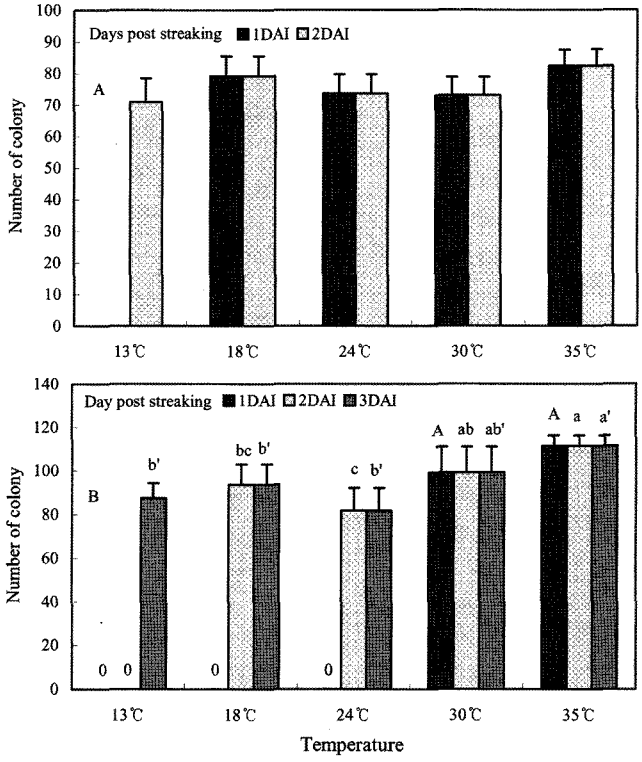

Fig. 4. Effect of temperature on the growth of symbiotic bacteria, *Xenorhabdus poinarii* (**A**) and *Xenorhabdis* sp. (**B**), on MacConkey agar.
Vertical bars represent standard error of the means. The same uppercase (1 DAI), lowercase (2 DAI), and lowercase with apostrophe (3 DAI) above the bars indicates no significant difference among means ($P<0.05$).

Table 2. Mortality of *Galleria mellonella* larvae after injection of the symbiotic bacterium, *Xenorhabdus poinarii* (Xp) or *Xenorhabdus* sp. (Xs).

Symbiotic bacteria	Time post injection (h)	13°C	18°C	24°C	30°C	35°C
Xp	12	0 b	0 b	0 b	0 b	92.5±4.8 a
	24	0 b	0 b	100 a	100 a	100 a
	36	0 b	0 b	100 a	100 a	100 a
	48	0 b	100 a	100 a	100 a	100 a
	56	100 a	100 a	100 a	100 a	100 a
Xb	24	0 b	0 b	0 b	0 b	0 b
	48	0 b	0 b	0 b	100 a	100 a
	72	0 b	0 b	100 a	100 a	100 a
	96	0 b	0 b	100 a	100 a	100 a
	120	0 b	0 b	100 a	100 a	100 a
	144	0 b	0 b	100 a	100 a	100 a
	168	0 c	67.5±4.8 b	100 a	100 a	100 a
	192	0 b	100 a	100 a	100 a	100 a
	240	7.5±4.7 b	100 a	100 a	100 a	100 a

Mean percentages of mortality followed by the same letters are not significantly different among temperatures (Student-Newman-Keul's Test, $P<0.05$).

to 35°C. The number of colonies of *X. poinarii* was not significantly different among temperatures at one day after streaking ($F=95.2$; df=4, 45; $P<0.0001$), two days after streaking ($F=99.04$; df=4, 45; $P<0.0001$), and three days after streaking ($F=5.51$; df=4, 45; $P<0.0001$) (Fig. 4). Colonies of *X. poinarii* were not observed at 13°C until one day after streaking, and those of *Xenorhabdus* sp. were not observed at 13°C until two days after streaking (Fig. 4).

Virulence of Bacterium

Virulence of *Xenorhabdus poinarii* and *Xenorhabdus* sp. was higher with increasing temperature. *Xenorhabdus* sp. was more virulent than *X. poinarii*. The percentage mortality was 92.5% by *Xenorhabdus* sp. at 35°C in 12 h ($F=373.36$; df=4, 15; $P<0.0001$) and 100% at >24°C at all tested temperatures in 56 h, whereas 100% mortality was obtained by *X. poinarii* at 30°C and 35°C in 48 h and at 18°C in 192 h (Table 2). However, the percentage mortality was 72.5% at 13°C in 432 h (data not shown).

DISCUSSION

Virulence, development, reproduction, and motility of nematode species, and growth and virulence of their symbiotic bacteria, varies with nematode and bacterial species. In our study with nematodes, the virulence of *S. glaseri* Dongrae and *S. longicaudum* Nonsan resulted in lower mortalities at low temperatures, but there were no differences in the virulence between the two nematodes at temperatures greater than 24°C. In general, *S. glaseri* Dongrae was more adaptive to high temperature compared with *S. longicaudum* Nonsan, which was more adaptive to low temperature. That is, mortalities by *S. longicaudum* Nonsan were higher than those by *S. glaseri* Dongrae at low temperatures (*i.e.*, 13°C and 18°C) at all concentrations. *Steinernema longicaudum* Nonsan was isolated from a cold area in central Korea, whereas *S. glaseri* Dongrae was isolated from a warmer area in the southern part of Korea. Accordingly, temperature responses of entomopathogenic nematodes may be related to the climatic conditions from where they were isolated. Thus, the optimal temperature for *S. rarum* was 25°C, which corresponds to the warm climate of its original isolation locality [20]. Similarly, *S. monticolum*, which was isolated from a high mountain in Korea, is adapted to low temperatures [21, Choo unpublished data].

The temperature ranges for infection and development of *S. glaseri* Dongrae and *S. longicaudum* Nonsan were broader than those for reproduction. Similar results have been reported by a number of researchers. For example, the thermal niche breadth for infection of *S. carpocapsae* was 12°C–32°C [10], but infection by this nematode was most rapid at 20°C to 24°C [26]. Infection by *S. carpocapsae* occurred between 8°C and 16°C but did not reproduce at 8°C. At high temperatures, reproduction with *S. glaseri* did occur at 35°C, even though the nematodes developed to the first generation adults [24]. Interestingly, with *S. carpocapsae* DD-136, the nematodes developed to adults at 30°C, but the nematodes failed to reproduce [14], demonstrating that strain differences occur. Hazir *et al.* [12] showed that nematode strains of *S. feltiae* could result in differences in various parameters measured including reproduction.

Henneberry *et al.* [13] and Choo *et al.* [6] suggested that the failure of nematode reproduction at low and high temperatures may be due to inability of the symbiotic bacteria to grow. On the other hand, Milstead [23] showed

that *H. bacteriophora* did not reproduce at 12°C and 30°C, whereas its symbiotic bacterium caused host mortality at 12°C and 33°C. In our current study, the symbiotic bacteria grew and killed their hosts at 13°C and 35°C, indicating that the bacteria may not be the reason for the failure of nematode reproduction at these temperatures. That is, although infective juveniles of *S. glaseri* Dongrae and *S. longicaudum* Nonsan developed to adults at 13°C and 35°C, they did not reproduce at these temperatures. The low or high temperatures may have affected nematode mating behavior and/or production of oocyte or sperm [24]. In addition, the growth and/or quality of the symbiotic bacteria might be responsible for the low yield of nematodes at low temperature. Hatab and Gaugler [11] observed that bacterial cells that were cultured in insect lipids at low temperature (15°C) had lower lipid content than at higher temperatures (25°C or 30°C). Overall, temperature extremes were unfavorable for nematode reproduction because the number of *S. glaseri* Dongrae and *S. longicaudum* Nonsan progenies was low at low temperatures or nonexistent at high temperatures.

Temperature usually influences nematode motility [15, 24], but nematode movement also varies with species. *Steinernema glaseri* Dongrae was relatively motile at high temperature compared with *S. longicaudum* Nonsan. As with other parameters that we measured, the motility of *S. glaseri* Dongrae might be traced back to the climate from which it was isolated, where the average temperature is 14.4°C, whereas that of *S. longicaudum* Nonsan is 12.0°C. Moreover, *S. glaseri* Dongrae was isolated from a golf course where temperatures are known to fluctuate between day and night, whereas *S. longicaudum* Nonsan was from the forest where soil temperatures do not vary as much because of the shade.

X. poinarii and *Xenorhabdus* sp. caused high mortality of *Galleria* larvae from 24 h after injection except at 13°C. As expected, it took longer to kill insects at 13°C and 18°C. In general, the symbiotic bacteria were highly virulent to their *G. mellonella* host. In our study, *X. poinarii* isolated from *S. glaseri* Dongrae had a lower virulence than *Xenorhabdus* sp. from *S. longicaudum*. In addition, LT_{50} values of *X. poinarii* were longer than those of *Xenorhabdus* sp. at all tested temperatures, especially 6 times longer at 13°C. Many studies stated that either *X. poinarii* or its nematode partner, *S. glaseri*, alone was less pathogenic than other species of symbiotic bacteria. However, insecticidal activity can be different depending on strains within the same species of symbiotic bacterium [28].

Our study has shown that the temperature range for bacterial growth and insect mortality was wider than that for nematode reproduction. The bacteria grew and killed their insect hosts between 13°C and 35°C, but the nematodes were unable to reproduce even though they killed the insects at these temperatures. Finding the temperature ranges of the Korean nematode-bacterium complex provides us with information on the optimal performance for production and their use under conditions in Korea.

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