Effects of Temperature on Insecticidal Activity of
an Entomopathogenic Nematode, *Steinernema kushidai*
(Nematoda: Steinernematidae), against *Anomala cuprea*
(Coleoptera: Scarabaeidae) Larvae¹

AZUSA FUJIE, Mineo TACHIBANA,² Yoshiyuki TAKATA,³
Tomoko YOKOYAMA, Nobukazu SUZUKI²
and Toshihito UECHI²

Chiba Prefectural Agricultural Experiment Station, Midori-ku, Chiba 266, Japan

²Advanced Technology Laboratory, Kubota Corporation, Ryugasaki 301, Japan

³Faculty of Horticulture, Chiba University, Matsudo 271, Japan

(Received 7 January, 1994; Accepted 12 October, 1994)

Insecticidal activity of *Steinernema kushidai* against *Anomala cuprea* larvae, feeding activity of *A. cuprea* larvae, survival of *S. kushidai* infective juveniles (J_{III}) and qualitative changes of the symbiotic bacteria were examined under six different temperature conditions (10–35°C at 5°C intervals). Mortality of *A. cuprea* larvae was 95, 100 and 85% at 20, 25 and 30°C, respectively, but significantly lower at 10, 15 and 35°C. Feeding amount of *Quercus acutissima* leaves by the 3rd instar larvae was significantly greater at 20, 25 and 30°C. Survival rate of J_{III} in water suspension was significantly higher at 10, 15, 20 and 25°C and that in soil was also higher at 10, 15, 20, 25 and 30°C, respectively. The color of colonies and antibiotic characters in relation to insecticidal activity of symbiotic bacteria were not influenced by the temperature conditions during 10 and 35°C.

Key words: *Steinernema kushidai*, *Anomala cuprea*, *Xenorhabdus japonicus*, temperature, insecticidal activity

INTRODUCTION

Steinernema kushidai (KUSHIDA et al., 1987) is known for high insecticidal activity against the larvae of scarabaeid beetles such as the cupreous chafer, *Anomala cuprea* (KOIZUMI et al., 1988; MAMIYA, 1989; FUJIE et al., 1993), which are difficult to control with conventional chemical pesticides.

We have observed that the activity of *S. kushidai* against scarabaeid beetle larvae was low in cooler temperature conditions. Previous reports also mentioned that temperature had a direct effect on survival and infection of nematodes (MOLYNEUX, 1985; KAYA, 1990). Therefore, we examined the role of temperature on the potential of *S. kushidai* as a biological control agent, and describe the related factors that affect insecticidal activity.

¹ A major part of this study was presented at the Annual Meeting of the Japanese Society of Applied Entomology and Zoology, held in Matsumoto, April, 1993.

MATERIALS AND METHODS

Nematodes and scarabaeid beetles. *S. kushidai* was maintained on a modified dog food (DF) medium, as in OGURA and MAMIYA (1989). DF medium consisted of 88 g of dog food (Ajinomoto General Foods Corp., Japan), 12 g of yeast extract (Difco Lab., U.S.A.), 4 g of lard and 3 g of agar (Wako Pure Chem. Ind. Ltd., Japan) solubilized to a 1-l quantity with 33 mM Sørensen phosphate buffer (45.4 g/l KH_2PO_4 : 47.4 g/l $\text{Na}_2\text{HPO}_4 = 4 : 6$, solution diluted 10 times), pH 6.47. The infective juveniles (J_{III}) were recovered from the medium within a day by the Baermann funnel method and stored at 15°C in shallow water until use after washing three times with deionized water.

About 200 *A. cuprea* adult individuals collected by light traps were used for oviposition. Adults were fed with leaves of chestnut *Castanea crenata*, and eggs were collected from 10 confined adults each kept in a plastic container (480 cm² base, and 8 cm depth) with 1 cm of volcanic ash soil. Ten eggs were placed in a Petri dish (9 cm dia.) filled with 1 : 1 mixture of volcanic ash soil and humus. The 2nd and 3rd instar larvae emerged in the Petri dishes were individually reared in plastic cups (6 cm dia., 3 cm depth) with 30 ml of volcanic ash soil and an adequate supply of powdered leaves of the deciduous oak, *Quercus acutissima*. Volcanic ash soil kept at 30–35% water content was used. Water was sprayed on the soil surface when the soil dried.

Effects of temperature on the insecticidal activity of nematodes

(1) Assay in cups: Third instar larvae were individually released into plastic cups (6 cm dia., 3 cm depth) with 30 ml of volcanic ash soil at 35% water content, and powdered leaves on the soil surface. One milliliter of suspension with 1,000 J_{III} was introduced into each plastic cup. The cups were kept at 10, 15, 20, 25, 30 or 35°C. Ten cups were exposed to each temperature. Numbers of dead larvae were counted every day for 20 days after the treatment. Larval cadavers were dissected under a binocular microscope to confirm the existence of nematodes. Four trials were conducted with 10 cups per treatment.

(2) Assay in pots: Strawberry plants (var. *Nyoho*), *Fragaria* × *ananassa*, were individually planted in 1/5,000 a Wagner pots with volcanic ash soil. After releasing 10 2nd and 3rd instar larvae of *A. cuprea* into each pot, 20 ml of nematode suspension with 500 J_{III} per ml was introduced. Pots were kept at 15, 25 and 35°C, respectively. Numbers of surviving and dead larvae were counted 30 days after treatment. Two trials were conducted with three pots per treatment.

Effects of temperature on the ingestion by 3rd instar larvae. After larvae were weighed, the 3rd instar larvae were individually released into plastic cups (6 cm dia., 3 cm depth) with 30 ml of volcanic ash soil at 35% water content and a dry *Q. acutissima* leaf piece, and kept at 10, 15, 20, 25, 30 and 35°C for 2 days, respectively. Before and after ingestion, feeding amounts were estimated by weighing leaf pieces after desiccation at 70°C for 2 days. Three trials were conducted with five larvae per treatment.

Effects of temperature on the survival of J_{III} . Fifty milliliters of suspension with 100 J_{III} per ml was put into each 100 ml conical beaker and kept at 10, 15, 20, 25, 30 and 35°C for 7 days, respectively. Numbers of survived J_{III} were counted by sampling of 5 ml of suspension from each conical beaker twice. Two trials were conducted with two beakers per treatment.

One milliliter of suspension with 1,000 J_{III} was inoculated into each 100 ml conical beaker containing 25 g of volcanic ash soil at 35% water content. The conical beakers

were kept at 10, 15, 20, 25, 30 and 35°C for 7 days each. Numbers of surviving J_{III} isolated by the Baermann funnel method from the soil for a day were counted three times. Two trials were conducted with two and four beakers per treatment.

Effects of temperature on colonization and antibiotic characters in relation to insecticidal activity of symbiotic bacteria. Symbiotic bacteria *Xenorhabdus japonicus* (NISHIMURA et al., 1994) that were isolated from the J_{III} kept at six different temperature conditions (as described above) were used. Two cultures of symbiotic bacteria, XC-119-P1 and XC-119-P2, possessing high and low insecticidal activities (TACHIBANA, unpublished data) were also used as controls. XC-119-P1 and XC-119-P2 were the original cultures of symbionts that were isolated from *S. kushidai* J_{III} and maintained weekly on BTB/TTC-YS plate in Petri dishes (9 cm dia.) in Kubota Corporation since 1991. BTB/TTC-YS was yeast extract salt agar (0.5 g $NH_4H_2PO_4$, 0.5 g K_2HPO_4 , 0.2 g $MgSO_4 \cdot 7H_2O$, 5.0 g NaCl, 5.0 g of yeast extract and 15 g of agar in 1 l of water; DYE, 1968) supplemented with 0.0025% bromothymol blue (BTB) and 0.002% 2,3,5-triphenyltetrazolium chloride (TTC).

(1) Isolation of symbiotic bacteria: J_{III} kept at each temperature for 7 days were separately collected from soil by the Baermann funnel method, washed with deionized water and surface sterilized with 0.1% formaldehyde solution and 0.1% benzetonic chloride solution by decantation three times each. The sterilized J_{III} were washed three times with sterile deionized water and sedimented up to 1,000 J_{III} per ml. Three hundred microliters of each nematode suspension was spread on each BTB/TTC-YS plate and incubated at 25°C. After 4 days, a single bacterial colony was transplanted from each plate on another fresh BTB/TTC-YS plate and incubated at 25°C for 3 days.

(2) Color of colonies: Color of bacterial colonies and BTB adsorptions were determined on the 7th day after transplantation.

(3) Antibiotic activity: One loopful of symbiotic bacteria on each BTB/TTC-YS plate was transferred into 50 ml of nutrient broth-yeast medium, NY medium (8 g of nutrient broth and 20 g of yeast extract in 1 l of water, pH 7.0), in 250-ml baffled flasks. The flasks were shaken at 160 r.p.m. at 25°C for 3 days. The culture was then centrifuged at 10,000 r.p.m. for 5 min and the supernatant was filtrated through a 0.22 μm (pore size) sterile filter. Ten microliters of each filtrate was spotted on each plate of nutrient agar, NA plate (8 g of nutrient broth and 15 g of agar in 1 l of water) in Petri dishes (9 cm dia.) spread with *Bacillus subtilis* (IFO No. 3007). Antibiotic activity was examined with the appearance of inhibition zone at 24 h after the incubation at 25°C.

(4) Breeding of nematodes with thermal processed symbiotic bacteria: Two thousand J_{III} suspended in 1 ml of water were inoculated onto DF medium in Petri dishes (9 cm dia.) and cultivated at 25°C for 4 days. After confirming that J_{III} had grown to adults and that females had many eggs in their bodies, 10 ml of sterile deionized water was poured onto each DF medium and allowed to stand for 10 min. The nematode suspension was transferred to a sterile 40 μm mesh nylon sieve with a pippete. Females trapped on the sieve were washed three times with sterile deionized water by decantation and kept in sterile deionized water for 10 min. Fifteen milliliters of sterile 0.4 N NaOH was added to the concentrated female suspension. After being shaken for 10 min, the suspension was neutralized with 50% phosphoric acid and transferred to a sterile 30 μm mesh nylon sieve. Cell debris of females were trapped on the sieve and the suspension with eggs was obtained. After appropriate dilution, the number of eggs was counted with a Peter's 1-ml eelworm counting slide. From one plate, $2-3 \times 10^5$ eggs were collected. The bacterial contamination of egg suspension was checked using BTB/TTC-YS plate on which 100 μl

of egg suspension was spread.

One loopful of each symbiotic bacteria was inoculated into 50 ml of yeast extract salt broth, YS broth (DYE, 1968) in 250 ml baffle flasks, and shaken at 160 r.p.m. at 25°C for 24 h. Four hundred microliters of YS broth was inoculated into 20 ml of SGLPY medium (OGURA and HARAGUCHI, 1993) in each Petri dish (9 cm dia.). After 24 h of incubation at 25°C, 2,000 eggs were inoculated onto each SGLPY plate and kept at 25°C for more than 20 days.

(5) Insecticidal activity of nematodes with several thermally processed symbiotic bacteria: Monoxenic J_{III}, which possess six temperature-treated symbiotic bacteria and two control cultures of symbiotic bacteria, were collected from the medium by the Baermann funnel method. The 2nd instar larvae of *A. cuprea* were individually placed into the plastic cups (6 cm dia., 3 cm depth) with 30 ml of volcanic ash soil at 35% water content and an adequate amount of powdered leaves. One milliliter of suspension containing 1,000 J_{III} was poured onto the soil and food surface in each plastic cup, which was then kept at 25°C. The number of dead larvae was counted on the 10th day after treatment. Larval cadavers were examined for nematodes under a binocular microscope. Four trials were conducted with 10 cups per treatment.

RESULTS

Effects of temperature on the insecticidal activity of nematodes

Figure 1 shows the effects of temperature on the mortality of *A. cuprea* by *S. kushidai* during 20 days after treatment in the cup test. The mortality and the insecticidal period before death were higher and shorter, respectively, at 20, 25 and 30°C, and larvae were killed especially quickly at 25°C. However, very few larvae were killed at the lowest temperature of 10°C and at the highest temperature of 35°C.

The mortalities of the 3rd instar larvae were 95, 100 and 85% at 20, 25 and 30°C, respectively, but significantly lower at 10, 15 and 35°C on the 20th day after treatment. Nematodes were detected from almost all cadavers of larvae under each temperature condition.

Fig. 1. Effects of temperature at 10, 15, 20, 25, 30 and 35°C on the mortality of *Anomala cuprea* by *Steinernema kushidai* J_{III} for 20 days after treatment.

Table 1. Insecticidal activity of *Steinemema kushidai* J_{III} against *Anomala cuprea* larvae under three different temperature conditions in Wagner pots planted with strawberries

Temp. (°C)	No. of larvae/pot ^a	
	Survival (mean ± SD)	Dead ^b (mean ± SD)
15	8.3 ± 1.5 c ^c	0 a
25	0 a	5.3 ± 2.0 b
35	3.7 ± 0.6 b	0 a

^a Number of larvae was examined on the 30th day after treatment.

^b Dead larvae due to *S. kushidai* infection were noted and others were omitted.

^c Means followed by the same letter are not significantly different at the 5% level by DUNCAN's multiple range test.

Table 2. Effects of temperature on the feeding on *Quercus acutissima* leaves by *Anomala cuprea* 3rd instar larvae

Temp. (°C)	Amount of feeding ^a (mg/day/g of larva) (mean ± SD)
10	8.10 ± 8.61 a ^b
15	34.29 ± 20.84 b
20	37.63 ± 19.36 bc
25	43.71 ± 27.35 bc
30	54.23 ± 25.77 c
35	10.01 ± 12.12 a

^a Feeding on leaves is the weight of dry matter and examined on the 2nd day after treatment.

^b Means followed by the same letter are not significantly different at the 5% level by DUNCAN's multiple range test.

Table 1 shows the insecticidal activity of *S. kushidai* J_{III} against *A. cuprea* larvae at 15, 25 and 35°C in Wagner pots. Dead larvae were observed only at 25°C. The numbers of surviving and dead larvae were significantly less and more, respectively, at 25°C than at 15 and 35°C.

Effects of temperature on feeding amount of leaves by 3rd instar larvae

Table 2 shows the effects of temperature on the feeding of *Q. acutissima* leaves by 3rd instar larvae of *A. cuprea*. The amount of ingestion at 30°C, 54.23 mg/day/g of larva, was the greatest, but not significantly different from the values at 20 and 25°C. The values were significantly lower at 10 and 35°C.

Effects of temperature on the survival rate of J_{III}

Table 3 shows the effects of temperature on the survival rate of *S. kushidai* J_{III}, which was examined by directly counting surviving J_{III} in water. The survival rates of J_{III} were significantly higher at 10, 15, 20 and 25°C than at 30 and 35°C. The survival rate tended to decrease at higher temperature.

In soil, the survival rate estimated by the number of nematodes extracted with the Baermann funnel method, was also significantly lower at 35°C (Table 4).

Effects of temperature on color of colonies and antibiotic characters in relation to insecticidal activity of symbiotic bacteria

Colonies of all the cultures of *X. japonicus* isolated from J_{III} that had been kept under six different temperature conditions were blue, as shown in Table 5. Moreover, the supernatant of liquid medium after culturing these cultures indicated antibiotic activity. These two characters were similar to XC-119-P1, a culture with high insecticidal activity. On the other hand, the colonies of XC-119-P2, a culture with poor insecticidal activity, were red on media, and the supernatant indicated no antibiotic activity.

Mortalities of *A. cuprea* larvae from monoxenically cultured J_{III} with inoculated symbi-

Table 3. Effects of temperature on the survival of *Steinernema kushidai* J_{III} in water

Temp. (°C)	Survival ^a (%) (mean ± SD)
10	81.6 ± 10.7 c ^b
15	86.3 ± 8.9 c
20	77.0 ± 10.0 c
25	70.0 ± 11.4 c
30	27.9 ± 15.1 b
35	0.4 ± 0.8 a

^a Survival was examined on the 7th day after treatment.

^b Means followed by the same letter are not significantly different at the 5% level by DUNCAN's multiple range test.

Table 4. Effects of temperature on the survival of *Steinernema kushidai* J_{III} in soil

Temp. (°C)	Survival ^a (%) (mean ± SD)
10	36.5 ± 19.6 b ^b
15	44.6 ± 17.2 b
20	31.0 ± 24.1 b
25	36.5 ± 22.1 b
30	38.9 ± 17.0 b
35	10.0 ± 8.6 a

^a Survival was examined on the 7th day after treatment and shown as the daily rate of nematodes extracted by the Baermann funnel method.

^b Means followed by the same letter are not significantly different at the 5% level by DUNCAN's multiple range test.

Table 5. Effects of temperature on the colonization and antibiotic character in relation to insecticidal activity of symbiotic bacteria, *Xenorhabdus japonicus*

Culture ^a	Color of colonies on BTB/TTC-YS medium	Antibiotic activity ^b	Mortality ^c (%)
Bacteria-10	Blue	+	100 b
Bacteria-15	Blue	+	100 b
Bacteria-20	Blue	+	100 b
Bacteria-25	Blue	+	100 b
Bacteria-30	Blue	+	100 b
Bacteria-35	Blue	+	100 b
XC-119-P1	Blue	+	100 b
XC-119-P2	Red	—	26.5 a

^a Bacteria-10, 15, 20, 25, 30 and 35 indicate the cultures of *X. japonicus* isolated from *Steinernema kushidai* J_{III} kept under 10, 15, 20, 25, 30 and 35°C, respectively, for 7 days. XC-119-P1, a highly insecticidal culture, and XC-119-P2, a weakly insecticidal culture, were used as controls.

^b Antibiotic activity of supernatant of liquid medium after *X. japonicus* culture.

^c Mortality of *Anomala cuprea* larvae by monoxenically cultured *S. kushidai* J_{III} with inoculated *X. japonicus* cultures. Means followed by the same letter are not significantly different at the 5% level by DUNCAN's multiple range test.

otic bacteria cultures had no significant difference among the six cultures and XC-119-P1, although the mortality by XC-119-P2 was significantly lower. Nematodes were also detected on almost all of the insect larvae killed by these six cultures and XC-119-P1, and on a few larvae killed by XC-119-P2.

DISCUSSION

S. kushidai is said to have characteristics suitable for warm areas (KUSHIDA et al., 1987). We also know from experience that the insecticidal efficacy of *S. kushidai* against scarabaeid beetles is lower in cooler temperatures. Efficacy is also low under sweltering

hot conditions, as in greenhouses in summer. Direct effects of temperature on survival and infection of nematodes were mentioned in previous reports (MOLYNEUX, 1985; KAYA, 1990). This impression was proven by the experiment that used cups and Wagner pots. The insecticidal activity of *S. kushidai* against *A. cuprea* was directly affected by temperature.

We investigated if this insecticidal activity dependence on temperature was affected by amount of ingestion by *A. cuprea* and survival rate of *S. kushidai* J_{III}. Feeding activity of *A. cuprea* larvae that was indicated by the amount of ingestion was higher at 20, 25 and 30°C than at 10, 15 and 35°C. A higher feeding activity possibly increases the opportunity for infection by J_{III}. In water and soil the survival rate of J_{III} on the 7th day was not significantly different at 10, 15, 20 and 25°C. However, the survival rate of J_{III} in soil did not drop as it did in water at 30°C. We estimated that lack of oxygen proved fatal to most J_{III} in water. A temperature as high as 35°C was a fatal condition in water and soil. In the soil experiment, the survival rate was indicated as relative values that were estimated by the Baermann funnel method, because the extracted rate for nematodes was usually low and was affected by several factors (MINAGAWA, 1979).

Colony color of *X. japonicus* originating from J_{III} kept at 10–35°C was blue, characteristic for the highly insecticidal culture XC-119-P1. Moreover, insecticidal activity of J_{III} that held the *X. japonicus* cultures was higher than that of J_{III} that held the weakly insecticidal culture, XC-119-P2. This result suggests that the change from primary form to secondary form, known as dimorphism in *Xenorhabdus* spp. (AKHURST, 1980), was not affected by low or high temperatures such as 10 or 35°C, at least *in vivo*. On the other hand, it has been reported that supernatant of *X. nematophilus* symbiont culture medium lost pathogenicity against *Spodoptera litura* from boiling heat treatment (YAMANAKA et al., 1992).

KAYA (1990) described that the temperature range for nematode infection depended on the native home temperatures, and that the range in which more than 70% infection occurred agreed with the range of native home temperature. Temperature in Shizuoka, adjacent to the location (Hamakita) where *S. kushidai* was first isolated, ranged from 0 to 30°C. The range of temperatures required by *S. kushidai* for higher insecticidal activity is limited to higher temperature conditions between 20 and 30°C, which are in the upper third of native home temperatures. The appropriate temperature for the storage of *S. kushidai* J_{III} is also higher than that of other infective stages, *S. carpocapsae* and *S. feltiae* (= *S. bibionis*) (DUTKY et al., 1964; BEDDING, 1984; KUSHIDA et al., 1987; FUJIE et al., 1993). For example, temperature below 5°C, appropriate for the storage of *S. feltiae*, allows survival of *S. kushidai* J_{III} that is moderate in soil and poor in water (FUJIE et al., 1993). This must be considered when we use *S. kushidai* as a biological control agent.

We concluded that insecticidal activity of *S. kushidai* was dependent on temperature and possibly on other factors, such as feeding activity of *A. cuprea* and survival rate of *S. kushidai* J_{III}. As shown in this experiment, these factors defined effective temperature range.

REFERENCES

- AKHURST, R.J. (1980) Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *J. Gen. Microbiol.* **121**: 303–309.
- BEDDING, R.A. (1984) Large scale production, storage and transport of the insect parasitic nematodes *Neoaplectana* spp. and *Heterorhabditis* spp. *Ann. Appl. Biol.* **104**: 117–120.

- DUTKY, S.R., J.V. THOMPSON and G.E. CANTWELL (1964) A technique for the mass propagation of the DD-136 nematode. *J. Insect Pathol.* **6**: 417–422.
- DYE, D.W. (1968) A taxonomic study of the genus *Erwinia*. I. The 'amylovora' group. *N. Z. J. Sci.* **11**: 590–607.
- FUJIE, A., T. YOKOYAMA, M. FUJIKATA, M. SAWADA and M. HASEGAWA (1993) Pathogenicity of an entomogenous nematode, *Steinemema kushidai* MAMIYA (Nematode: Steinernematidae), on *Anomala cuprea* (Coleoptera: Scarabaeidae). *Jpn. J. Appl. Entomol. Zool.* **37**: 53–63 (in Japanese with English summary).
- KAYA, H.K. (1990) Soil ecology. In *Entomopathogenic Nematodes in Biological Control* (R. GAUGLER and H.K. KAYA, eds.). CRC Press, Boca Raton, Florida, U.S.A., pp. 93–115.
- KOIZUMI, C., T. KUSHIDA and J. MITSUHASHI (1988) Preliminary field tests on white-grub control by an entomogenous nematode, *Steinemema* sp. *J. Jpn. For. Soc.* **70**: 417–419.
- KUSHIDA, T., Y. MAMIYA and J. MITSUHASHI (1987) Pathogenicity of newly detected *Steinemema* sp. (Nematode) to scarabacid larvae injurious to tree seedlings. *Jpn. J. Appl. Entomol. Zool.* **31**: 144–149 (in Japanese with English summary).
- MAMIYA, Y. (1989) Comparison of the infectivity of *Steinemema kushidai* (Nematode: Steinernematidae) and other Steinernematid and Heterorhabditid nematodes for three different insects. *Appl. Entomol. Zool.* **24**: 302–308.
- MINAGAWA, N. (1979) Efficiencies of two methods for extracting nematodes from soil. *Appl. Entomol. Zool.* **14**: 469–477.
- MOLYNEUX, A.S. (1985) Survival of infective juveniles of *Heterorhabditis* spp., and *Steinemema* spp. (Nematode: Rhabditida) at various temperatures and their subsequent infectivity for insects. *Rev. Nematol.* **8**: 165–170.
- NISHIMURA, Y., A. HAGIWARA, T. SUZUKI and S. YAMANAKA (1994) *Xenorhabdus japonicus* sp. nov. associated with the nematode *Steinemema kushidai*. *World J. Microbiol. Biotechnol.* **10**: 207–210.
- OGURA, N. and N. HARAGUCHI (1993) Xenic culture of *Steinemema kushidai* (Nematoda; Steinernematidae) on artificial medium. *Nematologica* **39**: 266–273.
- OGURA, N. and Y. MAMIYA (1989) Artificial culture of an entomogenous nematode, *Steinemema kushidai* (Nematoda: Steinernematidae). *Appl. Entomol. Zool.* **24**: 112–116.
- YAMANAKA, S., A. HAGIWARA, Y. NISHIMURA, H. TANABE and N. ISHIBASHI (1992) Biochemical and physiological characteristics of *Xenorhabdus* species, symbiotically associated with entomopathogenic nematodes including *Steinemema kushidai* and their pathogenicity against *Spodoptera litura* (Lepidoptera: Noctuidae). *Arch. Microbiol.* **158**: 387–393.