Artificial Culture of an Entomogenous Nematode, *Steinernema kushidai* (Nematoda: Steinernematidae)

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An entomogenous nematode, *Steinernema kushidai*, which is conspicuously lethal on white grubs, was cultured in artificial media. The nematode propagation was vigorous in the dog food agar medium supplemented with peptone, but poor in the non-supplemented medium. Analysis of the ammonia content of nematode cultured media indicated that the nematodes and/or symbiotic bacteria metabolized large amount of protein to propagate themselves.

**INTRODUCTION**

A new steinernematid nematode was discovered from the soil collected in Hamakita, Shizuoka Prefecture (Kushida et al., 1986) and named *Steinernema kushidai* (Mamiya, 1988). The nematode has a conspicuous lethal effect on the larvae of several species of scarabaeid beetles, known as white grubs (Kushida et al., 1987; Koizumi et al., 1988). Although *S. kushidai* was maintained on either dog food agar or chicken offal medium, its reproductive rate on these media was far lower than that of *S. feltiae* (Mamiya, 1988). In this paper, we examine the causes of this low reproductive rate by comparing ammonia contents of media where *S. kushidai* or *S. feltiae* were cultured. A simple method to maintain *S. kushidai* in a semifluid dog food agar supplemented with peptone and the importance of protein for the nematode propagation are presented.

**MATERIALS AND METHODS**

*Medium.* Ten kinds of media were used (Table 1); all media were semifluid after autoclaving at 120°C for 20 min. Five ml of the sterilized media was pipetted into a culture bottle (45×85×25 mm).

*Nematode culture.* Infective juveniles (*J*₃₃) of *S. kushidai* and *S. feltiae* (Mexican strain) were prepared from the dog food agar (Kondo and Ishibashi, 1984). *J*₃₃ were washed three times with sterilized distilled water (DW). Five hundred *J*₃₃ suspended in 100 μl DW were inoculated to each culture bottle, plugged with a silicone cap (Shinetsu Polymer Ltd., Japan), and incubated at 25°C for up to 40 days. Average numbers and standard deviation (SD) were examined for the *J*₃₃ per bottle.

*Measurement of ammonia content in exhausted media.* Ten to 40 days after nematode inoculation, DW was added to the culture bottle to a total volume of 30 ml. The mixture was centrifuged at 12,000 g for 30 min. The supernatant was passed through
a 0.2 μm pore size filter (Millipore Corp., U.S.A.). Appropriate quantity of the supernatant (200 μl to 1 ml) was added to DW to become 4 ml. To this solution was added 1 ml of 1.2 M sodium potassium tartrate and 0.2 ml of Nessler's solution (Wako Pure Chem. Indus. Ltd., Japan). Absorbance at 425 nm was measured 20 min after the reaction began. Differences in ammonia contents in media were measured before and after nematode inoculation. To compare the ammonia production in Medium A by *S. kushidai* and *S. felttiae*, the ammonia content increased in the culture bottle was divided by the number of J_{III} produced. Results are indicated by ng of nitrogen (ng N).

**RESULTS**

**Nematode propagation and ammonia content in Medium A**

Numbers of J_{III} of *S. kushidai* and *S. felttiae* were examined 10 to 40 days after nematode inoculation in Medium A (Fig. 1). *S. kushidai* J_{III} did not increase in number during the first 20 days of culture but did to 31,500±27,597 per culture bottle during the next 20 days. *S. felttiae*, however, reproduced promptly and numerously; 476,400±72,510 J_{III} per bottle 40 days after inoculation.

Figure 1 also shows the ammonia content of Medium A in which *S. kushidai* or *S. felttiae* had been cultured. Averages and SD of the values, which were obtained after 40 days' incubation, were 32.6±11.0 ng N in *S. kushidai* and 5.3±1.2 ng N in *S. felttiae*, respectively.

**Culture of S. kushidai and S. felttiae in Medium F**

Both species of nematode were cultured in the dog food agar supplemented with peptone (3.6%). Numbers of J_{III} of *S. kushidai* increased promptly during the first 10 days, and reached 172,125±38,322 by 20 days. The increase in numbers of J_{III} of *S. felttiae* occurred mainly from 10 to 20 days after inoculation, and 20 days' incubation yielded 173,750±27,837 J_{III} (Fig. 2).
Fig. 1. Changes in numbers of infective juveniles produced (circles connected with single line) and ammonia content (circles with double line) in the dog food agar without additive peptone (Medium A) in which nematodes had been cultured. Solid and open circles indicate *S. kushidai* and *S. feltiae*, respectively. Vertical lines indicate standard deviation (5 culture bottles).

**Culture of *S. kushidai* in Medium B, C, D, E, and F**

*S. kushidai* was cultured in the dog food agar containing various amounts of peptone. In Medium B (peptone: 0%), J_{III} were scarcely produced. One hundred and seventy adults were observed in one bottle after 40 days' incubation. In Medium C (peptone: 0.45%) and D (peptone: 0.9%), numbers of J_{III} increased. Numbers of J_{III} 40 days after incubation were 20,250±33,585 in medium C and 60,625±10,043 in medium D. In Medium E (peptone: 1.8%) and F (peptone: 3.6%), 40 days' incubations yielded 162,500±37,541 J_{III} and 162,875±20,344 J_{III}, respectively (Fig. 3).

**Culture of *S. kushidai* in Medium G, H, I, and J**

The effects of varying the amount of dog food in medium supplemented with peptone (1.5%) on reproduction of *S. kushidai* were examined. Inoculated J_{III} did not grow in the Medium G (Dog food: 0%). J_{III} were produced in Medium H (Dog food: 4.5%) though the number of J_{III} did not grow beyond 100,000. Numbers of J_{III} in Medium I (Dog food: 6.5%) increased during the first 15 days to 128,480±19,063, but the numbers did not increase thereafter. Medium J (Dog food: 8.5%) yielded about 180,000 J_{III} 20 or more days after inoculation (Fig. 4).

**DISCUSSION**

An entomogenous nematode, *S. kushidai*, was shown to reproduce in high numbers on the dog food agar supplemented with peptone.
Artificial media commonly used to rear steinernematid and heterorhabditid nematodes were dog food agar (House et al., 1965; Hara et al., 1981) and the homogenates of offals taken from various domestic animals (Bedding, 1981). Although S. kushidai can propagate on these media, its reproductive rate was far lower than that of S. feltiae (Mamya, 1988). In the present study, the propagation of S. kushidai was also low in the semifluid dog food agar (Medium A) which allowed the nematode to move freely and take nutrients. Therefore, we assumed that the dog food lacked specific nutrient(s) for S. kushidai. To test our assumption, we measured the ammonia content of exhausted media. Ammonia content in the medium with S. kushidai was about six times more than that with S. feltiae after 40 days incubation. This result suggests that the nutrient(s) deficient for S. kushidai is(are) some specific metabolite(s) of protein. This possibility is supported by the fact that addition of peptone to dog food enabled S. kushidai to propagate vigorously.

All entomogenous nematodes in Steinernematidae and Heterorhabditidae have specific symbiotic bacteria (Akhurst, 1980). These bacteria proliferate in the host hemocoel and also in artificial media. The nematodes feed these bacteria and reproduce many off-springs. Therefore, it is possible that the nutrients may be deficient for these bacteria rather than specifically for the nematode. When 1/30 M Sørensen buffer (pH 6.47) instead of distilled water was used as solvent of dog food agar without peptone, infective larvae (JIII) of S. kushidai did not increase in number. This might be due to an unsuitable pH for the bacterial proliferation along with the nutrient deficiency.

Experiments to examine the effect of peptone indicate that suitable peptone concentration in the dog food agar is between 0.9 and 1.8%. When peptone (1.5%) was added to the media (Dog food: 4.5 to 8.5%), numbers of JIII produced increased in proportion to the concentration of dog food. However, medium was easier to handle when lower concentrations of dog food were used. For this reason, it seems appropriate to use Medium I for the culture of S. kushidai. S. kushidai can be maintained by transferring medium containing 500 JIII to 5 ml of the fresh medium every 40 days.

Several species of steinernematid and heterorhabditid nematodes are produced at
low cost using offal of various domestic animals (Bedding, 1981). Some improvements in these techniques are now in progress to establish S. kushidae production at low cost.

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REFERENCES


