RESEARCH NOTES

Pristionchus lheritieri as a Carrier of Rhizobium japonicum[1]

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We have found no reports of relationships of saprozoic nematodes and beneficial bacteria. Our study involved a saprozoic nematode and a nitrogen-fixing bacterium to determine whether a beneficial role existed. Eggs, larvae, and adults of *Pristionchus lheritieri* (Maupas) Paramonov, were collected on a 400-mesh sieve from an active culture of nematodes and *Pseudomonas* sp. on nutrient agar. Individual eggs were isolated, aspirated with a micro-pipette, placed in a 50-ml beaker containing an aqueous solution of 1:1000 $HgCl_2$ in sterile distilled water. After stirring 3 min the contents were drawn through a sterile micro-syringe millipore filter-holder to collect the eggs on No. 1 filter paper. Additional sterile distilled water was drawn through this collecting device for another 3 min to remove $HgCl_2$ residue. The filter paper barrier containing the eggs was cut aseptically into four equal sections, each of which was incubated at 24 C on an agar (2) plate culture of *Rhizobium japonicum* Kirts (48 h old). The nematodes thrived and were maintained by weekly transfers.

Seeds of soybean [*Glycine max* (L.) Merr. '*Lee*'] were surface-sterilized in a 1:1000 aqueous solution of $HgCl_2$ for 3 min rinsed five times in sterile distilled water and added to petri plates and test tubes containing water agar on White's medium (5) minus a nitrogen source. Six days after germination, roots that formed in petri plates were excised at the base of the hypocotyl. Seedlings that developed in the test tubes were left intact. Approximately 50 gravid *P. lheritieri* females from the *R. japonicum* cultures were surface-sterilized 3 min in 1:1000 aqueous $HgCl_2$ (a treatment lethal to exposed bacteria) and rinsed five times in sterile distilled water. Then the nematodes were crushed aseptically in a small watch glass of sterile distilled water and the contents were poured into petri plates and test tubes containing excised roots or seedlings, respectively. Another 50 gravid females were surface-sterilized as described previously and were added alive to excised roots or the roots of intact seedlings.

Two types of controls were used, one received sterile distilled water only and the other received an *R. japonicum* suspension in sterile distilled water. The plates and test tubes containing these four treatments were incubated at 24 C for 20 days and examined periodically for contamination.

In a similar experiment, the contents of the watch glass containing crushed or live nematodes were added to agar plates, enriched with special media (2) to grow *R. japonicum*, and examined later for colony formation. There were 10 replications per treatment and the experiment was repeated three times.

P. lheritieri originating from laboratory cultures with *Pseudomonas* sp. thrived on the new host *R. japonicum*. Also *R. japonicum* survived passage through the alimentary tract of *P. lheritieri* and, after defecation, produced colonies on agar plates enriched with *R. japonicum* media. Contents, crushed or defecated, from the nematode's alimentary tract caused nodulation on excised or intact soybean roots in water-agar or N-free modified White's medium. Small nodules formed on soybean roots 7-10 days after inoculation with bacterial suspensions, crushed nematode suspensions, and live nematodes. Nodulation did not occur in the control treatment which received only sterile distilled water. There were no major comparative differences in root nodulation among the remainder of the treatments, including the other control with the bacterial suspension. Percentages (obtained from all replications or 30 root systems per treatment) of those roots showing nodulation only differed slightly between excised roots (18-19)

Received for publication 27 December 1973.

[1] Oregon Agricultural Experiment Station Technical Paper No. 3720.

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and the roots of intact seedlings (19-20) among the treatments from the water agar plates. Percentages did increase substantially, however, on N-free White's medium where percentages of nodulation were approximately two times higher for intact roots (59-61) when compared with excised roots (28-30). Nodules formed on roots in each experiment were tested for nitrogen fixation using the method of Koch and Evans (4). Reduction of acetylene to ethylene by nodules taken from all treatments confirmed nitrogen-fixing capabilities.

R. japonicum or its metabolic by-products supported enormous populations of *P. lheritieri* in laboratory cultures. A superabundance of a bacterial food source may account for passage of bacteria. Nematodes also can transport bacteria which adhere to their external body surface (3) as well as by ingestion and defecation (1). Since our data were not obtained from treatments beginning with equal concns of *R. japonicum*, we are unable to compare the efficiency of nematode dissemination with a suspension inoculation. However, an association of *P. lheritieri* and *R. japonicum* can result in nodulation of soybean roots. Thus, saprozoic nematodes can disseminate beneficial bacteria.

LITERATURE CITED

1. CHANTANAO, A., and H. J. JENSEN. 1969. Saprozoic nematodes as carriers and disseminators of plant pathogenic bacteria. J. Nematol. 1:216-218.
2. DE HERTOGH, A. A., P. A. MAYEUX, and H. J. EVANS. 1964. The relationship of cobalt requirement to propionate metabolism in Rhizobium. J. Biol. Chem. 241:2446-2453.
3. HAWN, E. J. 1963. Transmission of bacterial wilt of alfalfa by Ditylenchus dipsaci (Kühn). Nematologica 8:65-68.
4. KOCH, B., and H. J. EVANS. 1966. Reduction of acetylene to ethylene by soybean root nodules. Plant Physiol. 41:1748-1750.
5. WHITE, P. R. 1954. The cultivation of animal and plant cells. Ronald Press, New York. 239 p.