In-vitro interrelationships between rhizosphere bacteria and *Heterodera schachtii*

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SUMMARY

The influence of rhizobacteria on hatching, migration, and penetration of *Heterodera schachtii* was evaluated under laboratory

A-3, A-57, A-59, T-58, P-510, P-523, P-76, P-741, and SR-3. Isolates A-59, T-58, and P-523 have been identified as *Pseudomonas fluorescens* (Trevisan) Migula. SR-3 is a Streptomycin-resistant mutant of A-59. All isolates are Gram-negative rods.

All isolates were isolated from the rhizosphere of sugar beet. They were selected for their ability to reduce *H. schachtii* root penetration in greenhouse experiments when used as a seed treatment (Oostendorp & Sikora, 1989).

Bacteria were grown for 18-24 hours at 25° C on the media and at the pH values given in Table 1. The bacterial cells were scrapped off the surface of the agar plates with a sterile spatula and suspended in sterile $MgSO_4$ (0.1 M/l). The controls were treated with 0.1 M/l $MgSO_4$ only.

Table 1

Growth medium and pH of media used for inoculum production of bacterial isolates.

Isolate	*Medium*	*pH*	*Isolate*	*Medium*	*pH*
A-3	St-I	7.2	A-57	St-I	5.6
A-59	St-I	5.6	T-58	TS	5.6
P-76	KB	7.2	P-741	KB	7.2

resulting suspensions were diluted and aliquots were streaked onto Standard I - Agar with and without Streptomycin. The number of colonies was counted after 48 hours of incubation.

HATCHING TEST

Root exudates were collected by placing 100 six-week-old sugar beet seedlings into a beaker with 1 l of deionized water. The plants were removed after 24 hours and the solution containing the root exudates was sterilized by filtration through a 0.45 µm Millipore filter. Five times nine milliliters of the solution were each mixed with 1 ml of a suspension containing approximately 10^7 cells of each bacterial isolate tested. After 24 hours the bacteria were removed by filtration and the hatch promoting activity of the treated exudates was measured by mixing equal volumes of exudate and a *H. schachtii* egg suspension. Untreated root exudates and water, both amended with the same amount of $MgSO_4$ used to add the bacteria, served as controls. After four days, the number of hatched juveniles and remaining eggs were counted and the hatch rate was calculated according to hatch rate = hatched juveniles × 100/eggs + juveniles.

ATTRACTION TEST

For the attraction tests, the bacteria were inoculated in

The seedlings were planted at one end of 5-cm long × 2-cm wide × 0.5-cm deep block of fine sand (Kerstan & Röpke, 1977). One thousand *H. schachtii* juveniles were then pipetted onto the opposite end of the sand strip (Fig. 1 A). The sand blocks were then moistened with 2 ml of distilled water and incubated in a closed Petri dish at 20° C with 16 h photoperiod for 4 days. The sand blocks were separated into five 1-cm-long sections with a scalpel. The number of juveniles per section was determined by separating the nematodes from the sand by flotation and decanting.

The average distance covered by one juvenile was calculated from juveniles per section and distance travelled from site of inoculation. The number of juveniles that penetrated the root at the end of the block was counted after staining the roots with acid-fuchsin in lactic acid (Ferris, 1985). Untreated plants and sand blocks without plants were used as controls.

Penetration test

The techniques used were the same as in the attraction test. The plant roots, however, were buried along the entire length of the block (Fig. 1 B). Furthermore, 500 nematodes were spread over the entire surface of the block. Juveniles inside the root and in the sand were counted after two days.

Test for toxin production

For the test on possible production of toxic or inhibitory substances, the bacteriophagous nematode *Panagrellus redivivus* was used. The nematode was extracted and inoculated onto agar plates on which

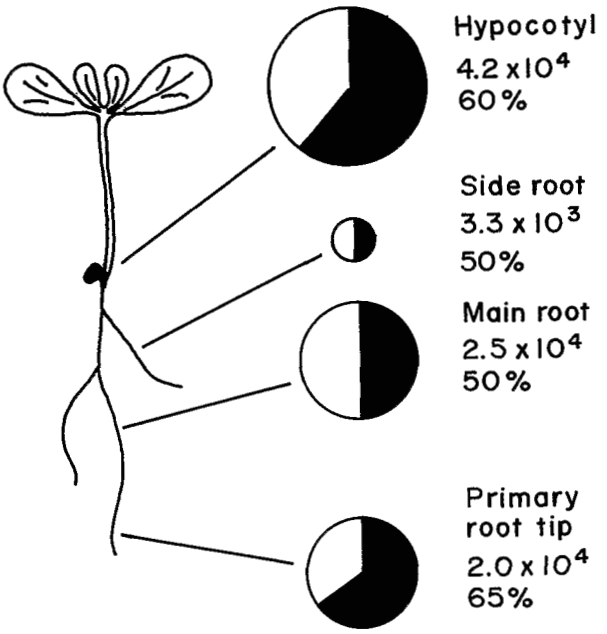
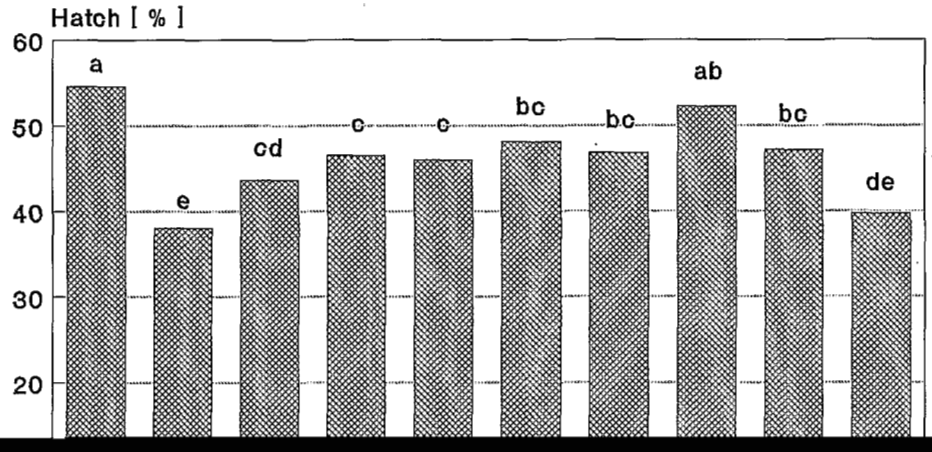


Fig. 2. Density of rhizobacterial colonization of sugar beet roots in cfu/mm and in percent of the total bacterial flora 22 days after seeding.

10^5 cfu/mm of root, the latter representing 100 % of the total bacterial population measured. Examination of the tips of the primary roots revealed bacterial densities that ranged between 2.7×10^3 and 10^5 cfu/mm root tip. The average numbers of bacteria and percentage SR-3 on


Hatch [%]
60
50
40
30
20
a
e
cd
c
c
bc
bc
ab
bc
de

treated controls (Fig. 5). Penetration was reduced ($P \leqslant 0.001$) 55 % and 68 % under the control level when roots were treated with isolates A-57 and P-523, respectively. Isolates P-76 and P-741 did not alter penetration levels.

Toxin production

None of the bacteria tested produced metabolites inhibitory or toxic to *Panagrellus redivivus*. There were no indications that the bacteria altered the movement or behavior of the nematode.

Discussion

Fluorescent pseudomonads are frequently used in the biological control of soil-borne plant-pathogenic fungi. The production of antibiotics and the competition for

the wildtype strain A-59 may be more competitive than the mutant strain SR-3, we believe that an inoculation technique must be developed that extends bacterial survival and aids a more reliable colonization.

The number of antagonistic rhizobacteria detected 22 days after planting agrees with the findings of Suslow and Schroth (1982). From these numbers and from an estimate of the root length observed in this experiment, we concluded that 10^7 cfu per root system is well in the range that occurs in unsterilized soil. Therefore, one plant or its exudates were treated with approximately this number of bacteria in the experiments on the mode-of-action to reduce possible side effects of the presence of untypically high numbers of bacteria.

Root exudate hatch stimulation was reduced ($P \leqslant 0.05$) by incubation with seven of the eight isolates. Reduction to the level considered to be spontaneous hatch occurred with isolate P-741. There was no re-

site for penetration (Zuckerman & Jansson, 1984). The

Kerstan, U., & Röpke, S. (1977). Einfluss von Systemnema-