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Antagonism of Azotobacter chroococcum isolates to Rhizoctonia solani

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Abstract

Antagonism of isolates of *Azotobacter chroococcum* to *Rhizoctonia solani* on agar plates was studied, and isolates were tested for their ability to control *R. solani* infection of potato sprouts in sterilized and unsterilized soil. The degree of antagonism exhibited varied strongly among the isolates and was also found to be temperature-dependent.

At 25, 20 and 15 °C, all but one strongly antagonistic *Azobacter* isolate effectively prevented infection of sprouts of potatoes planted in a soil heavily infected with a pathogenic isolate of *R. solani*. At 10 °C none was effective.

Introduction

Since 1930, bacterial fertilizers *(Rhizobium* sp., Azotobacterin, Phosphobacterin and blue-green algae) have been used in some countries to increase crop yields. Among these organisms is *Azotobacter chroococcum,* a free-living nitrogen-fixing bacterium, that is rather common in (not too acidic) soils. It thrives profusely in the rhizosphere of plants as their root exudates support its growth. The population of *A. chroococcum* in some Dutch soils in the plough layer was found to vary from $13 \cdot 10^2$ to $7 \cdot 10^3$/g soil, and seldom reached 10^4 cells.

The positive effect of *A. chroococcum* on yield and plant growth after inoculation of the seeds or seedlings has been attributed to a multiple action of *A. chroococcum* in soil (Brown, 1974; Shende et al., 1975), viz. nitrogen fixation, suppression of plant pathogens, production of plant growth-promoting substances, effect on other beneficial microorganisms, and mobilization of soil phosphate.

As *A. chroococcum* has been found to exhibit antagonistic action against pathogenic soil organisms, it is worthwhile to examine its possible role in biological control of plant diseases.

The aim of the present study was to find antagonistic activities of isolates of *A. chroococcum* against *Rhizoctonia solani* on agar plates and to test isolates in soil for their ability to control or minimize infection of potato sprouts with *R. solani.*

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Materials and methods

Cultures. A pathogenic isolate of *R. solani* was used. *A. chroococcum* isolates were obtained from a loamy sand, containing shell fragments, from the North-East Polder.

Test of antagonism on agar plates. The antagonism of *A. chroococcum* isolates to *R. solani* in vitro was examined on agar. Sterilized filter paper discs dipped in 7-day-old broth cultures of *A. chroococcum* were placed on potato dextrose agar plates which were then inoculated with a culture of *R. solani.* After incubation for 5 days at 25 ± 1 °C, the diameter of the inhibition zones (including the filter paper disc) was measured. Each measurement was made in duplicate and each treatment was replicated three times.

Test of antagonism in soil. For the test of antagonism in soil five isolates of *A. chroococcum* were selected on the basis of the maximum inhibition zone they had formed on agar against the growth of *R. solani.* Ten-day-old cultures of these *A. chroococcum* isolates were prepared in Ashby's liquid medium; the density of *A. chroococcum* ranged from 10^7 to 18^8 cells per ml. A 15-day-old submerged culture of *R. solani* with formation of sclerotia and grown in malt biotone medium at 25 °C was used. After fragmentation the culture contained 10^7 propagules per ml.

Half of the number of the pots was filled with 1 kg of air-dried and sieved soil, a holocene sandy loam with a pH of 6.5. The other half was filled with the same soil after it had been autoclaved at 120 °C for 1 h on 3 consecutive days.

The soil of each pot was mixed with 60 ml broth culture of *A. chroococcum* isolates and with 40 ml broth culture of fragmented *R. solani* hyphae. For a treatment with a mixture of five isolates, aliquots of 12 ml broth culture of each *A. chroococcum* isolate were mixed. The control pot received 40 ml broth culture of *R. solani* and 60 ml of sterile Ashby's medium. Two healthy pre-germinated potato tubers (cv. Irene) were planted at 12 cm depth. Four living sclerotia of *R. solani* grown on malt biotone agar were transferred to each tuber. Soil moisture was maintained at 25%. Treatments were in duplicate. Pots were covered with aluminium foil and kept at 25 °C in an incubator. The same procedure was followed for 20, 15, and 10 °C. After each sprout had been washed with tap water, the presence of disease and its intensity on each sprout was recorded 18 days after inoculation. Numerical ratings were given from 0 to 5 based on the percentage of the sprout area that was diseased, as indicated in Table 1.

Table 1. Rating of infestation of sprouts.

Rating	Disease (% of sprout area)
0	0
1	1 -10
2	10.1-20
3	20.1-40
4	40.1-50
5	>50

Tabel 1. Waardering van de aantasting van de spruiten.

The disease index (DI) was calculated with this formula:

$$DI = \frac{\text{summation of all ratings}}{\text{number of sprouts observed} \times \text{maximum rating (5)}} \times 100 = \frac{\Sigma \text{ rating}}{N \times 5} \times 100$$

Results

Table 2 shows that not all isolates of *A. chroococcum* could suppress the growth of *R. solani* on agar plates. The antagonistic properties of *A. chroococcum* proved to be highly variable. Five strongly inhibitory (J2, J4, J5, J6 and SM6) and seven non-inhibitory isolates occurred, the others were intermediate. The action of some effective isolates is shown in Fig. 1.

In the pot experiments *R. solani* occurred on most sprouts, and numerous irregular brown and dark spots were observed, especially on the lower parts of the sprouts. Severe infection resulted in death of the sprouts. The symptoms were similar to those of damping-off and stem rot.

Table 3 shows that inoculation with *R. solani* of sterilized soil produced an infection of more than 50%. Infection was virtually absent following inoculation with *Azotobacter* isolates J4 and J6 at 25, 20 and 15 °C. Isolates J2 and J5 were effective at 25 and 20 °C. Isolate SM6 showed little or no antagonistic effect. The effect of a combined inoculation of all isolates was, in general, weaker than that of most individual inoculations.

The results obtained with unsterilized soil were similar to those of sterilized soil. Isolate J4 was slightly more effective than J6, even at 10 °C (Fig. 2). Isolates J2 and J5 were effective at 25 and 15 °C. The DI generally increased with decreasing temperature in the control, both in sterilized and unsterilized soil.

Table 2. Inhibition of growth of *R. solani* by the isolates of *A. chroococcum.*

Isolates of *A. chroococcum*	Average diameter of inhibition zone (cm)	Isolates of *A. chroococcum*	Average diameter of inhibition zone (cm)
G1	1.9 ± 0.4	K1	0.0
G2	2.1 ± 0.3	K2	0.0
G3	0.0	S1	1.5 ± 0.1
G4	2.1 ± 0.3	S2	1.7 ± 0.3
G5	1.9 ± 0.5	S3	2.2 ± 0.2
J1	0.0	S4	2.4 ± 0.2
J2	3.0 ± 0.3	S6	2.4 ± 0.7
J3	2.0 ± 0.1	SM1	2.9 ± 0.3
J4	4.0 ± 0.1	SM2	1.5 ± 0.2
J5	3.9 ± 0.2	SM2	2.5 ± 0.4
J6	3.9 ± 0.1	SM4	2.6 ± 0.3
J7	2.1 ± 0.3	SM5	0.0
J8	0.0	SM6	3.1 ± 0.4
J9	0.0	Control *(R. solani)*	0.0

Tabel 2. Remming van de groei van R. solani door 27 isolaten van A. chroococcum.

Fig. 1. Antagonism (antibiosis) of four *Azotobacter chroococcum* isolates against *Rhizoctonia solani.*

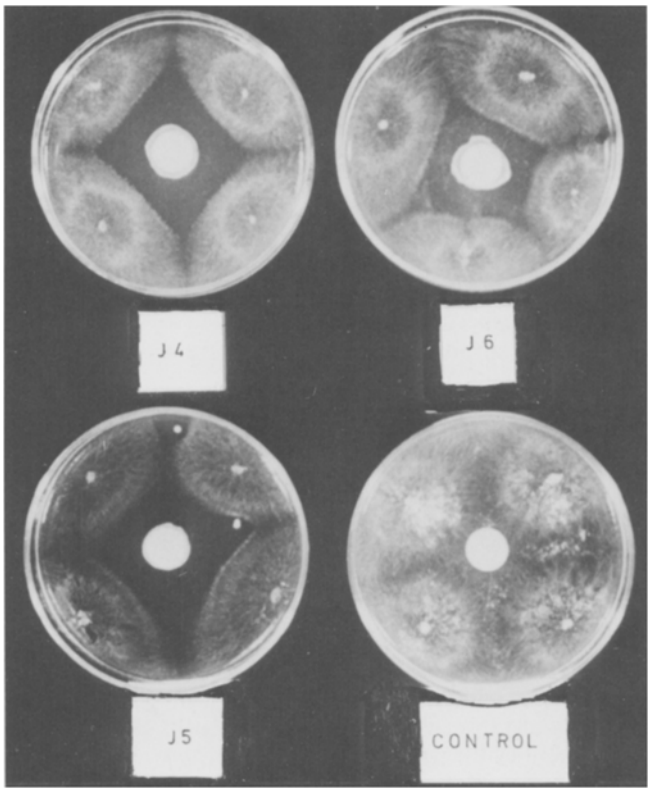

Fig. 1. Antagonisme (antibiose) van vier isolaten van Azotobacter chroococcum tegen Rhizoctonia solani.

Discussion

Antifungal action of *A. chroococcum* against *Aspergillus glaucus, Penicillium* spp., *Fusarium* spp. and *Alternaria* spp. has been reported (Mishustin, 1966; Lakshmi Kumari et al., 1972). Less infection with *Phytophthora infestans* and *Streptomyces scabies* on potato has been noticed (Sidorov, 1954) due to application of *Azotobacter.* According to Linchevskaya and Kaliberda (1958), late blight of potato could be minimized or reduced by applying *Azotobacter.*

A number of workers reported that seed inoculation with *Azotobacter* helped in inhibiting and preventing the occurrence of viral, fungal and bacterial diseases of some agricultural crops (Dorosinskii, 1962; Khudyakov and Marschunova, 1966).

The antagonistic effect of *Azotobacter* isolates J4 and J6 on *R. solani* was considerable at 25, 20 and 15 °C. However, none of the isolates was effective at 10 °C. This may be due to a too low inhibitory metabolic activity. In addition, the pathogen

Table 3. Antagonistic effect of five selected *Azobacter chroococcum* isolates in sterilized and unsterilized soil.

Treatment	Sterilized soil				Unsterilized soil			
	10°C	15°C	20°C	25°C	10°C	15°C	20°C	25°C
Control *(R. solani)*	82[1]	63	87	53	91	57	54	50
Azotobacter J2 + R. solani	51	48	13	9	47	10	26	6
Azotobacter J4 + R. solani	76	0	4	0	40	0	0	0
Azotobacter J5 + R. solani	70	20	9	3	70	9	23	0
Azotobacter J6 + R. solani	76	0	0	3	63	3	0	3
Azotobacter SM6 + R. solani	96	58	51	47	91	24	60	46
Azotobacter J2, J4, J5, J6, SM6 + *R. Solani*	62	24	20	13	88	30	20	14

[1] Average disease index.

Tabel 3. Het antagonistisch effect van vijf geselecteerde isolaten van Azobacter chroococcum in gesteriliseerde en niet-gesteriliseerde grond uitgedrukt in gemiddelde ziekte index.

Fig. 2. Sprouts of potatoes inoculated with *Azotobacter chroococcum* isolates J4 and J6 and *Rhizoctonia solani* in unsterilized soil at 15 °C.

Fig. 2. Aardappelspruiten geïnoculeerd met isolaten J4 en J6 van Azotobacter chroococcum en met Rhizoctonia solani in niet-gesteriliseerde grond bij 15 °C.

showed exceptional tolerance to all isolates of *Azobacter* at 10 °C in the sandy loam soil used. Isolates J4 and J6 performed well in a clay loam with pH 8.1 at 10 °C in vitro (unpublished results).
The inhibitory effect of *Azobacter* on *R. solani* on agar plates may be attributed to antibiotic substances produced by the isolates; the ineffectiveness of isolate SM6 to suppress *R. solani* in soil may be due to the specific nature of its antibiotics. It is possible that antibiotic substances produced by *A. chroococcum* SM6 are inactivated following adsorption by soil particles.

To determine whether the 'J' series of *A. chroococcum* has practical value in the biological control of *R. solani*, pot and field trials will be conducted.

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Samenvatting

Antagonisme van isolaten van Azotobacter chroococcum tegen Rhizoctonia solani

Op agarvoedingsbodems en in steriele en niet-steriele grond werd de antagonistische (antibiotische) activiteit van de bacterie *Azotobacter chroococcum* t.o.v. een pathogene stam van *Rhizoctonia solani* getoetst. In grond, een lichte zavel met pH 6,5 dienden spruiten van 'Irene' pootaardappelen als indicatoren voor de activiteit van *R. solani,* waarmee de grond en de poters kunstmatig waren besmet.

De antagonistische activiteit van de isolaten van *A. chroococcum* bleek op agar zeer sterk uiteen te lopen. Van de isolaten die op agar actief waren, bleek er één in grond bijna geen remmende activiteit te vertonen. In niet-steriele grond was het beeld gelijk aan dat van steriele grond: een snelle biologische inactivering van remstof(fen) trad dus niet op.

Er was een grote temperatuursinvloed. Bij 25, 20 en 15 °C konden sterk antogonistische stammen van *A. chroococcum* aantasting van aardappelspruiten door *R. solani* afdoende voorkomen. Bij 10 °C trad bijna geen bescherming op. Groei en stofwisseling van *A. chroococcum* zijn dan sterk gereduceerd.

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