Growth inhibition of rhizobia by certain antagonistic organisms

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INTRODUCTION

The beneficial effect of inoculating leguminous plants, even in fertile soils, with the proper effective strains of rhizobia is well known, (Ibrahim 1960, Loutfi, El-Sherbini and Ibrahim 1966 a, b and Taha, Mahmoud and Salem 1967 a, b).

Rhizobia however, are subjected to deleterious conditions in soils which inhibit their activities in nodule formation, and consequently disturb the symbiotic relationship between them and their hosts, (Alexander 1961 and Russell 1961). This results in the production of poor plant growth, unless adequate amounts of nitrogenous fertilizers are added. In this respect, various investigators indicated that the decline of the number of legume bacteria could be attributed to the presence of antagonistic organisms in soils, (Konishi and Fukuchi 1935, Nakimosckaia 1937, Krassilnikov and Koreniako 1944, Campillo 1947, 1949, Thornton and Brian 1949, Abdel-Ghaffar and Allen 1950, Harris 1953, Wieringa 1963, Visona and Tardieux 1964, Van Schreven 1964, Gabre 1967, and Kamel, Ibrahim and Shata 1970).

Hence, it was found of interest to study the antagonistic action exerted by certain organisms on the growth of some strains of rhizobia. Studies included also the effect of these organisms on nodule formation, nitrogen fixation and growth of clover, as well as rate of seed germination.

MATERIAL AND METHODS

Rhizobial strains:

Locally isolated strains of *R. leguminosarum* and *R. trifolii* were used. They were grown on glucose peptone yeast-extract agar medium (Allen 1961).

Antagonistic organisms:

Streptomyces sp. R2 and R8, *Aspergillus* sp. R9 and R14, and *Bacillus subtilis* R3 and S14, isolated from the soil and rhizosphere of bean plants (Kamel, Ibrahim and Shata 1970) were used in this experiment. Stock cultures were maintained on glucose peptone yeast-extract agar medium.

The antagonistic organisms were cultured in conical flasks containing 50 ml glucose peptone yeast-extract medium. The submerged culture technique was adopted, in which all flasks were shaken using a reciprocal shaker with 80 strokes per minute. After 14 days, filtrates were aseptically collected using EK Seitz filters.

RESULTS AND DISCUSSION

1. Effect of antagonistic organisms on growth of rhizobial strains

Crude filtrates of the antagonistic organisms were added to broth base medium 79, (Allen 1961, no calcium carbonate was added), prepared in test tubes at the rate of 0.2 ml per 10 ml medium, just before inoculation with the rhizobial strains. Four days later (28°C) growth of rhizobia was estimated by turbidimetry using a Lumetron colorimeter at 480 m μ . Results are shown in Table 1.

Table 1

Effect of crude filtrates of the antagonistic organisms on the growth of rhizobial strains

Organisms	Relative turbidity, (control = 100)	
	<i>R. trifolii</i>	<i>R. leguminosarum</i>
<i>Streptomyces</i> sp. R2	70.2	72.8
<i>Streptomyces</i> sp. R8	52.4	60.0
<i>Aspergillus</i> sp. R9	78.1	80.3
<i>Aspergillus</i> sp. R14	72.8	79.6
<i>Bacillus subtilis</i> R3	84.5	89.4
<i>Bacillus subtilis</i> S10	72.8	78.0

In general, growth filtrates inhibited the growth of rhizobial strains. Rate of inhibition varied according to the source of filtrate as well as the strain of rhizobia. In this respect, Robinson (1945) and Abdel-Ghaffar and Allen (1950) showed that the response of antibiotic-producing bacteria, fungi and actinomycetes commonly found in the soil varied greatly with the: a) strain of rhizobia, b) antagonist, and c) composition of the medium. Kamel, Ibrahim and Shata (1970) found glucose to be more suitable as a source of carbon for the activity of antagonistic organisms grown on peptone yeast-extract agar medium. *Streptomyces* filtrates showed the highest rate of antagonism followed by those of *Aspergillus* and *Bacillus subtilis*. This however, confirm the observations recorded from the streak method. In addition, *R. leguminosarum* was found to be more tolerant to the antagonistic action exerted by growth filtrates.

2. Effect of antagonistic organisms on nodule formation and growth of clover plants

A fertile clay loam soil (1.10% organic matter, 0.072% total nitrogen and pH 8.2) obtained from The University Farm at Mustorod, was added to earthenware pots 15 cm in diameter at the rate of 1 kg per pot. Clover seeds (*T. alexandrinum*) inoculated with rhizobial culture 3 days old were planted. Crude filtrates were added

at the rate of 20 ml per pot. Two weeks later, plants were thinned to keep 10 per pot. After 45 days, they were taken off carefully from the soil and number of nodules per plant was determined. Later, they were weighted (oven dry, 80°C) and used for the determination of total nitrogen (Kjeldahl method, Jackson 1958). Results are recorded in Table 2.

Table 2

Effect of crude filtrates of the antagonistic organisms on number of nodules, dry weight and nitrogen content of clover plants

Organisms	mean number of nodules per plant	mean dry weight, g	mean total nitrogen	
			%	mg
Control	24	2.768	4.128	114.3
<i>Streptomyces</i> sp. R2	26	2.662	4.112	107.8
<i>Streptomyces</i> sp. R8	20	2.432	4.122	100.2
<i>Aspergillus</i> sp. R9	22	2.790	4.122	115.0
<i>Aspergillus</i> sp. R14	28	2.556	4.120	106.3
<i>Bacillus subtilis</i> R3	28	2.538	4.128	104.8
<i>Bacillus subtilis</i> S10	26	2.780	4.122	113.6

Crude filtrate of *Streptomyces* sp. R8 was found to be the most inhibitory one. Number of nodules was found to be in the average of 20 nodule per plant. However, no significant differences were recorded between the control and other treatments regarding rate of nodulation, where, mean number of nodules ranged between 24 and 28 per plant. Similar results were recorded concerning the effect of these filtrates on the dry matter yield of plants and their nitrogen contents, where, *Streptomyces* sp. R8 slightly decreased the dry matter yield and total amount of nitrogen with no significant differences. The slight effect of these filtrates on rate of nodule formation and nitrogen fixation led to the following experiment: filtrates were added to the previous soil moistened with sterile distilled water, at the rate of 2 ml per 10 g soil. Two sets of tubes were prepared, where one of them was sterilized. The antagonistic activity of the recovered filtrates was determined periodically on the growth, of *R. trifolii* grown on glucose peptone yeast-extract agar medium. The hole test method was adopted, in which holes of 5 mm in diameter were made. Recovered filtrates were obtained by adding to each tube 10 ml of sterile distilled water and shaken for 1 hr. (Szegei 1960). After sedimentation aliquots of 0.1 ml of the clear solution were used for the test. Inhibition zones were recorded in mm after 48 hr. (28°C). Results are shown in Table 3.

Crude filtrates completely lost their inhibitory action on the growth of *R. trifolii* after 3 days. This could be attributed to their inactivation as a result of their adsorption on organic and inorganic soil colloids, chemical reactions as well as decomposition by soil microflora. Their inactivation however, was found to be more pronounced in the non-sterilized soil, indicating the significant role of soil microflora in this process. This is in accordance with Szegei (1960) who pointed out to the inactivation of antibiotics when added to fertile soil. About

Table 3

The antagonistic activity of recovered filtrates on the growth of *R. trifolii*

Organisms	Time in days							
	0		1		3		7	
	not sterilized	sterilized	not steri- lized	steri- lized	not steri- lized	steri- lized	not steri- lized	steri- lized
<i>Streptomyces</i> sp. R2	++	++	—	+	—	—	—	—
<i>Streptomyces</i> sp. R8	+++	+++	+	+	—	—	—	—
<i>Aspergillus</i> sp. R9	+++	++	+	+	—	—	—	—
<i>Aspergillus</i> sp. R14	++	++	—	+	—	—	—	—
<i>Bacillus subtilis</i> R3	+	+	+	—	—	—	—	—
<i>Bacillus subtilis</i> S10	++	+	+	+	—	—	—	—

+ inhibition zone 10 mm

++ inhibition zone 20 mm

+++ inhibition zone 30 mm

El-Fadl et al. (1963) failed to prove the responsibility of bacteriophage or antibiotic-producing organisms in the failure of nodule formation in garden bean plants. Visona and Tardieux (1964) also showed that approximately 1/4 of the total micropopulation in the rhizosphere of studied plants were antagonists to rhizobia in vitro. In the natural habitat however, they seemed to have no effect on nodulation. On the other hand, lack of nodules was attributed by many investigators to the activities of antagonistic organisms in the soil and rhizosphere of plants, (Robinson 1945, Campillo 1947, 1949, and Abdel-Ghaffar and Allen 1950, Harty and Bygatt 1965, and Gabre 1967).

3. Effect of antagonistic organisms on germination of clover seeds

The effect of these filtrates on germination rate of clover seeds was also studied. Previously sterilized seeds in 0.1 % HgCl solution were soaked in the filtrates for

Table 4

Effect of crude filtrates of the antagonistic organisms on the germination of clover seeds

Organisms	germination rate, %	embryo length, cm
Control	99.1	1.54
<i>Streptomyces</i> sp. R2	90.0	1.28
<i>Streptomyces</i> sp. R8	92.8	1.17
<i>Aspergillus</i> sp. R9	93.0	1.29
<i>Aspergillus</i> sp. R14	95.2	1.34
<i>Bacillus subtilis</i> R3	90.1	1.49
<i>Bacillus subtilis</i> S10	91.1	1.29

12 hours. Meanwhile, seeds of control were soaked in sterile distilled water. Seeds were allowed to germinate in sterilized petri-dishes on moistened filter papers at 18°C. After 48 hours, 200 seeds from each treatment were examined for germination rate and length of embryo. Results are recorded in Table 4.

In general, germination rate of clover seeds, as well as length of embryo were inhibited by the crude filtrates of the antagonistic organisms. Rate of germination ranged from 90.0 and 95.2%, and length of embryo ranged from 1.17 and 1.49 cm, as compared with the control values which were found to be in the order of 99.1% and 1.54 cm respectively.

SUMMARY

Growth of root-nodule bacteria *R. trifolii* and *R. leguminosarum* was antagonized, in vitro, by the crude filtrates of certain antagonistic organisms isolated from the rhizosphere of bean plant when grown in glucose peptone yeast-extract medium, and the highest rate of antagonism was recorded with *Streptomyces* sp. R8. In vivo however, where clover (*T. alexandrinum*) was grown in a fertile clay loam soil inoculated with *R. trifolii*, growth filtrates (20 ml/1 kg soil) showed no significant effect in decreasing nodulation, nitrogen fixation and dry matter yield of plants.

Recovered filtrates from the soil, completely lost their inhibitory action on the growth of *R. trifolii* after 3 days.

Their inactivation however, was found to be more pronounced in the non-sterilized soil.

In addition, germination of clover seeds as well as length of embryo were partially inhibited by the filtrates of the antagonistic organisms.

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Hamowanie wzrostu bakterii Rhizobium przez niektóre antagonistyczne organizmy

Streszczenie

Wzrost bakterii brodawkowych *Rhizobium trifolii* i *R. leguminosum* *in vitro* hamowany był przez surowe filtry z hodowli niektórych antagonistycznych organizmów, wyizolowanych z rizosfery fasoli.

Na pożywe z glukozą, peptonem i ekstraktem z drożdży wzrost tych bakterii najsilniej był hamowany przez filtrat z hodowli *Streptomyces* sp. R8. Natomiast *in vivo*, w hodowli *Trifolium alexandrinum* na żyznej glebie gliniastej, inkulowanej *R. trifolii*, filtry z hodowli organizmów antagonistycznych (20 ml/kg gleby) nie wykazywały istotnego wpływu na modulację wiązania azotu i na plon suchej masy roślin.

Otrzymane z gleby filtry po trzech dniach traciły całkowicie właściwości hamujące wzrost *R. trifolii*. Okazało się, że inaktywacja tych filtratów jest wyraźniejsza przy hodowli w glebie nie sterylizowanej. Stwierdzono poza tym, że pod wpływem filtratów z organizmów antagonistycznych zarówno kiełkowanie nasion koniczyny, jak i długość ich zarodków były częściowo hamowane.