Proc. Indian natn. Sci. Acad. **B 45**, No. 2, pp. 150-153 (1979)
Microbiology

THE EFFECT OF MICROBIAL CULTURE FILTRATES ON THE GROWTH BEHAVIOUR OF DIFFERENT STRAINS OF RHIZOBIA

S. N. SHUKLA* *and* R. S. DWIVEDI
Botany Department, Banaras Hindu University, Varanasi 221 005

(*Received* 22 *July* 1978)

Effect of metabolites of 15 fungi and 5 actinomycetes on growth of 5 different strains of *Rhizobium* was studied *in vitro*. Amongst the metabolites of fungi, those of *Cunninghamella bertholletiae*, *Trichoderma lignorum*, *Aspergillus flavus*, *A. niger*, *Penicillium citrinum*, *Fusarium oxysporum* and *Myrothecium roridum* inhibited the growth of all the rhizobia tested. The metabolites of *Streptomyces aureofaciens* and *Streptomyces* strain 9 inhibited the growth of all the test rhizobia.

INTRODUCTION

Antagonism with strains of *Rhizobium* by other soil-inhabiting micro-organisms has long been known to occur. Such antagonism as well as lysis by bacteriophage or by other means has often been known as a causal factor in cases of faulty nodulation of legumes (Allen & Allen, 1950). Hely *et al.* (1957) noted that the reason for faulty nodulation in subterranean clover is due to microbial antagonism by other soil micro-organisms inhabiting the clover rhizosphere. They observed proper nodulation with autoclaved soil having effective strain of *Rhizobium*. There is paucity of information regarding the interaction between soil-inhabiting microfungi and actinomycetes, and different strains of rhizobia. In this study, we conducted investigations on the effect of culture filtrates of 15 fungi and 5 actinomycetes, which were dominant in the rhizosphere and rhizoplane region of a leguminous plant, i.e. *Trifolium alexandrinum* Linn., on growth of 5 different *Rhizobium* strains.

MATERIALS AND METHODS

For the preparation of metabolites, equal size (4 mm) of blocks of fresh cultures of selected fungi (Table I) were inoculated aseptically in separate sterile flasks containing 100 ml liquid Czapek's medium of the following composition: K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 1.0 g; $FeSO_4$, trace; Yeast powder, 0.5 g; $NaNO_3$, 2.0 g and distilled water 1000 ml. Metabolites of selected actinomycetes (Table II) were prepared in the same manner as above by adding equal blocks (4 mm) of fresh culture in separate sterile flasks containing 100 ml liquid medium (Kuster & William, 1964) of the following composition: Soluble Starch, 10 g;

**Present address:* Scientist S-1, Division of Plant Pathology, Central Rice Research Institute, Cuttack 753 006, Orissa.

TABLE I

In vitro *studies on effect of fungal metabolites on growth of rhizobia*

Metabolites of	*Rhizobium trifolii*	*R. legumi-nosarum*	*R. phaseoli* strain 1	*R. phaseoli* strain 2	*Rhizobium* sp. (cow-pea group)
Cunninghamella bertholletiae	—	—	—	—	—
Penicillium japonicum	—	+	—	—	+
Chaetomium bostrychodes	—	—	—	+	+
Trichoderma lignorum	—	—	—	—	—
Aspergillus fumigatus	+	—	+	—	+
A. flavus	—	—	—	—	—
A. niger	—	—	—	—	—
A. candidus	+	—	—	—	+
Penicillium citrinum	—	—	—	—	—
Paecilomyces varioti	—	+	—	—	—
Acrophialophora fusispora	—	—	+	—	+
Cladosporium herbarum	—	+	+	+	+
Curvularia lunata	+	+	+	+	+
Fusarium oxysporum	—	—	—	—	—
Myrothecium roridum	—	—	—	—	—
Control	+	+	+	+	+

+ indicates growth of rhizobia — indicates inhibitory effect

TABLE II

In vitro *studies on effect of metabolites of actinomycetes on growth of rhizobia*

Metabolites of	*Rhizobium trifolii*	*R. legumi-nosarum*	*R. phaseoli* strain 1	*R. phaseoli* strain 2	*Rhizobium* sp. (cow-pea group)
Nocardia fructifera	+	+	+	+	+
Streptomyces albus	—	—	—	—	+
S. aureofaciens	—	—	—	—	—
Streptomyces sp. strain 9	—	—	—	—	—
Micromonospora sp.	+	+	—	—	+
Control	+	+	+	+	+

+ indicates growth of rhizobia — indicates inhibitory effect

Casein, 0.3 g; KNO_3, 2.00 g; NaCl, 2.0 g; K_2HPO_4, 2.0 g; $MgSO_4$, 0.05 g; $CaCO_3$, 0.025 g; $FeSO_4.7H_2O$, 0.019 g and distilled water 1000 ml (*p*H 7–7.2). The inoculated flasks of fungi and actinomycetes were incubated at 25°C and 30°C respectively for 15 days and thereafter the contents filtered through separate sterile, folded muslin cloth. The culture filtrates were finally filtered through Seitz filter and concentrated to half of the original volume on water bath at 40°C.

Five different *Rhizobium* strains, e.g. *R. phaseoli* strain 1, *R. phaseoli* strain 2, *R. trifolii*, *R. leguminosarum* and *Rhizobium* sp. (cow-pea group) were isolated from nodules of *Phaseolus mungo*, *P. aureus*, *Trifolium alexandrinum*, *Pisum sativum* and *Vigna catajang* respectively. The rhizobia were maintained on yeast mannitol agar medium. The growth behaviour of above mentioned rhizobia was studied *in vitro* in presence of metabolites of selected fungi and actinomycetes by streak method. Equal amount of cooled (40°C) sterilized yeast mannitol agar medium was poured separately into sterile Petri dishes containing 2 ml of individual metabolite of fungi and actinomycetes. The Petri dishes were rotated to make a homogeneous solution of metabolites with the basal medium. The medium was allowed to solidify. Streaks were made on the agar disc from different strains of *Rhizobium* separately. Three replicates were prepared for each strain. Agar discs without metabolites served as control. The Petri dishes were incubated at 25°C for 48 hr and thereafter the observations were recorded (see Tables I & II).

Results and Discussion

Certain fungi and actinomycetes were noted to inhibit the growth of rhizobia (Tables I & II). Amongst the metabolites of fungi, those of *Cunninghamella bertholletiae*, *Trichoderma lignorum*, *Aspergillus flavus*, *A. niger*, *Penicillium citrinum*, *Fusarium oxysporum* and *Myrothecium roridum* inhibited growth of all rhizobia. Robinson (1946) studied the antagonistic action of *Aspergillus wentii* towards *Rhizobium*. Ruschmann (1951) noted *Aspergillus niger*, *Penicillium claviforme* and *P. notatum* to be antagonistic towards strains of *Rhizobium*. *Fusarium lini* and *F. culmorum* exerted antibiotic action against rhizobia (Dorn, 1956). Certain fungi have been noted as inhibitory, stimulatory or ineffective towards growth of rhizobia and nodule development (Holland & Parker, 1966; Chhonkar & Subba Rao, 1966). Lim (1961) observed that certain root surface fungus, viz. *Verticillium* sp. decreased the infection of root hairs of legume without affecting the population of *Rhizobium* in the rhizosphere while some fungi like *Paecilomyces* were stimulatory.

Amongst the metabolites of actinomycetes, those of *Streptomyces aureofaciens* and *Streptomyces* sp. strain 9 inhibited growth of all the test rhizobia. Metabolites of *Nocardia fructifera* and *Micromonospora* sp. were ineffective. *Rhizobium* sp. (cow-pea group) was relatively less susceptible to the action of metabolites of the test fungi and actinomycetes. Some actinomycetes, especially species of *Streptomyces*, were noted to have antirhizobial effect (Thornton *et al.*, 1949; Landerkin & Lockhead, 1948; Fogle & Allen, 1948; Abdel-Ghaffar, 1950). Damirgi and Johnson (1966) tested susceptibility of *Rhizobium japonicum* against 24 isolates of actinomycetes and suggested that antirhizobial soil micro-organisms in a particular soil played an important role in the establishment of specific rhizobial strains.

Vanschreven (1964) tested the antagonistic activity of 22 actinomycetes against a number of rhizobia. He further noted that the repeated exposure of *Rhizobium meliloti* and *R. trifolii* strains to antagonistic actinomycetes resulted in occurrence of a small number of variants producing either no nodules, ineffective nodules or less effective nodules. The reason for the antagonistic reaction of culture filtrates against the tested rhizobia may be due to presence of toxic chemicals produced by particular fungus or actinomycetes during their growth in the culture medium. The fungi and actinomycete which were found antagonistic to the rhizobia may also affect adversely the various stages of nodulation such as root hair curling, infection thread formation, etc., directly or indirectly resulting into faulty nodulation in legumes.

ACKNOWLEDGEMENT

The authors are grateful to the Head of Botany Department for providing laboratory facilities.

REFERENCES

Abdel-Ghaffar, A. S. (1950). Microbial antagonism against *Rhizobium lupini*. M. S. Thesis, Univ. Wiscon. Madison, p. 65.

Allen, E. K. & Allen, O. N. (1950). Biochemical and symbiotic properties of the rhizobia. *Bacteriol. Rev.*, **14**: 273-330.

Chhonkar, P.K. & Subba Rao, N.S. (1966). Fungi associated with legume root nodules and their effect on rhizobia. *Can. J. Microbiol.*, **12**: 1253-1261.

Damirgi, M.S. & Johnson, H.W. (1966). Effect of soil actinomycetes on strains of *Rhizobium japonicum*. *Agron. J.*, **58**: 223-224.

Dorn, M. (1956). Uber den Einfluz phytopathogenen pilze aub die entwicklung und infektion kfraft von drei *Rhizobium*. *Arten. Zentr. Bakteriol. Parasitol. Abstr.*, **109**: 120-139.

Fogle, C. E. & Allen, O. N. (1948). Associative growth of actinomycetes and rhizobia, *Proc. 48th Gen. Meet. Soc. Amer. Bact.* Minneapolis, pp. 1-53.

Hely, F.W., Bergersen, F.J. & Brockwell, J. (1957). Microbial antagonism in the rhizosphere as a factor in the failure of inoculation of subterranean clover. *Aust. J. agric. Res.*, **8**: 22-44.

Holland, A.A. & Parker, C.A. (1966). Studies on microbial antagonism in the establishment of clover pasture. II. The effect of saprophytic soil fungi upon *Rhizobium trifolii* and growth of subterranean clover. *Pl. Soil*, **23**: 329-340.

Kuster, E. & William, S.T. (1964). Selection of media for isolation of *Streptomycetes*. *Nature, Lond.*, **202**: 928-929.

Landerkin, G.B. & Lockhead, A.G. (1948). A comparative study of the activity of fifty antibiotic actinomycetes against a variety of soil bacteria. *Can. J. Res.* C. **26**: 501-506.

Lim, G. (1961). Microbial factors influencing infection of clover by nodule bacteria. Doctoral Thesis, University of London.

Robinson, R. S. (1946). The antagonistic action of by-products of a culture of *Aspergillus wentii*. *J. Bacteriol.*, **51**: 129.

Ruschmann, G. (1951). Neue Forschung sergbnisse uber Antibiotika und ihre *Bedontung fur* die Bodenfruchtbarheit Nitt. I. 2 *Pflanzenernahr. Dung. Bodenk.* **55**: 201-235.

Thornton, G.D., Alencar, J. & Smith, F.B. (1949). Some effect of *Streptomyces albus* and *Penicillium* spp. on *Rhizobium meliloti*. *Soil Sci. Soc. Am. Proc.*, **14**: 188-191.

Vanschreven, D. A. (1964). The effect of some actinomycetes on rhizobia and *Agrobacterium radiobactor*. *Pl. Soil*, **21**: 283-302.