Greenhouse evaluation of rhizobia as biocontrol agent of root-infecting fungi in okra

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Summary

Nine rhizobial strains isolated from the root nodules of *Cicer arietinum*, *Vigna radiata*, *V. mungo*, *Samanea saman*, *Sesbania sesban*, *Leucinia* sp., *Prosopis cineraria* and *Medicago sativa* were used to study their effects on root-infecting fungi viz., *Macrophomina phaseolina*, *Fusarium solani* and *Rhizoctonia solani*. In dual culture plate assay, strains of *Bradyrhizobium* sp., and *R. meliloti* were found to inhibit radial growth of *M. phaseolina*, *F. solani* and *R. solani* producing zones of inhibition. *Bradyrhizobium* sp., and *R. meliloti* either used as seed dressing or as soil drench significantly suppressed root-rot infection caused by *M. phaseolina*, *F. solani* and *R. solani* in okra, a non-leguminous crop under greenhouse conditions. Biomass of plants was also higher in the presence of rhizobia.

Key words: biological control, root-rot, rhizobia

INTRODUCTION

Bacteria of the genus *Rhizobium* are of considerable scientific and economic interest because of their ability of nitrogen-fixation in leguminous plants (Hynes et al., 1990) and in the control of soil-borne root-infecting fungi both in leguminous and non-leguminous plants (Ehteshamul-Haque and Ghaffar, 1993; Siddiqui et al., 1998a; 1998b). Seed treatment is an attractive method for introducing biocontrol agents into the soil root environment since it protects seed from seed-borne and soilborne pathogens and enables the seed to germinate and become established as a healthy seedling (Chang and Kommehal, 1968). Antagonists applied to the

seed may have the opportunity to be the first colonizer of the roots (Chao et al., 1986). Similarly, control of soil-borne plant pathogens by the addition of antagonistic microorganisms to the soil is also a potential non-chemical mean for the plant disease control. It has been hypothesized that if soil was amended with antagonists that decrease the rapid saprophytic development of the pathogens during early stages of soil recolonization, less infection would occur and severity of the disease would be reduced (Marios et al., 1981). An experiment was therefore carried out to examine the efficacy of different strains of *Rhizobium* and *Bradyrhizobium* in the biocontrol of root-infecting fungi viz., *Macrophomina phaseolina*, *Fusarium solani* and *Rhizoctonia solani* on okra (*Abelmoschus esculentus* L.).

MATERIALS AND METHODS

Isolation of the Rhizobial and Bradyrhizobial strains: From the root nodules of leguminous plants viz., *Cicer arietinum* (*Bradyrhizobium* sp., KUCC-811); *Vigna radiata* (*Bradyrhizobium* sp., KUCC-823); *Vigna mungo* (*Bradyrhizobium* sp., KUCC-819); *Samania saman* (*Bradyrhizobium* sp., KUCC-824); *Sesbania sesban* (*Bradyrhizobium* sp., KUCC-818); *Leucinia* sp., (*Bradyrhizobium* sp., KUCC-839); *Prosopis cineraria* (*Bradyrhizobium* sp., KUCC-821) and *Medicago sativa* (*Rhizobium meliloti* KUCC-815 & KUCC-139) were isolated using modified method of Subba Rao, (1977) where nodules picked from the roots and surface sterilized by treatment with 70% ethanol for 3 min., then with 2% sodium hypochlorite for 3 min. The nodules were crushed between forceps in sterilized distilled water and the contents plated onto Yeast Extract Mannitol Agar medium supplemented with Congo red. After 3 days, cream coloured gummy colonies which appeared on the medium was purified by streaking on YEMA.

In vitro test against root-infecting fungi: The rhizobial strains were streaked on one side of the Petri dish, containing Czapek's Dox Agar, pH 7.2. On other side of the Petri dish a 5-mm-diam., disc from actively growing colonies of the test fungi viz., *M. phaseolina*, *Fusarium oxysporum*, *F. solani* or *R. solani* was inoculated. The dishes were incubated at 28°C and after one week zone of inhibition (if any) was recorded (Drapeau et al., 1973).

In vivo testing:

Seed dressing: Seeds of okra were surface sterilized with 1% Ca(OCl)₂ for 3 min., rinsed thoroughly in running tap water and dried aseptically. Eight surface disinfested seeds were treated with 5-day-old rhizobial strains using 1% gum arabic. Population of rhizobial strains per seed are given in Table 1. Eight treated seeds were sown in 8-cm-diam., plastic pots, each containing 250 g soil. Seed treated with sterile distilled water used as control. The soil had a natural infestation of 3-7 sclerotia g⁻¹ of soil of *M. phaseolina* as estimated by a wet sieving and diluting technique (Sheikh and Ghaffar, 1975); 6.5% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955); and 2800 cfu g⁻¹ of soil of a mixed population of *Fusarium* spp., as found by soil dilution technique (Nash and Snyder, 1962). Treatments were

replicated three times and the pots were kept in a randomized fashion on the screen house bench of the Soil-borne Diseases Research Laboratory, Department of Botany, University of Karachi where soil was kept at 50% Water Holding Capacity (K e e n and R a c z k o w s k i, 1921).

Soil drench: A 25 ml aqueous cell suspension of 5-day-old culture of rhizobial strains were drenched in 8-cm-diam., plastic pots each containing 250 g soil. Population of rhizobial isolates are shown in Table 1. The population of pathogens in soil was the same as described for the first experiment. Experimental design was also the same as described for seed dressing.

Plants were uprooted after 30 days of emergence and growth parameters in terms of plant height and fresh weight of shoot were recorded. To determine the incidence of fungi, one-cm-long root pieces after washing in tap water were surface sterilized with 1% $\text{Ca}(\text{OCl})_2$ and transferred onto Potato Dextrose Agar plates supplemented with penicillin (100,000 units/l.) and streptomycin sulfate (0.2 g/l.) at 5 pieces per plate. Plates were incubated at room temperature (28°C) and after one week, infection of roots by fungi was recorded.

Data were analysed and subjected to one way or two way Analyses of Variance (ANOVA) depending upon experimental design followed by Least Significant Differences (LSD) according to G o m e z and G o m e z (1984).

Results:

In vitro: In dual culture, rhizobial strains showed variability in antagonistic activity against root-infecting fungi. Of the 9 rhizobial isolates used, *Rhizobium meliloti* (KUCC-139) inhibited all the test fungi viz., *M. phaseolina*, *F. oxysporum*, *F. solani* and *R. solani*. A zone of inhibition of 7 and 4 mm was produced by *Bradyrhizobium* sp., (KUCC-811) against *M. phaseolina* and *F. solani* respectively whereas *Bradyrhizobium* sp., (KUCC-823) produced a zone of inhibition of 8, 15 and 10 mm respectively against *M. phaseolina*, *F. oxysporum* and *F. solani*. A zone of 10 mm was produced by *Bradyrhizobium* sp., (KUCC-819) against *F. oxysporum* while *Bradyrhizobium* sp., (KUCC-824) produced a 3 mm zone of inhibition against *F. oxysporum*. *Bradyrhizobium* sp., (KUCC-818) produced a zone of inhibition of 6 and 2 mm against *M. phaseolina* and *F. solani* respectively. *R. meliloti* (KUCC-139) produced zones of inhibition of 12, 10, 12 and 11 mm against *M. phaseolina*, *F. oxysporum*, *F. solani* and *R. solani* respectively (Table 1).

In vivo:

Seed dressing: *Bradyrhizobium* sp., (KUCC-818) produced maximum plant height and fresh weight of shoot of okra (Table 2). A complete suppression in *M. phaseolina* infection was observed following treatment with *Bradyrhizobium* sp., (KUCC-818) whereas *Bradyrhizobium* sp., (KUCC-819) produced >80% inhibition in *M. phaseolina* infection. *F. solani* infection was completely prevented following application with *Bradyrhizobium* sp., (KUCC-839) and *R. meliloti* (KUCC-815 or KUCC-139). Root infection caused by *R. solani* was completely inhibited in the treatment where *Bradyrhizobium* sp., (KUCC-811, 819 & 839) and strain of *R. meliloti* were used (Table 3).

Soil drench: Maximum plant height was recorded in the treatment where *Bradyrhizobium* sp., (KUCC-821) was used whereas highest fresh weight of shoot was observed in the treatment where *Bradyrhizobium* sp., (KUCC-818) was used (Table 2). *Bradyrhizobium* sp., (KUCC-824) exhibited complete suppression of *M. phaseolina* infection. Similarly, use of *Bradyrhizobium* sp., (KUCC-823), *Bradyrhizobium* sp., (KUCC-821) and *R. meliloti* (KUCC-815) resulted in >75% inhibition in *M. phaseolina* infection. Apart from *Bradyrhizobium* sp., (KUCC-811 & 821) all the rhizobial isolates showed complete suppression of *F. solani* infection. *Bradyrhizobium* sp., (KUCC-819, 818 and 839) and *R. meliloti* (KUCC-139) showed complete suppression of *R. solani* infection (Table 3).

DISCUSSION

In the present study, 9 rhizobial strains were isolated from root nodules of *Cicer arietinum*, *Vigna radiata*, *Vigna mungo*, *Samania saman*, *Sesbania sesban*, *Leucinia* sp., *Prosopis cineraria* and *Medicago sativa* and identified. In dual culture, strains of *Bradyrhizobium* sp., and *R. meliloti* were found to inhibit the radial growth of *M. phaseolina*, *F. oxysporum*, *F. solani* and *R. solani* producing zones of inhibition. Different strains of rhizobia showed different inhibitory effects against different root-infecting fungi, since some rhizobial strains were found to inhibit the growth of one or two pathogens. Such a similar observation has been made by Frey and Linda (1988) who found inhibition of *R. solani* by 9 rhizobial strains out of 34 strains used. Similarly, Chao (1990) reported tremendous variability among the strains of rhizobia in antagonistic ability against fungi. A single strain of rhizobia can infect certain species, or even certain varieties within a species of legumes (Allen, 1971). Like nodule forming ability, antagonistic effects of rhizobia presumably depend upon the strain.

Some strains of rhizobia which fix atmospheric nitrogen in association with leguminous plants showed significant control of *M. phaseolina*, *R. solani* and *Fusarium* spp., infection on both leguminous plants like soybean and mungbean and non-leguminous plants like sunflower and okra (Ehteshamul-Haque and Ghaffar, 1993). In the present study also seed dressing or soil drench with different strains of rhizobia showed promising results in the control of root-infecting fungi viz., *M. phaseolina*, *F. solani* and *R. solani* in okra. Bacterization of soybean seeds with rhizobia significantly reduced *F. oxysporum* infection (Mew and Howard, 1969). At a given population of *Phytophthora megasperma* and *F. oxysporum*, higher level of *R. meliloti* reduced root-rot of lucerne (Tu, 1980). In the present study, *Bradyrhizobium* sp., (KUCC-839 & 811) and *R. meliloti* (KUCC-815) although did not show antagonistic activity on root-infecting fungi *in vitro*, while *in vivo* they showed significant control of root-rot pathogens. This is in agreement with the findings of Chakraborty and Chakraborty (1989), who reported bacterization of pea seeds with *R. leguminosarum* showed good control of *F. solani* with no inhibitory effects *in vitro*. Presumably, rhizobia, instead of a direct effect on root-infecting fungi, might have stimulated other rhizospheric microorganisms deleterious to *M. phaseolina*, *F. solani* and *R. solani*. It is also speculated that metabolites produced in agar medium was also different in both quality and quantity than that occurring in the rhizosphere.

In the present study, rhizobia not only significantly suppressed root-rot infection but also enhanced plant top biomass. This growth-promoting effect may be direct or indirect. Direct mechanism may involve the production of plant growth regulators such as auxins, cytokinins and gibberellin like substances (Even sen and Ble v i n s, 1981; S h e n g, 1993) which act directly on the plant itself and affect growth. Indirect mechanism may involve the production of toxic metabolites (Ch a k r a b o r t y and P u r k a y a s t h a, 1984) which have their inhibitory effect on soil-borne plant pathogens, thereby increased plant growth. Rhizobia which live as a saprophyte, migrate towards the roots through chemotactic response and this attraction was not specific as the rhizobia were attracted to other legumes as well as to non-leguminous plants (D o w l i n g and B r o u g h t o n, 1986). It would appear that rhizobia which is a good rhizosphere organism for leguminous or non-leguminous plants presumably prevent the contact of pathogenic fungi on roots by covering the hyphal tip of the fungus and by parasitizing it (T u, 1978). In electron microscopy, rhizobia were found inside the hyphae of *F. oxysporum*, *Phytophthora megasperma*, *Pythium ultimum* and *Ascochyta imperfecta* (T u, 1979). Besides parasitizing the hyphae, rhizobia also produce antibiotics (M a l a j c z u k et al., 1983) which resulted in lysis of the fungal hyphae (M a l a j c z u k, 1984). Differences in the efficacy of rhizobia on different host may also be due to the quality of root exudates which influence the colonization by bacteria (P a r k e, 1991).

The results of the present study suggest that rhizobium not only interacts with the roots of legumes but may also interact beneficially with non-leguminous plants. This is inherently logical since any bacterium present in the rhizosphere, including rhizobium, would benefit by improving nearby plant growth, as plant roots exude nutritional compounds. Most rhizobium strains are likely to have been exposed for extensive period during their evolution to non-legume rhizospheres. As rhizobium may be found as a free-living rhizobacterium and appear to promote growth of non-legumes as well as host legume species, the term plant growth-promoting rhizobacterium or PGPR seems to apply. Given the current ecological and economical importance of rhizobium in growing legumes, the PGPR ability of rhizobium may be of considerable agronomical importance (N o e l et al., 1995).

CONCLUSION

Results from the present study would therefore suggests that rhizobia has great potential as a biological control agent of root-infecting fungi and application of an effective rhizobial isolate may result in better crop production.

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Table 1.

In vitro inhibition of *Macrophomina phaseolina*, *Fusarium oxysporum*, *Fusarium solani* and *Rhizoctonia solani* by species of *Rhizobium* and *Bradyrhizobium*

<i>Rhizobium</i> spp	Source	Locality	Zone of inhibition (mm)/ type of reaction			
			<i>M. phaseolina</i>	<i>F. oxysporum</i>	<i>F. solani</i>	<i>R. solani</i>
<i>Bradyrhizobium</i> sp. (KUCC-811)	<i>Cicer arietinum</i>	Malir	7	**	4	*
<i>Bradyrhizobium</i> sp. (KUCC-823)	<i>Vigna radiata</i>	Karachi University	8	15	10	**
<i>Bradyrhizobium</i> sp. (KUCC-819)	<i>Vigna mungo</i>	Karachi University	**	10	**	**
<i>Bradyrhizobium</i> sp. (KUCC-824)	<i>Samania saman</i>	Karachi University	*	3	*	*
<i>Bradyrhizobium</i> sp. (KUCC-818)	<i>Sesbania sesban</i>	Karachi University	6	*	2	*
<i>Bradyrhizobium</i> sp. (KUCC-839)	<i>Leucaena</i> sp.	Karachi University	*	*	*	*
<i>Bradyrhizobium</i> sp. (KUCC-821)	<i>Prosopis cineraria</i>	Malir	**	**	*	*
<i>Rhizobium meliloti</i> (KUCC-815)	<i>Medicago sativa</i>	Malir	*	*	**	**
<i>R. meliloti</i> (3DOA1) (KUCC-139)	<i>Medicago sativa</i> USDA†		12	10	12	11

* - No inhibition

** - Test fungus inhibited but later over grew

† - United States Department of Agriculture

Table 2

Effect of different strains of *Rhizobium* and *Bradyrhizobium* used as seed dressing or as soil drench on growth of okra

Treatments	Seed dressing			Soil drench		
	Population of rhizobia per seed (cfu)	Plant height (cm)	Shoot weight (g)	Population of rhizobia per ml (cfu)	Plant height (cm)	Shoot weight (g)
Control	—	13.0	2.7	—	14.0	2.8
<i>Bradyrhizobium</i> sp. (KUCC-811)	3.2×10^6	13.3	3.0	1.3×10^8	15.9	3.3
<i>Bradyrhizobium</i> sp. (KUCC-823)	2.9×10^6	14.6	2.9	3.8×10^8	15.2	2.6
<i>Bradyrhizobium</i> sp. (KUCC-819)	2.5×10^6	13.7	2.5	4.2×10^8	14.5	2.9
<i>Bradyrhizobium</i> sp. (KUCC-824)	2.9×10^6	13.5	2.7	2.4×10^8	14.9	2.1
<i>Bradyrhizobium</i> sp. (KUCC-818)	3.1×10^6	16.3	3.6	3.1×10^8	14.6	3.8
<i>Bradyrhizobium</i> sp. (KUCC-839)	1.2×10^6	14.2	2.5	3.6×10^8	15.7	2.2
<i>Bradyrhizobium</i> sp. (KUCC-821)	2.5×10^6	14.2	2.4	4.6×10^8	16.5	3.2
<i>Rhizobium meliloti</i> (KUCC-815)	2.7×10^6	15.8	1.7	1.6×10^8	13.2	2.0
<i>R. meliloti</i> (KUCC-139)	1.9×10^6	14.6	2.3	2.1×10^8	13.2	2.6
LSD $p < 0.05$	—	2.1	1.1	—	3.2	1.4

Data represent mean of three replicates

Table 3

Effect of different strains of *Rhizobium* and *Bradyrhizobium* used as seed dressing or as soil drench in the control of *Macrophomina phaseolina*, *Fusarium solani* and *Rhizoctonia solani* in okra

Treatments	Seed dressing			Soil drench		
	<i>M. phaseolina</i>	<i>F. solani</i>	<i>R. solani</i>	<i>M. phaseolina</i>	<i>F. solani</i>	<i>R. solani</i>
	Infection%					
Control	31	19	31	60	15	13
<i>Bradyrhizobium</i> sp. (KUCC-811)	0	6	0	29	8	6
<i>Bradyrhizobium</i> sp. (KUCC-823)	21	8	0	13	0	50
<i>Bradyrhizobium</i> sp. (KUCC-819)	6	13	13	25	0	0
<i>Bradyrhizobium</i> sp. (KUCC-824)	17	13	0	0	0	8
<i>Bradyrhizobium</i> sp. (KUCC-818)	13	13	0	50	0	0
<i>Bradyrhizobium</i> sp. (KUCC-839)	8	0	8	25	0	0
<i>Bradyrhizobium</i> sp. (KUCC-821)	19	21	8	6	13	13
<i>Rhizobium meliloti</i> (KUCC-815)	25	0	6	13	0	6
<i>R. meliloti</i> (KUCC-139)	8	0	8	25	0	0
LSD p<0,05	Treatment = 17.7 Pathogen = 9.7			Treatment = 19.9 Pathogen = 10.9		

Data represent mean of three replicates

***Rhizobium* jako czynnik biologicznej ochrony przed grzybami atakującymi system korzeniowy okry w warunkach szklarniowych**

Streszczenie

Badano działanie dziewięciu linii *Rhizobium* wyizolowanych z brodawek korzeniowych *Cicer arietinum*, *Vigna radiata*, *V. mungo*, *Samania saman*, *Sesbania sesban*, *Leucinia* sp., *Prosopis cineraria* i *Medicago sativa* na grzyby infekujące system korzeniowy: *Macrophomina phaseolina*, *Fusarium solani* i *Rhizoctonia solani*. *Bradyrhizobium* sp. i *R. meliloti* w badaniach laboratoryjnych hamowały wzrost *M. phaseolina*, *F. solani* i *R. solani*, a użyte do zaprawiania nasion lub doglebowo istotnie ograniczały infekcję korzeni okry powodowaną przez *M. phaseolina*, *F. solani* i *R. solani* w warunkach szklarniowych. Obecność *Rhizobium* zwiększała produkcję biomasy roślin okry.