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In vitro Antagonism of *Rhizobium* Strains Isolated from Various Legumes

Cigdem Kucuk* and Cenap Cevheri

Harran University, Faculty of Arts and Science, Department of Biology, Sanliurfa, Turkey.

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The effects of 20 *Rhizobium* strains isolated from *Phaseolus vulgaris* L., *Trifolium repens* var. *repens, Cicer arietinum* L., *Lens culinaris* Medik., *Vigna unguiculata* L. and *Phaseolus vulgaris* L. 'Red Kidney' plants on mycelium growth of *Fusarium oxysporum, F. moniliforme, F. solani, F. culmorum, F. oxysporum* F1 strain, *F.oxysporum* F2 strain, *F.oxysporum* F3 strain, *F.oxysporum* F4 strain and *Trichoderma harzianum* strains were observed on solid medium. The most effective *Rhizobium* strain against the *Fusarium* species was *R. phaseoli* 4.5 strain. *Rhizobium* sp. 3.5, *R. trifolii* 2.3a, *R. trifolii* 2.16 and *R. trifolii* 2.25a strains didn't affect the growth of *T. harzianum* T1. *R. phaseoli* 1.17, *R. phaseoli* 1.114, *R. ciceri* N7 and *R. ciceri* N28 strains didn't affect the growth of *T. harzianum* T15 and *R. ciceri* N7 strain of *Rhizobium* didn't affect the growth of *T. harzianum* T3. In this study IAA production of *Rhizobium* strains was assayed colorimetrically. The results showed that IAA production by *Rhizobium* strains ranged from 2.19 to 129.7 μg ml^{-1}. Phosphate solubilization and siderophore production by the strains were also tested. Amount of IAA, P solubilization and siderophore in *R. phaseoli* 4.5 strain was the highest. Potential use of this strain needs further tests in *planta*.

Key words: Antagonistic effect, *Rhizobium,* strains, Fungi, *in vitro*.

The diseases caused by soilborne plant pathogens pose serious threats to the production of several crops such as bean, wheat, tomato and chickpea[2, 23]. Although chemical control of pathogens reduces the disease to some extent, it is not cost effective and environment-friendly. An alternative and in several cases effective method to control plant pathogens is the use of biological control agents[5,10,16, 22, 39].

Rhizobium bacteria promote plant growth directly by affecting symbiotic N_2 fixation, nodulation or nodule occupancy[37]. Various strains of *Rhizobium* spp. and *Trichoderma* spp. have been described as effective biological control agents antagonistic to many fungal plant pathogens[10, 17, 22, 23, 24,29]. *Rhizobium* sp. significantly reduced wilt and root rot on common bean and chickpea caused by *Fusarium* sp.[2,3, 15, 23]. Various traits (like toxic and/or and stimulatory metabolites, indole acetic acid, exopolysaccharides, antimicrobial and bacteriocin like substances) expressed synchronously, or in a controlled sequence, are considered responsible for the action of *Rhizobium* strains as biological control agents[2,6, 20, 23, 28, 29]. The ability to produce an antibiotic-like substance was found in a *R. trifolii* strain[14, 26, 29]. Strains of *R. leguminosarum* biovar *viciae*, *R. tropici*, *R. trifolii*, *R. ciceri* and *Sinorhizobium meliloti* were found to produce siderophores[7,13,32]. Siderophore producing strain of *R.meliloti* was able to inhibit *Macrophomina phaseolina in vitro*[4]. These compounds would reduce the available iron in other microorganisms[27]. Competition for nutrients and rhizosphere colonization ability were also considered as possible mechanisms of

* To whom all correspondence should be addressed.
Tel: +90 414 3183567 ;
E-mail adress: cdmkucuk@yahoo.com

antifungal activity in *R. trifolii* and *R. lupini*[29,30,36].

Rhizobia produce siderophores[4,7] and its indole acetic acid (IAA)[11].They are antagonistic to deleterious or phytopathogenic fungi[14]. Siderophore production and utilization in rhizobia is of particular interest due to the dominant role of iron in the nitrogen fixation and assimilation process[13]. IAA produced by bacteria improves plant growth by increasing the number of root hairs and lateral roots[18,33]. Microbial biosynthesis of IAA in soil is enhanced by tryptophan from root exudates or decaying cells[11]. Phosphate solubilization is important to plant growth because P an assential element and has low availability to plants due to its chemical properties[12]. A large number of *Rhizobium* strains are able to solubilize insoluble P compounds but this ability is variable among strains[9].

Fusarium spp. are soil borne fungi that specifically attack many agricultural crops causing wilts or foot and root rots that result in significant yield losses[3,22,23]. Many studies have proved the potential of *Trichoderma* spp. as biological agents antagonistic to several soilborne plant pathogens including *Fusarium* spp.[14,21,22]. The soil bacteria such as *Bacillus* sp., *Azotobacter* sp., *Pseudomonas* sp., *Micrococcus* sp., *Rhizobium* sp. plays an important role in the growth and development of plants by altering the availability of nutrients. *Rhizobium* spp. are also known as bacteria that colonize the rhizosphere [24,26,27]. The objectives of this study were to determine : (i) IAA and siderophore production[7,13,18] and (ii) P solubilization and *in vitro* antagonistic activity of *Rhizobium* strains against plant pathogenic *Fusarium* spp. and beneficial *T. harzianum* strains.

MATERIALS AND METHODS

Microorganisms

The strains of *Rhizobium* used in this study were isolated from roots of different Leguminous plants as listed in Table 1. Fungal cultures (*Fusarium solani, F. culmorum, F. moniliforme, F.oxysporum, F. oxysporum* F1, *F. oxysporum* F2, *F. oxysporum* F3 and *F.oxysporum* F4, *Trichoderma harzianum* T1, *T. harzianum* T3, *T. harzianum* T7, *T. harzianum* T8, *T.harzianum* T15, *T.harzianum* T18, *T.harzianum* T20 and *T.harzianum* T22 strains) were obtained from the culture collection of the Microbiology Laboratory of Harran University in Turkey.

Interactions between fungi and *Rhizobium* strains

Rhizobium strains were grown in 100 ml Yeast Extract Broth (YEM) at 30°C in a rotary shaker at 120 rpm for 2 days [38]. Two ml of the culture suspension (10^5 cell ml^{-1}) was mixed into 20 ml of Yeast Glucose Mineral Agar (YGMA) (mannitol, 5g; glucose, 5 g; K_2HPO_4, 0.5 g; $MgSO_47H_2O$, 0.8 g; NaCI 0.1 g; $ZnSO_47H_2O$, 0.1 mg; $CuSO_47H_2O$, 0.05 mg; $FeSO_47H_2O$, 0.2 mg; $MnSO_45H_2O$, 0.5 mg; Na_2MoO_4, 0.05 mg; H_3BO_3, 0.25 mg and yeast extract (MERCK) 2.5 g; agar, 15 g in 1000 ml distilled water). After the agar solidified, a mycelial plug was placed on the agar surface[29,34]. Radial mycelial growth was recorded after five days. Cultures without *Rhizobium*, inoculated only with fungus served as control.

Inhibition of fungi by *Rhizobium* free filtrates

Rhizobium isolates were grown in 100 ml YGM broth at 30°C in a rotary shaker at 120 rpm for 2 days. Sterile culture extracts were obtained by 0.45 µm pore size of cellulose filters. Wells (7 mm diameter), made in the YGMA plates previously inoculated with 10^6 fungal spores ml^{-1} were filled with sterile *Rhizobium* filtrates[35]. Inhibition zones around the wells were observed after 3 days of incubation when the growth of the fungus around the wells cleared[30,35]. The cultures without *Rhizobium* filtrates comprised the control.

Lysis of fungi by *Rhizobium* strains

YGMA plates were mixed with a suspension of *Rhizobium* strains (10^5 cell ml^{-1}) and incubated at 30°C for 2 days. Filtrates (100 µl) of 5 day cultures of fungal strains (10^6 spore ml^{-1}) were added to wells made in the medium, lytic activity was demonstrated when the growth of fungal cultures arround the wells cleared[35]. Cultures without *Rhizobium* strains inoculated only by fungi comprised the control.

Determination of phosphate solubilizing ability

The *Rhizobium* strains were tested for their ability to dissolve phosphate under *in vitro* conditions using Pikovaskaya's medium (glucose, 10 g; tricalcium phosphate, 5 g; $(NH_4)_2SO_4$, 0.5 g; KCl, 0.2 g; $MgSO_47H_2O$, 0.1 g; $MnSO_47H_2O$, 0.001 g; $FeSO_47H_2O$, 0.001 g; yeast extract, 0.5 g; agar, 15 g; distilled water, 1000 ml)[9,12]. The strains were spot-inoculated on the medium, and P solubilization was checked by measuring the zone of clearance.

Indole Acetic Acid (IAA) production

The IAA production in liquid culture was determined colorimetrically[19]. *Rhizobium* strains were grown in 100 ml YEM for 72 h at 30 °C at 120 rpm. Cultures were centrifuged at 5000 rpm for 30 min. The supernatant (50 µl) was mixed with 450 µl phosphate buffer. From this mixture 60 µl was added to 440 µl of phosphate buffer in a tube containing 500 µl of Salkowski reagent (12 g of $FeCl_3$ per liter of 7.9 M H_2SO_4)[25]. Development of pink-red indicates IAA production[18]. Red color formation was quantified as the absorbance at a wavelenght of 540 nm in a spectrophotometer. The amount of IAA produced per milliliter of culture was estimated using a standard curve[19]. Specific productivity was calculated according to the equation by Ghosh and Basu[18].

Specific productivity= (Absorbance $_{540}$ / IAA)

In vitro siderophores production by *Rhizobium* strains

Siderophore production by *Rhizobium* was evaluated only for strains antagonistic to *Fusarium* strains. The *Rhizobium* strains were grown on Yeast Extract Agar[38] supplemented with $FeCl_3$ (1µM). A fungal spore suspension (0.1 ml, 10^6 spore ml^{-1}) was spread on the surface of YGMA plates and wells 7 mm in diameter were punched into the agar medium and filled with 150 µl of *Rhizobium* (10^5 cell ml^{-1}) culture in YEM. The petri dishes were incubated at 30°C and antifungal activity was evaluated after 7 days by measuring the growth inhibition zones that formed around each well[7,13].

Statistical analyses

Duncan's multiple range (DMR) test was applied for the difference between microorganisms[1].

RESULTS AND DISCUSSION

Rhizobium strains isolated from root nodules of different plants showed different interactions with the tested fungi. In this study, some *Rhizobium* strains inhibited the growth of some *Fusarium* spp., so they may have the potential to act as biocontrol agents. *F.oxysporum* strain F4 was inhibited by *Rhizobium ciceri* N28, *F. oxysporum* strain F3 was inhibited by *R. leguminosarum* strain 6.1a, *F.culmorum* was inhibited by *R.ciceri* strain N7 and *F.moniliforme* and *F.culmorum* were inhibited by *R.phaseoli* 5.1a (Table 2).

R.leguminosarum 2.18 was inhibited *F.oxysporum* strain F3, *F.oxysporum* strain F4, *F.moniliforme, F.culmorum, R.ciceri* N22 strain was inhibited *F.oxysporum* strain F1, *F.oxysporum* strain F3, *F.oxysporum* strain F4, *F.oxysporum, F.solani, R.phaseoli* 1.20 strain was inhibited *F.oxysporum* strain F3, *F.moniliforme, F.solani* and *F.culmorum*. Inhibition of microorganisms was most likely due to excretion of essential metabolites produced in excess by the interacting microorganisms in a medium where it is in limited supply[3,10,27]. *R. phaseoli* strain 4.5 which minimized fungus growth was significantly different from other *Rhizobium* strains. Growth of all fungi were greatest in the presence of *R.phaseoli* strain 1.114. Therefore, this strain was less effective against fungal growth. *R.phaseoli* 4.5 and *R.phaseoli* 1.20 strains were found to suppress the growth of various *Fusarium* spp. *in vitro*. The inhibition zones between *Fusarium* spp. and the strains tested were up to 20 mm for *F. oxysporum* F1, *F. oxysporum* F2 and *F. oxysporum* F3 and up to 25 mm for *F. solani* and *F. culmorum* (data not shown).

Table 1. *Rhizobium* strains and host plants

Strain	Host Plant	Strain	Host Plant
1.17	Common bean (*Phaseolus vulgaris* L.)	2.10	Clover (*Trifolium repens* var. *repens*)
1.15	Common bean (*Phaseolus vulgaris* L.)	N7	Chickpea (*Cicer arietinum* L.)
1.20	Common bean (*Phaseolus vulgaris* L.)	N27	Chickpea (*Cicer arietinum* L.)
1.114	Common bean (*Phaseolus vulgaris* L.)	N28	Chickpea (*Cicer arietinum* L.)
5.1a	Common bean (*Phaseolus vulgaris* L.)	N22	Chickpea (*Cicer arietinum* L.)
5.1b	Common bean (*Phaseolus vulgaris* L.)	6.2a	Lentil (*Lens culinaris* Medik.)
2.18	Clover (*Trifolium repens* var. *repens*)	6.1a	Lentil (*Lens culinaris* Medik.)
2.25a	Clover (*Trifolium repens* var. *repens*)	6.1c	Lentil (*Lens culinaris* Medik.)
2.3a	Clover (*Trifolium repens* var. *repens*)	3.5	Cowpea (*Vigna unguiculata* L.)
2.16	Clover (*Trifolium repens* var. *repens*)	4.5	Red bean (*Phaseolus vulgaris* L.'Red Kidney')

Significant differences in the antagonistic activity of the strains were observed (Table 2).

After 5 days of growth, sterile filtrate of *R.ciceri* N28 the growth on *F.culmorum* and *F.oxysporum* and inhibited the growth of *F.oxysporum* F1. However, after 7 days of incubation, this sterile filtrate inhibited all tested fungi. Usually the growth rate of the soil and rhizosphere microorganisms such as *Pseudomonas* spp., *Bacillus* spp. appeared faster on agar medium as compared to *Rhizobium* strains[23,27]. In this way so the rhizobial biomass produced was possibly insufficient for active amounts of inhibitors to be produced. This inhibition effect on *Fusarium* spp. might be the result of possibility antifungal compounds released by the antagonist into the culture medium.

Lytic activity of many strains was demonstrated by the clearing of fungal growth around the bacterial filtrates due to possible

Table 2. *Fusarium* growth (mm) under treatment with *Rhizobium* strains and DMR test for the comparison of microorganisms

Rhizobium strains	*Fusarium* species[a]							
	F1	F2	F3	F4	F.m	F.o	F.s	F.c
1.17	30± 0.01*	10± 0.02	10± 0.01	25±0.01	21±0.01	10±0.02	20±0.01	29±0.01
1.15	23± 0.02	10± 0.6	23± 0.01	10±0.01	20±0.01	10±0.04	30±0.01	9±0.01
1.20	10± 0.02	31± 0.1	10± 0.01	27±0.04	10±0.01	21±0.02	10±0.01	10±0.00
1.114	30± 0.03	31± 0.02	32± 0.01	64±0.01	30±0.01	35±0.02	40±0.01	51±0.08
5.1a	30± 0.05	30± 0.02	23± 0.01	23±0.01	19±0.01	30.5±0.001	21±0.01	14±0.01
5.1b	10± 0.02	23± 0.03	10± 0.01	10±0.00	21±0.01	31±0.00	10±0.07	26±0.03
2.18	25± 0.02	30± 0.00	10± 0.01	10±0.03	10±0.01	18±0.13	16±0.01	10±0.02
2.25a	20.5±0.02	30± 0.01	20± 0.01	21±0.01	29±0.01	22±0.01	20±0.01	10±0.02
2.3a	10± 0.01	25± 0.01	26± 0.01	10±0.01	21±0.01	10±0.01	31±0.05	28±0.01
2.16	25± 0.01	30± 0.02	25± 0.01	30±0.05	10±0.01	10±0.01	22±0.01	30±0.01
2.10	10± 0.05	22± 0.02	24± 0.01	22±0.01	29±0.01	29±0.01	20±0.01	20±0.01
N7	24± 0.03	25± 0.02	10± 0.01	10±0.01	21±0.01	10±0.01	10±0.05	7±0.04
N27	26± 0.01	10± 0.00	25± 0.01	10±0.01	10±0.01	30±0.00	22±0.10	35±0.01
N28	26± 0.01	10± 0.01	41± 0.01	8±0.01	27±0.01	10±0.03	10±0.01	36±0.09
N22	10± 0.02	26± 0.04	10± 0.01	10±0.01	22±0.01	10±0.04	10±0.01	29±0.05
6.1a	35± 0.05	34± 0.02	9± 0.01	30±0.02	10±0.01	35±0.02	10±0.01	20±0.02
6.1c	25± 0.07	10± 0.02	10± 0.01	31±0.06	20±0.01	10±0.12	26±0.04	31±0.01
6.2c	10± 0.03	25± 0.01	21± 0.01	25±0.12	25±0.01	30±0.01	20±0.02	25±0.03
3.5	20± 0.1	20± 0.01	10± 0.01	10±0.17	25±0.01	25±0.05	10±0.09	20±0.01
4.5	10± 0.02	10± 0.01	10± 0.01	10±0.02	10±0.01	20±0.07	21±0.11	10±0.01
Control	80± 0.01	80± 0.03	80± 0.01	80±0.01	79±0.01	80±0.01	80±0.01	80±0.01

Rhizobium strains		*Rhizobium* strains		*Fusarium* species	
1.17	19.75cde	2.10	20.70 cd	F1	19.75 bc
1.15	17.16 gh	N7	14.58 j	F2	22.35 a
1.20	16.33 1j	N27	18.91 defg	F3	18.11 d
1.114	39.37 a	N28	20.79 cd	F4	20.10 bc
5.1a	24.54 b	N22	15.66 1j	F.m	19.37 cd
5.1b	17.66 efgh	6.1a	23.54 bc	F.o	20.02 bc
2.18	14.95 j	6.1c	19.41 def	F.s	18.38 d
2.25a	21.79 c	6.2c	20.58 cd	F.c	21.00 b
2.3a	19.12 defg	3.5	17.45 fgh		
2.16	22.95 bc	4.5	12.37 k		

[a]F.m.: *Fusarium moniliforme*, F.o: *F. oxysporum*, F.c: *F. culmorum*, F.s: *F.solani;* F1, F2, F4: *Fusarium oxysporum* (isolated from chickpea), F3: *Fusarium oxysporum* (isolated from lentil). * Values are the means ± standard deviations.

antimicrobial substance production such as the one reported by Chao[10] and Özkoç and Deliveli[29]. Estevez de Jensen et al[15] suggested that in order to avoid competition in the rhizosphere, bacteria and fungi populations may colonize different habitats. Consequently, the authors suggested that a combination of appropriate bacteria and fungi would provide better control capability than either of them used alone. Harman et al[21] reported that when pea seeds were treated with both *Rhizobium* sp. and *T.hamatum*, the former had no effect on the protective ability of the latter. In this study, growth of *T.harzianum* strain T22 (Table 3) was inhibited by some of the *Rhizobium* strains (*R.phaseoli* 4.5, 3.5, *R.leguminosarum* 6.2c, *R.trifolii* 2.25a, *R.trifolii* 2.18, *R.ciceri* N7, *R.ciceri* N27) tested. Symbiosis between *Rhizobium* and legumes can reduce the dependence of host plants on nitrogen fertilizer and the correct usage of *Trichoderma* strain can decrease the amount of fungicide needed. Both of these organisms are highly desirable in organic farming[8,16,24]**.** For this reason, potential biocontrol agents or plant growth inducers should be selected carefully, taking in

Table 3. Trichoderma harzianum strains growth (mm) under treatment of *Rhizobium* strains and DMR test for the comparison of microorganisms

Rhizobium strains	*Fusarium* species[a]							
	F1	F2	F3	F4	F.m	F.o	F.s	F.c
1.17	57 ± 0.01*	60 ± 0.03	45± 0.03	60± 0.03	72± 0.01	62±0.01	63±0.01	42±0.01
1.15	62 ± 0.03	52 ± 0.02	50± 0.02	62± 0.03	68 ± 0.01	60±0.02	58±0.02	40±0.01
1.20	50 ± 0.02	69 ± 0.02	50± 0.01	68± 0.02	60± 0.01	62±0.02	55±0.02	42±0.01
1.114	48 ± 0.04	65 ± 0.01	45± 0.01	52± 0.01	72 ± 0.00	67±0.01	50±0.03	52±0.01
5.1a	52 ± 0.01	58 ± 0.02	60± 0.01	55± 0.02	70 ± 0.01	52±0.03	52±0.02	57±0.01
5.1b	50 ± 0.02	60 ± 0.02	60 ± 0.01	55± 0.01	65 ± 0.02	57±0.04	64±0.02	38±0.02
2.18	60 ± 0.02	50 ± 0.01	42± 0.01	55± 0.02	60 ± 0.02	55±0.02	64±0.02	35±0.01
2.25a	71 ± 0.01	48 ± 0.01	50± 0.03	62± 0.05	60 ± 0.03	55±0.02	62±0.02	32±0.01
2.3a	72 ± 0.03	48 ± 0.01	52± 0.03	50± 0.01	68 ± 0.01	63±0.02	60±0.03	44±0.01
2.16	70 ± 0.06	52 ± 0.02	68± 0.04	50± 0.01	63 ± 0.01	65±0.02	60±0.01	40±0.02
2.10	68 ± 0.02	60 ± 0.03	68± 0.04	48± 0.02	62 ± 0.00	62±0.01	60±0.01	42±0.01
N7	48 ± 0.03	72 ± 0.02	57± 0.01	40± 0.02	70 ± 0.01	62±0.01	62±0.01	35±0.01
N27	50 ± 0.01	58 ± 0.03	55± 0.02	40± 0.03	75 ± 0.02	60±0.03	60±0.03	37±0.00
N28	52 ± 0.02	50 ± 0.03	55± 0.03	55± 0.02	72 ± 0.03	60±0.03	50±0.01	42±0.01
N22	56 ± 0.02	62 ± 0.02	55± 0.02	57± 0.01	65 ± 0.01	58±0.06	50±0.03	44±0.01
6.1a	62 ± 0.03	60 ± 0.01	60± 0.02	56± 0.01	60 ± 0.02	54±0.02	62±0.03	40±0.01
6.1c	62 ± 0.04	68 ± 0.01	62± 0.03	60± 0.04	58 ± 0.02	55±0.01	63±0.04	40±0.02
6.2c	60 ± 0.02	52 ± 0.04	65± 0.02	45± 0.02	60 ± 0.03	53±0.02	57±0.02	33±0.03
3.5	75 ± 0.01	68± 0.05	47± 0.02	40± 0.02	62± 0.01	55±0.02	68±0.01	32±0.05
4.5	38 ± 0.01	40± 0.02	40± 0.04	35± 0.03	55± 0.02	52±0.03	48±0.01	28±0.02
Control	80± 0.01	80± 0.01	80± 0.01	80± 0.01	80± 0.02	80±0.01	80±0.01	68±0.03

Rhizobium strains		*Rhizobium* strains		*Fusarium* species	
1.17	41.667cd	2.10	42.33bcd	T1	58.45b
1.15	39.00cde	N7	35.00def	T3	56.28bc
1.20	42.00cd	N27	37.66cdef	T7	54.43c
1.114	52.00ab	N28	42.00cd	T8	52.35d
5.1a	57.00a	N22	44.00bcd	T15	64.86a
5.1b	38.00cdef	6.1a	39.66cde	T18	58.71b
2.18	35.33def	6.1c	42.00cd	T20	56.73bc
2.25a	32.00ef	6.2c	32.00ef	T22	39.93e
2.3a	45.33abc	3.5	32.00ef		
2.16	40.66cde	4.5	29.00f		

*Values are the means ± standard deviations.

account their effects on other beneficial microorganisms as well.

Many bacteria were shown to encourage plant growth by promoting the outbreak of secondary roots, acting as protectors against phytopathogenic microorganisms via plant growth regulations such as auxin, gibberellin, siderophore, HCN and antibiotic production[18,37]. IAA is one of the most important auxins. The IAA production and specific productivity by *Rhizobium* strains given at in Table 4. The amount IAA, *R. phaseoli* 4.5 strain was the highest (129.7 µg ml^{-1}), while the lowest IAA amount was found in *R. phaseoli* strain 1.114 (2.12 µg ml^{-1}).

Specific productivity in these strains were 65.8 and 1.9, respectively. This could be due to better utilization of medium components for IAA production by *R. phaseoli* strain 4.5, as well as by strains *R. phaseoli* 5.1a and *R. ciceri* N28, compared to the others. The *Rhizobium* strains were varied grately regarding both the growth and IAA production (Table 4). Similar results on *Rhizobium* strains were reported by Data and Basu[11], Perrine et al.,[31]. IAA produced by bacteria improves plant growth by increasing the number of root hairs and lateral roots[18,25]. Perrine et al.,[31] suspected that IAA production by *R. leguminosarum* plays a role in the growth promotion of plants. Our strains were IAA producers and they may be involved in plant growth.

R.phaseoli 4.5 strain had the highest P solubilization activity on culture media (Table 5).

Table 4. IAA production by *Rhizobium* strains on YEM

Strains	Growth (OD at 540 nm)	IAA (µg ml^{-1}) production	Specific (IAA production /growth) productivity
1.17	1.34	37.2	27.8
1.15	1.43	28.6	19.5
1.20	1.68	47.7	28.3
1.114	1.12	2.12	1.9
5.1a	1.72	90.4	52.6
5.1b	0.65	14.1	21.7
2.18	1.72	61.2	35.6
2.25a	1.80	48.8	28.1
2.3a	1.82	35.6	19.6
2.16	1.69	29.2	17.3
2.10	1.84	58.7	31.9
N7	1.75	6.25	31.6
N27	1.67	22.4	13.4
N28	1.90	75.2	39.6
N22	1.43	30.03	21.0
6.1a	1.57	66.6	42.4
6.1c	1.68	30.6	18.2
6.2c	1.42	27.8	19.6
3.5	1.25	25.6	20.5
4.5	1.97	129.7	65.8

Table 5. Phosphate solubilization of strains

Strain	P-solubilization *(mm)	Strain	P-solubilization *(mm)
1.17	-	2.10	1 ± 0.01
1.15	6 ± 0.04	N7	2 ± 0.4
1.20	6 ± 0.02	N27	7 ± 0.7
1.114	2 ± 0.10	N28	5 ± 0.00
5.1a	7 ± 0.01	N22	4 ± 0.03
5.1b	-	6.2a	3 ± 0.04
2.18	-	6.1a	5 ± 0.12
2.25a	-	6.1c	3 ± 0.02
2.3a	-	3.5	4 ± 0.01
2.16	1 ± 0.00	4.5	9 ± 0.01

*Radius of the clarification zone around colonies on agar. Values are the means ± standard deviations

R. ciceri N27 was showed solubilization activity similar to that of *R. phaseoli* 5.1a (Table 5). These results also suggest that our *Rhizobium* strains might be affective in solubilizing of phosphates in the soil. This increases the potential applicability of rhizobial inoculants in agriculture. However, *R. phaseoli* 1.17, *R. trifolii* 2.18, *R. trifolii* 2.25a, *R. phaseoli* 5.1b and *R. trifolii* 2.3a strains were not effective as P-solubilizing. A further work is required to consider their role in enhancing plant growth.

In this study, it was noted that inhibition recorded may be due to substances synthesized by these Rhizobial strains. *R. ciceri* N22 and *R. phaseoli* 1.15 strains in absence of sufficient $FeCI_3$ were exhibited siderophore production. Similarly, siderophore producing strain of *R. meliloti* has also been shown to inhibit the plant pathogen *Macrophomina phaseolina* and hence reported to act as a potential biocontrol agent[4]. Siderophore producing bacteria are promoting plant growth indirectly by sequestering the iron in the rhizosphere, especially in neutral and alkaline soils, and thereby its availability for the growth of pathogens[13].

Many other root nodule bacteria have been tested for siderophore production using Chrome Azurol S (CAS) reagent[7,13]. A survey of four strains of *Rhizobium loti*, 35 strains of *Rhizobium ciceri* and three strains of *R. leguminosarum* bv. *viciae* were found CAS-positive[13]. 50% of the *Rhizobium* strains (*R.phaseoli* 4.5, *R. phaseoli* 1.15, *R.ciceri* N22, *R.ciceri* N28, *R.ciceri* N7, *R. phaseoli* 5.1a, *R.leguminosarum* 6.2c, *R.trifolii* 2.25a, *Rhizobium* sp. 3.5) tested in this study were siderophore positive.

The extrapolation of microbial interactions on agar, in relation to soil or rhizosphere situation is difficult and conclusion should be made cautiously. Apart from the obvious effect of environment, variations in interaction produced by changing the agar test procedure, complicates the field interpretations. The amount and variety of nutrients available to the interacting organisms in the soil are very different to those of culture media. *Rhizobium* strains might protect the plant with which they make symbiotic contact against the *Fusarium* species. The antagonistic microorganisms used as agents of biological control must have a neutral or synergic relationship with the beneficial bacteria as *Rhizobium* that based the plant root. *Rhizobium* sp. 3.5, *R. trifolii* 2.16, *R. trifolii* 2.3a and 2.25a didn't affect the growth of *T. harzianum* T1. *R. phaseoli* 1.17, *R. phaseoli* 1.114, *R. ciceri* N7 and *R. ciceri* N28 strains didn't affect the growth of *T. harzianum* T15 and *R. ciceri* N7 strain of *Rhizobium* didn't affect the growth of *T. harzianum* T3. Mixture of *Rhizobium* and *Trichoderma* can be used against *Fusarium* spp.

CONCLUSIONS

20 *Rhizobium* strains screened were produced IAA, 15 of those positively solubilized phosphate and nine of them possibly produced siderophores. In future work, it will be easier to determine the appropriate *Rhizobium* strains to be proposed as a potential biocontrol agents. Inoculation with mixture of *T. harzianum* and *Rhizobium* strains selected for biocontrol can be used against *Fusarium* spp. in a rhizosphere environment.

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