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Expression of *Serratia* and *Pseudomonas* Chitinase Genes in *Rhizobium* Via Horizontal Gene Transfer

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ABSTRACT

This study aimed to transfer chitinase genes into *Rhizobium leguminosarum* bv. *vicia* in order to enhance the defense of faba bean plants against soil pathogens, in addition to , improving symbiotic nitrogen fixation, as well. Toward this target, 12 transconjugants resulted from conjugation between *Pseudomonas flurocescens* and *Serratia marcescence* as a donors against four strains of *Rhizobium leguminosarum*. The donar strains were tested for chitinolytic activity depending on chitin hydrolysis zone appeared on chitin agar medium. Six out of eight matings between *Pseudomonas* and *Serratia* against *Rhizobium* were successeded. Some recombinants expressed significant amount of IAA production in both complete and minimal media. Some of *Rhizobium* transcojugants showed significant performance for chitinase activity above the mid-parent. Cell culture and cell – free filterate of some *Rhizobium* transconjugants showed higher antagonistic activity against *Rhizoctonia solani* in relation to the mid-parent, because they were able to produce higher amounts of antifungal metabolites. These transcojugants may inhibit the growth of various soil-born pathogens with a higher efficiency than their parents.

Keywords : Gene transfer, chitinase gene, chitin hydrolysis, *Rhizobium* transconjugants.

INTRODUCTION

Chitin, is a polymer of N-acetyl glucosamine, it was an important structural component of insects, fungi, and nematodes. Application of chitinase reduced plant diseases caused by certain soil fungi and nematodes (Shapira *et al.* 1989). This beneficial effect of chitinase has been attributed to secrete chitin-degrading enzymes (Mercer *et al.* 1992). *Serratia marcescens*, a gram-negative bacterium, is very efficient in the degradation of chitin because of its ability to produce different chitinolytic enzymes. Two chitinase genes (*chiA* and *chiB*) have been isolated from *S. marcescens* (Brurberg *et al.* 1995). Wherease, some of *Pseudomonads fluorescens* have currently received world-wide attention due to the production of a wide range of antifungal compounds viz., fluorescent pigments, side rophores, volatile compounds such as hydrocyanic acid (HCN), antibiotics and lytic enzymes.

Lytic enzymes (chitinase, β-1,3-glucanase and protease) are responsible for the lysis and hyper parasitism of antagonists against deleterious fungal pathogens (Ramyasmruthi *et al.* 2012). In addition, the acquisition of DNA by horizontal gene transfer is one of the evolutionary strategies that contribute to the formation of genetics variants in the environments (Cruz and Davies 2000). Horizontal gene transfer plays an important role in many biological aspects including the emergence and spread of virulence (Dorbindt and Hacker 2001), symbiosis (Sullivan and Ronson 1998), the degradation of xenobiotic compounds (Tsuda *et al*. 1999) and resistance to antibiotics.

Rhizospheric bacteria may influence plant growth by secreted phytohormones, such as auxins. Production of the auxin indole acetic acid (IAA) is wide spread among plant-associated bacteria. Beneficial bacteria synthesize IAA predominantly by an alternate tryptophan-dependant pathway, through indole pyruvic acid (Patten and Glick 1996). Many of recent reports indicated that IAA was a signaling molecule in bacteria and therefore have a direct effect on bacterial physiology (Spaepen *et al*. 2008). Some important key concerns for *Rhizobium* adaptability to various soil conditions as inoculants which as follows ; production of indole acetic acid (IAA), resistance to antibiotcs and tolerance to variable pH . Production of IAA increased with the age of the culture (Shweta *et al.* 2018) .

This study aimed to induce *Rhizobium* transconjugants harboring chitinase genes from other bacterial sources to enhance the defense of faba bean plants against soil borne pathogens in addition to improving symbiotic nitrogen fixation , as well.

MATERIALS AND METHODS

Materials

Genetic materials : The genetic materials used in this study is a bacterial strains listed in Table 1 which including their sources, as well as, their designation.

Media and growth conditions

Yeast extract mannitol medium (YEM) :*Rhizobium* strains were grown at 28 C^o in yeast extract mannitol medium (YEM) according to Vincent (1970). It was supplemented with 0.1 mg ml^{-1} L-tryptophan for IAA assay. Strains were maintained at 4 C° on slants of this medium.

Yeast extract-mannitol-congo red agar (YMCRA): This medium was used to ensure *Rhizobium* strains according to Pattison and Skinner (1974).

Minimal medium: This medium was consists per liter; mannitol 5g, KH_2PO_4, 1g ; K_2HPO_4,1g; $MgSO.7H_2O$, 0.18g; NH_4CL, 0.5g; Na glutamate, 0.02 g; Ferric ammonium citrate, 0.004g ; $FeCl_3$, 0.004g ; Biotin, 0.3µg and $CaSO_4$, 0.13g. finaly, pH was adjusted to 6.8. This medium was used for IAA assay according to Balassa (1963).

King's medium: It was used for the maintenance of *Pseudomonas* strain according to Merck (1994).

Colloidal chitin media: It was used for screening chitinase producing strains on colloidal chitin agar medium according to Elsayed and Edrees (2014).

Table 1. Bacterial strains used in this investigation

Strains	Sources or References	Designation
Rhizobium leguminosarum (3841)	Kindly provided by Prof . J P W Young, Department of Biology, University of Yourk, UK.	RL-3841
Rhizobium leguminosarum (ARC 207)	Agric. Res. Center, Dept. of Microbiology, Giza, Egypt.	RL-207
Rhizobium leguminosarum (USDA2074)	Kindly provided by Dr. Peter van Berkum, Microbiologyist,National Rhizobium culture collection, USDA, Baltimore Avenue Beltsville.	RL-2074
Rhizobium leguminosarum (12612)	IAM culture collection, Univ. of Tokyo, Japan.	RL- 12612
Pseudomonas fluorescences(NRRL B-23932)	National Center for Agriculture Utilization Research, USA.	PF-23932
Serratia marcescens	Microbiology Dept., Soil, Water and Environmental Research Institute, Agricultural Research Center (ARC) Giza, Egypt.	*Sm*
Rhizoctonia solani	Plant Pathology Institute, Agricultural Research Center, (ARC) Giza, Egypt.	*R. solani*

Intrinsic antibiotic resistance profiles: Resistance to antibiotics was tested on YEM agar supplemented with the antibiotics tested as shown in Table 2.

Potato Dextrose Agar (PDA) : This medium was used to grow *Rhizoctonia solani* strain according to Rieuf (1985) , as well as, in antagonism test .

Cultural filtrates of rhizobia : Rhizobial cultural filtrates were prepared as described by El-Batanony *et al*. (2007).

Table 2. Antibiotics and their concentrations used for genetic marking *Rhizobium* strains.

Antibiotics	Abbreviation	Concentratio(µg/ml)
Chloramphenicol	*Cm*	35
Ampicillin	*Ap*	50
Tetracycline	*Tc*	20
Penicillin	*Pc*	150
Neomycin sulphate	*Nm*	800
Erythromycin ethlsuccinate	*Eryth*	20
Rifampicin	*Rif*	150
Vancomycin	*Vc*	150
Streptomycin	*Str*	75
Amoxycillin	*Am*	400
Clindamycin	*CL*	50
Genamycin	*Gm*	20

Methodology

Testing the antagonism

The antagonism of bacterial strains and their transconjugants against *Rhizoctonia solani* was performed using well diffusion method. Culture filtrate was dropped in a prepared holes of the solid *Rhizoctonia* medium deep inoculated with the test microorganism. The antibacterial activity was measured from the zone appeared around the holes according to Nedialkova and Naidenova (2005).

Antibiotic susceptibility testing

Antibiotic susceptibility test was measured by plate diffusion method according to Collins and Lyne (1985) using strains grown to logarithmic growth phase in nutrient broth. Plates were incubated overnight at 28°C and the diameter of resulting zones of inhibition was measured according to Toda *et al*. (1989).

Bacterial mating

Matings were performed between *Pseudomonas* and *Serratia* as a donors against *Rhizobium* as recipients (Selvarathnan and Gealt 1993). Representative media were supplemented with appropriate antibiotics for each cross and the transconjugants appeared on selective medium were picked up for testing to chitinase production. Conjugation was performed using overnight culture grown at log-phase. Donors and recipients were mixed in a 1:2 ratio and incubated for the appropriate time. Samples of 0.1 ml from the serial dilutions of the mixture mating were plated on suitable selective media according to Lederberg and Lederberg (1952).

detection with the Salkowski colorimetric technique

Rhizobium , *Pseudomonas* strains and their transconjugants were grown overnight in YEM medium and Kings-B broth medium, respectively at 28°C. Production of IAA in the supernatant was assayed as described by Pilet and Chollet (1970). This method was shown to be the most sensitive and most specific as earlier reported by Glickmann and Dessaux(1995) .

Screening of chitinolytic activity

For enrichment of chitinase-producing bacteria, a mineral medium containing colloidal chitin as a sole carbon source was used. Chitinolytic activity was measured by observing the size of the halo zone formed around the colonies after seven days of incubation. When colloidal chitin media supplemented with bromocresol purple, the colored zone formation was observed (Someya *et al*. 2011).

Cultural filtrates of rhizobia

Rhizobium strains and their recombinants were grown in yeast extract manitol- broth medium using shaking incubator (200 rpm) for five days at 28-30^{0}C. Cells were harvested by centrifugation at 6000 rpm for 20 min and the supernatants were filter sterilized through 0.45µm bacterial filter. Three replicates were prepared for each rhizobial strain (El-Batanony *et al*. 2007).

Statistical analysis

Data were subjected to the analysis of variance according to Snedecor and Cochran (1955). Least significant difference (L.S.D.) was used to compare between means if the F-test was significant.

RESULTS AND DISCUSSION

Intrinsic Antibiotic Resistance Profiles

Four *Rhizobium* strains, *Serratia marcescens* and *Pseudomonas* strains were genetically marked using 12 antibiotics.The results recorded in Table 3 showed that tetracycline (*Tc*) was more effective to inhibit the growth of all bacterial strains than the other antibiotics except for *Serratia marcescens*. On the other hand, the rifampcillin (*Rif*) inhibited the growth of all bacterial strains except for *Pseudomonas flurocescens*. The resistance to tetracycline was due to harboring *tet* genes on the bacterial DNA. The characterized *tet* genes encode three mechanisms of resistance including: efflux pump, ribosomal protection or enzymatic inactivation (Chopra and Roberts 2001).

Tetracycline resistance was analyzed before in the involvement of *tetA* and *tetE* genes in 16 isolates of the genus *Aeromonas* using polymerase chain reactions which appeared that 37.5% of the isolates were positive for *tetA* and 37.5% were *tetE* positive, however one isolate was positive for both genes (Balassiano *et al*. 2007). The resistance to tetracyclin was agreed with Zahran *et al*. (2012), who found that *Rhizobium* isolates was greatly inhibited by tetracycline. Rhizobia isolated from cowpeas varied in their resistance to streptomycin, rifampicin, kanamycin and penicillin (Sinclair and Eaglesham 1984). All bacterial strains tested were resistance to ampicillin and penicillin.The presence of 16 R-plasmids in *R. leguminosarum* was also found to increase bacterial resistance toward ampicillin (Sikka and Kumar, 1984). All strains tested in this study showed resistance to erythromycin and cloroamphenecole except for *Pseudomonas flurocescens*. Antibiotic resistance was encodedby several genes, many of them can transfer between bacterial strains (Jessica *et al*. 2015).

Khanaka *et al*. (1981) found that fast-growing species of *Rhizobium* tested was resistant to > 32 µg/ml penicillin and to < 1 µg/ml tetracyclin. Meanwhile, most of the slow-growing *Rhizobium* were susceptible to penicillin concentrations < 16 µg/ml, while they were resistant to tetracyclin concentrations > 1 µg/ml.

Horizontal gene transfer

Horizontal gene transfer is the direct transfer of genetic material from one organism to another. In order to construct recombinant bacterial strains *Pseudomonas* and *Serratia marcescens* was used as a donors against *Rhizobium* strains (Table 4). The donor strains were selected on the basis of chitin –degradation.

All matings between, *Pseudomonas* and *Serratia marcescens* against *Rhizobium* were successed , except for , the mating between *P. fluorscence* against RL-3841 and *P. fluorscence* X RL-207, which failed to transfer genetic material from the donor to the recipients. This may be due to the differences between the donor and the recipient in nucleotide sequences, gene expression, codon usages, post translational modifications and protein interactions (Nielsen and Townsend 2001). In addition, the conjugated strains may be from the same Gram staining species. Different times were needed for different matings to appeared transconjugants on selective medium. Horizontal gene transfer via conjugation process may occur either as inter or intra species.

Table 3. Genotypes of different bacterial strains basesd on antibiotics sensitive or resistance markers.

Antibiotics	Strains					
	RL-3841	***RL-207***	***RL-2074***	***RL-12612***	***PF***	***Sm***
Cm	+	-	+	+	-	+
Ap	+	+	+	+	+	+
Tc	-	-	-	-	-	+
Pc	+	+	+	+	+	+
Nm	+	+	+	+	-	-
Eryth	+	+	+	+	-	+
Rif	-	-	-	-	+	-
Vc	+	+	+	+	-	-
Am	-	+	+	+	+	-
Stre	+	+	+	+	+	-
CL	+	+	+	+	-	+
Gm	+	+	+	+	-	-

+, – = Resistance and sensitive to antibiotics, respectively.

Table 4. Conjugal transfer between the donor strains *Serratia marcescens* and *Pseudomonas fluorescences* against *Rhizobium*.

Mating	Parental geneotypes	Mating time†	Time of mating††	Recombinant genotype
PF X *RL-3841*	*Cm-Rif+ X Cm+Rif-*	3	ND	No appeared
Sm X *RL-3841*	*Tc+ Stre- X Tc-Stre+*	3	3	*Tc+Stre+*
*PF*X *RL-207*	*Nm^- Rif^+ X Nm^+ Rif-*	4	ND	No appeared
Sm X *RL-207*	*Nm^- Cm^+ X Nm^+ Cm^-*	3	3	*Nm^+ Cm^+*
PF X *RL-2074*	*Cm- Rif+ X Cm+ Rif^-*	3	6	*Cm+ Rif+*
Sm X *RL-2074*	*Tc^+ Nm^- X Tc^- Nm^+*	3	4	*Tc^+ Nm^+*
PF X *RL-12612*	*Cm- Rif+ X Cm+, Rif^-*	3	3	*Cm^+Rif+*
Sm X *RL-12612*	*Tc+Nm^- X Tc-Nm+*	6	4	*Tc+ Nm^+*

† Time needed for genetic transfer (day).
†† Time needed to appeared transconjugants on selective medium (day).
ND = Not detected.

Chitinase and indole acetic acid production

All transconjugants resulted from the mating between *Sm* and *RL*-207 appeared insignificant differences in IAA production (Table 5). On the other hand , some of *Rhizobium* transconjugants (Tr_2 and Tr_4) appeared significant performance of chitinolytic activity above the mid-parent. This results are in agreement with Bahar *et al*. (2012) , who found that bacteria have been produced chitinase to degrade chitin polymer and produced metabolites that supported their growth on chitin as the only carbon and energy source without any nutrients. In addition, Monreal and Reese (1969) found that *Serratia marcescens* was the most active bacteria for chitinase production, which is extra cellular and composed of an endo chitinase, a chitobiase and a factor (CH1) required for the hydrolysis of "crystalline" chitin . Parani *et al*. (2011) found that maximum chitinase production of *S. marcescens* could observed at 96 h of incubation with pH 5.5 at 30°C under shaking conditions (120 rpm). Brurberg *et al*. (1995) reported that *Serratia marcescens* was efficient in degradation of chitin because it was produced different chitinolytic enzymes. The same trend was also shown by El- Adl *et al*. (2016), who found that *Serratia marcescens* appeared complete hydrolysis of colloidal chitin in a short time.

Table 5. Chitinase and IAA secretion by *Rhizobium* transconjugants resulted from the mating between *Sm* and *RL*-207 .

Strains	Chitinase production		IAA (µg/ml) (CM)	IAA (µg/ml) (MM)
	Diameter (cm) of clear zones	Diameter of the purple colored zone(cm)		
RL-207	1.0	1.3	3.9	1.2
Sm	1.6	1.7	1.2	0.7
Mid parent	1.3	1.5	2.55	0.95
Tr_1	1.3	1.4	4.2	0.3
Tr_2	1.8	2.9	1.9	1.5
Tr_3	0.0	0.0	4.0	0.7
Tr_4	2.3	2.3	7.8	0.8
Tr_5	0.0	0.0	4.5	1.5
Tr_6	0.0	0.0	6.1	1.1
Tr_7	0.0	0.0	6.1	1.0
Tr_8	1.4	1.5	7.7	0.9
Tr_9	0.0	0.0	3.3	1.4
Tr_{10}	1.2	1.0	6.8	1.6
F-test	**	**	NS	NS
LSD 0.05	0.21	0.39		
0.01	0.29	0.55		

CM,MM = Complete medium and minimal medium , respectively.

****,NS : Means significance at 0.01 probability level and insignificant differences, respectively.**

The data summarized in Table 6 appeared that transconjugant (Tr_{26}) showed significant clear hydrolysis zone on colloidal chitin agar medium above the mid-parent. On the other hand, some of transconjugant isolates (Tr_{27} and Tr_{30}) expressed significant IAA production in complete medium in relation to the mid-parent. These results are in harmony with those obtained by Jeuniaux (1966), who found that chitinase is a glucanohydrolase that degrades chitin polymer of *N*-acetylglucosamin, a major cell-wall constituent of many fungi into short dimers. Sitrit *et al*. (1993) indicated that *R. meliloti* colonies harboring the chitinase gene were identified by a clear halo zone of degraded chitin. Okay (2008) showed that chitinase secreted by *S. marcescens* was much higher than the other species of *Serratia* genus. While , Mazen *et al*. (2008) reported that IAA, exo polysaccharides and chitinase enzyme were secreted in all the tested rhizobia with different degrees. The results obtained here were agreed with Ghosh and Basu (2002), who found that *Rhizobium* isolated from the root nodules of *Dalber gia Lanceolaria* secreted high values of IAA at 2.5 mg / ml of L-tryptophan concentration. Wherease, Theunis *et al*. (2004) showed that bacterial indole acetic acid (IAA) has proposed to play a major role in the *Rhizobium* legume symbiosis. Morever, Ernstsen *et al*. (1987) found that rhizobia are known to produce a huge amounts levels of IAA both in free living conditions and also symbiotically in root nodules.

Table 6. Chitinase and IAA production by *Rhizobium* transconjugants resulted from the mating between *Sm* and *RL*-12612.

Strains	Chitinase production		IAA (µg/ml) (CM)	IAA (µg/ml) (MM)
	Diameter (cm) of clear zones	Diameter(cm) of the purple colored zone		
RL-12612	1.5	1.7	5.6	1.7
Sm	1.6	1.7	1.2	0.7
Mid parent	1.55	1.7	3.4	1.2
Tr_{21}	0.0	0.0	1.4	0.5
Tr_{22}	0.0	0.0	1.4	0.5
Tr_{23}	0.0	0.0	4.6	0.5
Tr_{24}	0.0	0.0	2.9	0.5
Tr_{25}	0.0	0.0	6.0	0.4
Tr_{26}	2.2	2.6	3.3	0.5
Tr_{27}	0.0	0.0	7.6	0.5
Tr_{28}	0.0	0.0	3.0	1.0
Tr_{29}	1.4	1.7	4.8	0.4
Tr_{30}	0.0	0.0	7.0	0.2
F-test	**	**	*	*
LSD 0.05	0.16	0.31	3.2	0.53
0.01	0.23	0.44	4.5	0.74

***,** : Means significance at 0.05 and 0.01 levels of probability, respectively.**

Some transconjugants appeared significant clear hydrolysis zone on colloidal chitin agar medium in relation to the mid-parent (Table 7). These results agreed with that obtained by Sridevi and Mallaiah (2008) , who found that *Rhizobium sp*. appeared maximum chitinolytic activity at 36 h of incubation at neutral pH. Sitrit *et al*. (1993) reported that *Rhizobium meliloti* harboring *Serratia marcescens* chitinase gene showed gene expressed efficiently that degraded hyphal tips of *Rhizoctonia solani*. However, Krishnan *et al*. (1999) showed that mobilized the chitinase gene constructed into *S. meliloti* and their transconjugants can produce chitinase. Whereas , all *Rhizobium* transconjugants produced insignificant amounts of IAA in minimal medium in relation to the paternal strains. This result agreed with that obtained by Sahasrabudhe (2011), who demonstrated that rhizobia isolates appeared red colour reaction with salkowaski reagent indicated their ability to secrete IAA.

Table 7. Chitinase and IAA production by *Rhizobium* transconjugants resulted from the mating between *Sm* and *RL*-3841.

Strains	Chitinase production		IAA (µg/ml) (CM)	IAA (µg/ml) (MM)
	Diameter (cm) of clear zones	Diameter (cm) of the purple colored zone		
RL-3841	1.6	3.1	7.8	1.7
Sm	1.6	1.7	1.2	0.7
Mid-parent	1.6	2.4	4.5	1.2
Tr_{31}	1.4	1.5	1.9	0.5
Tr_{32}	1.2	1.9	1.0	0.4
Tr_{33}	1.9	1.9	3.9	0.3
Tr_{34}	0.0	0.0	5.9	0.5
Tr_{35}	1.5	1.8	5.1	0.5
Tr_{36}	2.1	2.8	5.1	1.4
Tr_{37}	2.0	3.7	6.8	0.4
Tr_{38}	0.0	0.0	6.0	0.6
Tr_{39}	1.3	1.5	3.5	0.5
Tr_{40}	1.3	1.5	7.2	0.5
F-test	**	**	NS	**
LSD 0.05	0.18	0.8		0.51
0.01	0.25	1.1		0.72

CM,MM = Complete and minimal medium , respectively.
****, NS : Means significance at 0.01 probability level and insignificant differences, respectively.**

The data summarized in Table 8 appeared that some transconjugants resulted from the mating between *Pseudomonas* and *Rhizobium* produced significant amounts of chitinase in relation to the mid parent. These results agreed with Nanda kumar *et al*. (2007), who found that *Pseudomonas fluorescens* induced a visible zone around the paper disc on chitin containing medium, indicating that *Pseudomonas* strains could degrade and utilize chitin polymer for their growth. *Rhizobium* transconjugants showed insignificant levels of IAA produced in complete and minimal media. This agreed with Wahyudi *et al.* (2011) , who found that *Pseudomonas spp*. was able to produce IAA in various levels , as well as, appeared chitinolytic activity in chitin agar medium.

Table 8 . Chitinase and IAA production by *Rhizobium* transconjugants resulted from the mating between *PF* and *RL*-2074.

Strains	Chitinase production		IAA (µg/ml) (CM)	IAA (µg/ml) (MM)
	Diameter (cm) of clear zones	Diameter (cm) of the purple colored zone		
RL-2074	0.0	0.0	5.9	0.9
PF	1.5	1.7	5.2	0.9
Mid parent	0.75	0.85	5.55	0.9
Tr_{41}	1.1	3.0	4.9	0.6
Tr_{42}	1.7	3.1	4.5	1.0
Tr_{43}	0.0	0.0	2.2	0.4
Tr_{44}	0.0	0.0	5.8	0.4
Tr_{45}	0.0	0.0	2.9	0.4
Tr_{46}	0.0	0.0	5.0	0.6
Tr_{47}	0.0	0.0	1.7	0.6
Tr_{48}	0.0	0.0	8.7	0.6
Tr_{49}	0.0	0.0	3.7	0.4
Tr_{50}	0.0	0.0	5.6	0.2
F-test	**	**	NS	**
LSD 0.05	0.18	0.27		0.26
0.01	0.26	0.38		0.37

CM, MM = Complete and minimal medium , respectively.
****, NS : Means significance at 0.01 probability level and insignificant differences, respectively.**

The data presented in Table 9 appeared the antagonistic activity of *Rhizobium* transconjugants resulted from the mating between *Sm* and *RL-3841* on the radial growth of *Rhizoctonia solani* . The results showed that the cell culture of some transconjugants (Tr_{31} ,Tr_{33} , Tr_{35} ,Tr_{36} and Tr_{37}) appeared significant effect on the radial growth of *Rhizoctonia solani*. The results obtained herein agreed with Nautiyal (1997), who found that *Rhizobium sp*. suppress the growth of *F. oxysprum*f. sp. *ciceri, Rhizoctonia bataticola* and *pythium sp.* However, Kibria and Hossain (2000) showed that rhizobia inhibited significantly the growth of pathogenic fungi such as *Macrophomina phaseolina, Rhizoctonia spp, Fusarium sp*. and *Pythium spp* with both leguminous and non-leguminous plants. Krishnan *et al.* (1999) found that protein extracts from nodules initiated by chitinase-producing *Rhizobium* spp. were efficient to degrade the hyphal tips of *R. solani*. Safinaz and Al-Saman (2014) found that *Rhizobium leguminosarum* showed the least antagonistic effect against *R. solani*, where 65% of the seeds emerged and 69.2% of them were survived.

The data presented in Table 10 appeared the antagonistic activity of *Rhizobium* transconjugants resulted from the mating between *PF* and *RL- 2*074 on the radial growth of *Rhizoctonia solani*. The results obtained herein appeared that cell culture and cell-free filterate of most transconjugants (Tr_{41} ,Tr_{42},Tr_{43},Tr_{44},Tr_{45},Tr_{46} ,Tr_{48} and Tr_{50}) showed significant antagonism against *Rhizoctonia solani*. This agreed with Munazza and Fauzia (2012) , who demonstrated that *Pseudomonas fluorescens* inhibit the fungal growth compared to the control.

Siddiqui (2006) reported that many of bacterial strains such as *Bacillus, Pseudomonas* and recently *Rhizobium* were shown to effectively control different soil-borne plant pathogenic fungi under both green house and field conditions. However, Samavat *et al*. (2011) found that combined treatments of common bean seeds with rhizobia and *P. fluorescens* cultural filtrates reduced root rot and damping-off severity. Mery *et al*. (2013) found the greatest inhibitory activity of the supernatant of *P. fluorescens* . Charitha *et al.* (2003) showed that *Pseudomonas fluorescens* is antagonistic to various soil borne pathogens. Sindhu and Dadarwal (2001) demonstrated that *Pseudomonas* strains suppress the growth of *Rhizoctonia solani*. This suppress was clearly visible by very limited growth or the complete absence of fungal mycelium in the inhibition zone surrounding a bacterial colony. The bacterial cell culture, as well as, cell free culture filtrate used in this study appeared a strong antifungal activity against *Rhizoctonia solani*.

Table 9. Antagonistic activity of *Rhizobium* transconjugants resulted from the mating between *Sm* and *RL-3841* against the radial growth of *Rhizoctonia solani*.

Strains	Diameter of inhibition zone (cm)	
	Cell – culture	Cell-free filtrate
RL-3841	0.0	0.0
Sm	1.3	1.0
Mid- parent	0.65	0.5
Tr_{31}	2.0	0.9
Tr_{32}	0.0	0.0
Tr_{33}	1.6	1.2
Tr_{34}	0.8	0.8
Tr_{35}	1.5	0.9
Tr_{36}	2.2	1.2
Tr_{37}	1.9	1.5
Tr_{38}	0.0	0.0
Tr_{39}	0.0	0.0
Tr_{40}	0.0	0.0
F-test	**	**
LSD 0.05	0.35	0.58
0.01	0.49	0.81

** **: Significance at 0.01 level of probability.**

Table 10. Antagonistic activity of *Rhizobium* transconjugants resulted from the mating between *PF* and *RL-2074* against the radial growth of *Rhizoctonia solani*.

Strains	Diameter of inhibition zone (cm)	
	Cell- culture	Cell-free culture filtrate
RL-2074	0.8	0.7
PF	0.9	0.7
Mid- parent	0.85	0.7
Tr_{41}	2.3	1.9
Tr_{42}	2.3	1.3
Tr_{43}	1.5	1.5
Tr_{44}	2.2	1.5
Tr_{45}	1.8	1.5
Tr_{46}	1.8	1.5
Tr_{47}	0.9	0.9
Tr_{48}	1.6	1.6
Tr_{49}	0.8	0.8
Tr_{50}	1.4	1.4
F-test	**	*
LSD 0.05	0.47	0.56
0.01	0.66	0.79

,* **: significance at 0.05 and 0.01 levels of probability, respectively.

In conclusion**,** recombinant isolates of rhizobia harboring chitinase genes played a significant role in controlled plant pathogenic fungi, leading them to be used as inhibitor agents against the soil borne pathogenic fungi. Cell culture and cell-free filtrate of some transconjugants could be used as inhibitory agents against *Rhizoctonia solani*.

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التعبير الجينى لإنزيم الشيتينيز فى بكتيريا الرايزوبيم المنقول من السيراتيا والسيدوموناس بواسطة النقل الأفقى للجينات

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تهدف هذه الدراسة إلى نقل جينات الشيتينيزإلى سلالات رايزوبيم الفول البلدى لتحسين مقاومة هذا النبات ضد المسببات المرضية الفطرية الموجودة فى التربة ولتحسين عملية تثبيت النيتروجين. لتحقيق هذا الهدف تم تقييم السلالات الأبوية و 12 من الإتحادات الوراثية الجديدة الناتجة عن تزاوجات مختلفة أجريت بين سلالتى السراتيا والسيدوموناس كسلالات معطية مع سلالات من الرايزوبيم لإنتاج إنزيم الشيتينيزمما أدى إلى إحداث تحلل كامل للشيتين الموجود فى بيئة الآجار. أوضحت النتائج نجاح ست من التزاوجات الثمانية التى تم إجراؤها بين سلالات السراتيا والسيدوموناس مع سلالات الريزوبيم . أظهرت النتائج أيضا أن بعض الإتحادات الوراثية الجديدة أعطت زيادة معنوية فى إنتاج حامض الإندول اسيتيك على كلا من البيئة الكاملة والبيئة الحدية. كما أظهر كلا من المعلق الخلوى والراشح لبعض الإتحادات الوراثية الجديدة للرايزوبيم نشاط تضاد عالى ضد فطر الريزوكتونيا سولانى مقارنة بمتوسط الآباء وذلك يعكس قدرة الإتحادات الوراثية الجديدة على إنتاج كميات مرتفعة من المضادات الفطرية ، هذه الإتحادات الوراثية الجديدة ربما تمنع نمو العديد من المسببات المرضية الموجوده فى التربة نظراً لكفائتها المرتفعة فى إنتاج إنزيم الشيتينيزمقارنة بالسلالات الأبوية.