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Screening of effective antagonists from potato rhizosphere against bacterial wilt pathogen

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Abstract

Potato is the most consumed vegetable while stands 4th among food crops after wheat, rice, and maize in terms of production. Bacterial wilt caused by *Ralstonia solanacearum* is one of the most devastating diseases throughout the world causing substantial losses in potato and in other important crops. This study was embarked to search for biocontrol of pathogen, keeping in view recent reports of disease incidence in major potato growing areas of Pakistan. In this study, several potato growing areas of Punjab (Pakistan) were visited to study the bacterial community residing in potato rhizosphere and also screen bacterial antagonists against the virulent strains (Rs9, Rs17 and R43) of *R. solanacearum*. The results revealed that out of 221 rhizobacterial isolates, *Bacillus* spp. (101) were dominantly isolated from potato rhizosphere, followed by *Pseudomonas* spp. (76) and *Serratia* spp. (44). All these isolates were tested for antagonism using dual culture plate method which showed 11 isolates to be highly antagonistic against either isolate of *R. solanacearum*. Against Rs9, Rs17, Rs43 and GMI1000 highest zone of inhibition was recorded with B28 (12.3 mm), P11 (10.2 mm), B9 (9.9 mm) and B85 (11.7 mm) respectively. Culture filtrate (CF) of antagonists was also checked against live *R. solanacearum* cells which also showed inhibition in liquid medium. Antagonistic isolates were tested for plant growth promoting (PGP) traits i.e. indole acetic acid (IAA), siderophore production, P-solubilization, root colonization, and chitinase production. The isolates B28 and B85 were potential isolates possessing antagonistic activity along with several PGP traits.

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Introduction

Bacterial wilt (BW) was firstly observed in potato, tomato and eggplant (Smith, 1896). Since then, this disease is the most important bacterial disease and the pathogen (*R. solanacearum*) has been retrieved from over 200 plant species belonging to 53 botanical families with worldwide geographical distribution and yet new reports infecting the new hosts are not uncommon (Elphinstone, 2005). The presence of disease in six continents out of seven is not an unusual fact because of its host range and diversity among the isolates (Fegan and Prior, 2005). Huge economic losses are associated to this pathogen around the world (Elphinstone, 2005). Pathogenicity of *R. solanacearum* is dependent upon its densities and after reaching to certain level in plant it activates pathogenicity genes which in low densities would not be activated (Schell, 2000). Complications in controlling this pathogen are being faced because of the complex pathogenicity mechanism possessed by *R. solanacearum*, its ability to grow endophytically, perpetuation in soil, its deep layers, dispersion with water, and its association with weeds (Wang and Lin, 2005a).

In Pakistan, BW is well established and in recent surveys an average of 13.8% incidence was recorded all over the Pakistan in several vegetable crops, while in potato an average of 10.5 % incidence has been reported (Begum *et al.*, 2012). During detailed survey of major potato growing areas of Punjab (Pakistan), the maximum incidence has been reported upto 24.4% in Okara (Tahir *et al.*, 2014).

Keeping in view the economics of the losses caused by pathogen, several strategies have been adopted and investigated with focus on resistant cultivars (Fock *et al.*, 2000; Lin *et al.*, 2004), cultural measures (Adhikari and Basnyat, 1998; Katafiire *et al.*, 2005), chemical methods (Fortnum and Martin, 1998; Khanum *et al.*, 2005; Lin *et al.*, 2010), physical methods and biological control (Dong *et al.*, 1999; Guo *et al.*, 2004; Zhu and Yao, 2004; Götz *et al.*, 2006; Hu *et al.*, 2010) but to date no individual method has been found promising and if achieved, are not applicable to every region. With special reference to biological control, several studies have reported substantial control of disease but in field, several factors interfere with it which needs to be studied.

Plant growth promoting rhizobacteria (PGPR) are soil inhabiting beneficial microorganisms which are helpful for conducive plant environment. These are naturally present in rhizosphere in association to many plant species. PGPRs several functions and role in confering beneficial effects on plants have been described. The mechanisms of PGPRs have been categorized as direct and indirect with respect to plant-microbe interaction (Gupta *et al.*, 2000). Few functions and mechanisms that have been described in PGPR and can be active at one or other stage of plant life are; solubilization of mineral nutrients, suppression/inhibition of pathogens, enabling the plant to withstand drought, salinity or metal toxicity, synthesis of phytohormones, induced systemic resistance (ISR) (Gupta *et al.*, 2000). Some PGPRs have been used extensively as biological agents to control many soil-borne plant pathogens (Rajkumar *et al.*, 2005).

The present study focused at exploring the bacterial community residing in the potato rhizosphere and also screens bacterial antagonists against the virulent strains of *R. solanacearum*. Additionally the antagonistic isolates were also tested for possessing plant growth promoting traits for their usefulness.

Materials and methods

Collection of rhizospheric soil

Rhizospheric soil was collected by uprooting the plants and removing the excess soil adhering the roots, the soil in close proximity with roots was collected in polyethylene bag. The rhizosphere soil samples were collected from given areas (Table 1) and were processed for isolation of rhizobacteria.

Isolation of rhizobacteria

Isolation of Bacillus spp.

For isolating *Bacillus* spp., roots were cleaned off

adhering soil, small sections (1-2 cm length) of roots were placed in test tubes containing 5 ml of sterile distilled water (SDW), and test tubes were placed into a water bath at 80 °C for 20 min. After heating, a dilution series was plated onto Difco nutrient agar (NA) amended with 5% (w/v) sucrose and 200 ppm cycloheximide. Cultures were incubated at 28 °C for 48 h and individual colony types were selected. Individual isolates were dilution streaked to assure purity and strains were revived on NA when needed.

Isolation of Pseudomonas spp.

Isolation of *Pseudomonas* spp. was done on King's media B (KMB) (Peptone 20 g, K_2HPO_4 1.5 g, $MgSO_4.7H_2O$ 1.5 g, Glycerol 10 ml, Agar 15 g, water 1 l) amended with 200 ppm cycloheximide (King *et al.*, 1954). Using serial dilution, 0.1 ml aliquot from dilution 10^{-7} and 10^{-8} were placed on the KMB plates and incubated at 30 °C. The plates were observed for fluorescence under UV light.

Isolation of Serratia spp.

Caprylate thallous agar (CTA) medium was prepared (Atlas, 2005) for isolation of *Serratia* spp. from the collected samples. Serial dilution of samples was done and 0.1 ml aliquot from dilutions 10^{-7} and 10^{-8} was placed on the CTA plates and incubated at 30 °C for 5-7 days. The appearing colonies based on color, shape, and size were purified using dilution streaking method.

Screening of antagonists

For selection of efficient antagonistic rhizobacteria among the isolates of *Bacillus* spp., *Pseudomonas* spp. and *Serratia* spp., dual culture plate technique was used to initially identify the potential antagonists against highly virulent strains (Rs9, Rs17 and Rs43) of *R. solanacearum* (Tahir *et al.*, 2014).

Dual culture plate technique

The rhizobacterial and pathogenic isolates were revived on NA media 2 days before the test. Briefly, 0.1 % of suspension (OD_{600} = 0.1) containing 5 × 10^7 cfu/ml of each *R. solanacearum* isolate was added to semi-cooled NA and poured into the Petri plates. Bacterial suspension (OD_{600} = 0.1~ 5 × 10^7 cfu/ml) of each test rhizobacterial isolate was prepared in autoclaved de-ionized water and 2 µl of suspension was dropped on each sterile paper disc (4 mm) placed equidistantly on NA containing *R. solanacearum*. SDW treated plate served as a control. The plates were incubated for 3-4 days at 28 °C. Radius of zone of inhibition around the paper disc was observed and measured in millimeters (Sinclair and Dhingra, 1995).

Culture filtrate vs R. solanacearum

From the screened antagonists, the effect of culture filtrate (CF) of each antagonist against *R. solanacearum* growth was studied as described by Almoneafy *et al.* (2014). All effective antagonistic isolates were cultured in LB broth at 200 rpm for 48 h in incubated shaker at 30 °C. CF was obtained by centrifuging liquid culture at 14000 rpm at 4 °C for 15 min and sterilized twice through 0.22 µm membrane filter. To confirm sterilization of CF, 100 µl of CF was spread on NA plates and incubated. Subsequently, 500 µl *R. solanacearum* culture (10^7 cfu/ml) was added to a sterile 10 ml tube containing LB (3 ml) + CF (3 ml). The tubes were incubated on a rotary shaker at 200 rpm at 28 °C. A tube containing LB + *R. solanacearum* was used as a control. Growth of *R. solanacearum* was checked at 24 and 48 h by measuring OD_{600}.

Compatibility among antagonists

The obtained antagonists were checked for compatibility with each other using dual culture plate technique. Each antagonist was co-cultured with all other antagonists on NA and incubated for 3-4 days at 28 °C. Presence of inhibition zone was considered incompatible pair.

Plant growth promoting traits of antagonists

IAA Production

LB media was prepared and L-tryptophan (40 µg/ml) was added using 0.22 µm membrane filter. Each antagonistic isolate was cultured in LB media at 30 °C and 200 rpm for 48 h in shaking incubator. After 48 h, the cultures were spin at 12000 rpm for 10 min and 1 ml of CF was added to 1 ml Salkowski's reagent

($FeCl_3.6H_2O$ 1.5 ml of 0.5 M solution, in 80 ml of 60 % H_2SO_4). The mixture was allowed to stand at room temperature for 30 min and appearance of pink color was observed. The concentration of IAA synthesized by each isolate was determined spectrophotometrically at 550 nm and comparing with IAA standard curve (Gordon and Weber, 1951).

Siderophore production

Qualitative detection of siderophores by antagonistic isolates was determined on CAS blue agar medium. The medium was prepared (Louden *et al.*, 2011) and loop full bacteria was inoculated on the medium and the plates were incubated at 30 °C for 7-10 days. Appearance of orange halo zone was observed as an indicator of siderophore production. The isolates were categorized as strong (≥ 5 mm represented as '+++'), week (2-5 mm represented as '++'), slight (1-2 mm represented as '+') producers and non-siderophore producers (represented as '-').

Phosphorus solubilization

Phosphorus solubilization was studied using the National Botanical Research Institute Phosphate (NBRIP) growth medium (Nautiyal, 1999). A loop full of test bacteria was inoculated on the solidified media surface and kept in an incubator at 30 °C for 4-7 days. Appearance of halo zone around the colony was considered P solubilizer.

Root colonization

Root colonization of plants by the rhizobacteria is prerequisite for rhizocompetence and one of the most important tests to allow the rhizobacteria confer beneficial effects on plants through intact relationship with the plants. The test was carried out by culturing potato (cv Désirée) on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962). Young nodal sections from potato plant were cut and dipped in tap water for initial washing to get rid of any dust/soil followed by washing with 70 % ethanol (EtOH) for 2 min. The nodal sections were rinsed with SDW to wash out ethanol and then surface sterilized with 10 % bleach (chlorox) and subsequently washed with SDW successively for 6, 4, 3 and 1 min. Sterilized fragments were dipped in bacterial suspension (2.8×10^8 cfu/ml) for 1 minute and dried on sterilized blotting paper and placed on the media in covered test tubes. Test tubes were placed under the light and root colonization of bacteria was visually observed. The *in vitro* plants with roots were removed from the media and cut into small pieces for preparing serial dilutions. Colony forming units (CFU) were recorded from the dilution 10^{-7} on NA media.

Chitinase production

Cultures of antagonistic isolates (48 h old) were inoculated on the chitin minimal media (Dunne *et al.*, 1997) with Bromophenol Blue (BPB) as indicator and the plates were kept for incubation at 28 °C. Appearance of halo zone around the bacterial colony after 5-7 days was indicator of chitinase production.

Statistical analysis

All the experiments were performed with at least three replications and collected data was analyzed by statistical software STATISTIX (v 8.1). The difference in each treatment was analyzed by least significant difference (LSD) at P≥0.05 (Steel *et al.*, 1997).

Results and discussion

Isolation of rhizobacteria

From the dilutions plated on respective media, several isolates were obtained which were differentiated on the basis of their color, colony structure, elevation and the colonies appearing different were streaked separately to get purified isolates. The isolation of rhizobacteria resulted in total 221 isolates where *Bacillus* spp. (101) were dominantly isolated, followed by *Pseudomonas* spp. (76) and *Serratia* spp. (44). The isolates of each species from each area is given (Table 2).

We recorded that highest number of rhizobacterial isolates was obtained from Okara district where *Bacillus* isolates were dominating other isolates. Among total isolates from each area, number of *Bacillus* isolates were higher while, *Serratia* isolates were least retrieved from all areas and low in number among the total isolates obtained from each area

(Table 2).

Screening of antagonists

Dual culture plate technique

Screening of rhizobacterial isolates with dual culture technique revealed 11 potential isolates (zone of inhibition > 8 mm in radius) antagonistic to either isolate of *R. solanacearum* (Table 3). It was observed that *Bacillus* have greater number of isolates antagonistic to *R. solanacearum* as compared to *Pseudomonas* and *Serratia*, where only 2 and 1 isolates were antagonistic respectively. Against Rs9, highest inhibition zone (12.3 mm) was produced by B28, other isolates B14, B73, B98, P19, and S21 also showed significant results against Rs9. Similarly against Rs17, P11 was the best with highest inhibition zone (10.2 mm) followed by P19 (9.5 mm) and B55 (9.1 mm). Antagonism against Rs43 was recorded highest with isolate B9 where inhibition zone was 9.9 mm, isolate B47 and B85 also produce reasonable inhibition zone. Against the reference isolate GMI1000, B85 was found best antagonizing agent with mean zone of inhibition of 11.7 mm and other isolates (B28 and B98) were also effective.

Table 1. List of areas and locations of Punjab (Pakistan) visited for collection of rhizospheric soil.

District/Area	Sub-areas
Okara	Burj Jeeway Khan, Qadirabad, Bhuman Shah, Moza Ameer Aman, Salwal
Sahiwal	Chak 91/6R, Chak 95/6r, Chak 30/14L, Chak 86/6r
Sialkot	Chicharwali, Partan Wali, Khagga, Dhilam, Gunah, Sehjokala, Ghoinkey
Pakpattan	Shahu Baloch, Chak 39/SP, Chak 86/D
Kasur	Chitti Khui, Nizam pura, Dullay wali
Faisalabad	Sadhar, Pansara, Tandianwala, Chaba, Buraywala
Jhang	Chak 267, Chak Lailiana, Chak 460
Lahore	Mujaki, Mangaal, Lakhodair, Gurki, Awan, Ganja Sinduwa

Table 2. Rhizobacterial isolates retrieved from each potato growing area of Punjab.

Areas	*Bacillus* isolates	*Pseudomonas* isolates	*Serratia* isolates	Total isolates
Okara	20	10	4	34
Sahiwal	13	11	5	29
Sialkot	11	15	3	29
Pakpattan	10	7	8	25
Faisalabad	11	8	4	23
Jhang	10	11	9	30
Kasur	14	6	8	28
Lahore	12	8	3	23
Total isolates	101	76	44	221

Culture filtrate vs R. solanacearum

The effect of extracted CF of antagonistic isolates was studied in liquid medium which elucidated that the isolate antagonistic in dual culture plate method were also effective in liquid medium against *R. solanacearum* isolates. Their CF might be having some antibiotic compounds that inhibited the growth of all *R. solanacearum* isolates (Fig. 1-4). Highest antagonism was shown by CF of B28 against Rs9 (Fig. 1), P11 against Rs17 (Fig. 2), B9 against Rs43 (Fig. 3) and B85 against GMI1000 (Fig. 4).

Compatibility tests among antagonists

Compatibility test revealed that most isolates were not compatible with either one or more (Table 4). This was evident from the zone of inhibition that appeared in dual culture plate experiment. The isolates exhibiting zone of inhibition were considered non-compatible with each other. The test was useful to decide the application of isolates in consortium.

PGP traits of antagonists

IAA production

Almost all isolates produced IAA except S21. B28 synthesized the highest amount of IAA (40 µg/ml) (Table 5) which was clear by the color it produced and also the OD550 was calculated and maximum OD was obtained with this isolate (Table 5).

Table 3. Zone of inhibition by antagonistic isolates against virulent strains of *R. solanacearum*.

Isolates	Zone of Inhibition (mm radius)			
	Rs9	Rs17	Rs43	GMI1000
B9	0 e	2.5 ± 1 d	9.9 ± 3.2 a	0 c
B14	8.6 ± 1.5 bc	0 e	0 c	4.8 ± 1.5 b
B28	12.3 ± 2.3 a	5.3 ± 2.2 b	2.6 ± 1.5 b	10.6 ± 2.2 a
B47	0 e	3.7 ± 2.7 cd	8.5 ± 1.2 a	0 c
B55	0 e	9.1 ± 2.3 a	2.8 ± 1.2 b	6.4 ± 2.2 b
B73	9.6 ± 3.0 b	0 e	0 c	0 c
B85	3.4 ± 2.4 d	4.5 ± 2.2 bc	8.3 ± 1.9 a	11.7 ± 3.3 a
B98	7.9 ± 2.8 bc	0 e	0 c	10.3 ± 4.1 a
P11	0 e	10.2 ± 2.8 a	0 c	5.6 ± 3.4 b
P19	6.8 ± 1.9 c	9.5 ± 1.8 a	2.5 ± 1.0 b	0 c
S21	8.8 ± 2.8 b	0 e	0 c	0 c
Control	0 e	0 e	0 c	0 c

Values with different letters are significantly different at P≥0.05.

Table 4. Compatibility among antagonistic isolates.

Isolates	B9	B14	B28	B47	B55	B73	B85	B98	P11	P19	S21
B9		+	+	+	+	+	+	+	+	+	+
B14	-		-	-	+	-	-	+	+	+	-
B28	+	+		-	+	+	+	+	-	+	+
B47	+	+	+		+	+	+	+	-	+	+
B55	-	-	+	-		+	+	-	+	+	-
B73	+	+	+	-	+		-	+	+	+	-
B85	+	-	+	+	+	-		+	-	+	+
B98	+	+	+	+	+	+	+		+	+	+
P11	+	-	-	+	-	-	-	+		+	+
P19	+	-	+	+	-	+	+	+	+		+
S21	+	+	+	-	+	+	+	-	+	+	

'+' means compatible and '-' means incompatible.

Siderophore Production

Similarly siderophore production by the isolates was also observed, 8 isolates were capable of releasing siderophores with highest ability recorded with B28 as evident by the halozone surrounding the culture (Table 5). Three isolates were unable to release siderophores and no halo zone was observed around the colonies on CAS blue agar.

Phosphorus solubilization

P solubilization was only observed in 2 isolates i.e. B28 and B85 while the others were not showing any halozone (Table 5).

Table 5. Plant growth promoting traits of antagonists.

Isolates	*Siderophore production	**P-solubilization	IAA production (µg/ml)	**Chitinase production	Root colonization (CFU/ml in 10^{-7} dilution)
B9	++	-	10 ± 1.41 cd	-	4 ef
B14	-	-	4.50 ± 1.45 ef	-	6 de
B28	+++	+	40 ± 5.9 a	+	23 b
B47	+	-	8 ± 1.29 cde	-	4 ef
B55	-	-	20 ± 2.5 b	+	11 d
B73	-	-	5 ± 2.3 def	+	5 ef
B85	++	+	20 ± 3.25 b	+	31 a
B98	++	-	5.5 ± 0.21 cde	+	17 c
P11	+	-	3.8 ± 1.5 ef	-	8 de
P19	+	-	10.5 ± 2.8 c	-	6 de
S21	+	-	0 f	+	3 ef
Control	-	-	0 f	-	0 f

Values with different alphabets are significantly different at P≥0.05.

* Strong (≥ 5 mm halozone represented as '+++'), week (2-5 mm represented as '++'), slight (1-2 mm represented as '+') and non-siderophore producers as '-'

** '+' means present, '-' means absent

Root colonization

Root colonization by rhizobacterial isolates was checked on tissue cultured plants and phytagel was used to observe the colonization of roots. We found that the isolate B85 was efficient colonizer of the tissues. It rapidly colonized the roots and obtained from this was 31 cfu/ml (Table 5).

Chitinase production

Chitinase production test was conducted to see if the isolates antagonistic to *R. solanacearum* can also inhibit fungal growth by releasing chitinase enzyme that can hydrolyze fungal cell walls of which chitin is the main component. We observed that 6 out of 11 isolates synthesized chitinase enzyme. Five isolates of *Bacillus* spp. (B28, B55, B73, B85 and B98) and 1 isolate of *Serratia* spp. (S21) produced chitinase while no chitinase production was observed by pseudomonas isolates (Table 5).

Rhizosphere is the zone under the influence of the roots in account of higher activity of microorganisms because of the organic matter secreted by the plant roots (Hiltner, 1904). A great number of macroscopic and microscopic organisms such as fungi, bacteria, algae, and protozoa exist in the rhizosphere. Bacteria are the most plentiful among them. Plants select those bacteria helping most to their fitness by secreting organic compounds in exudate form (Lynch, 1990), making a very selective conditions where diversity is low (García *et al.*, 2001).

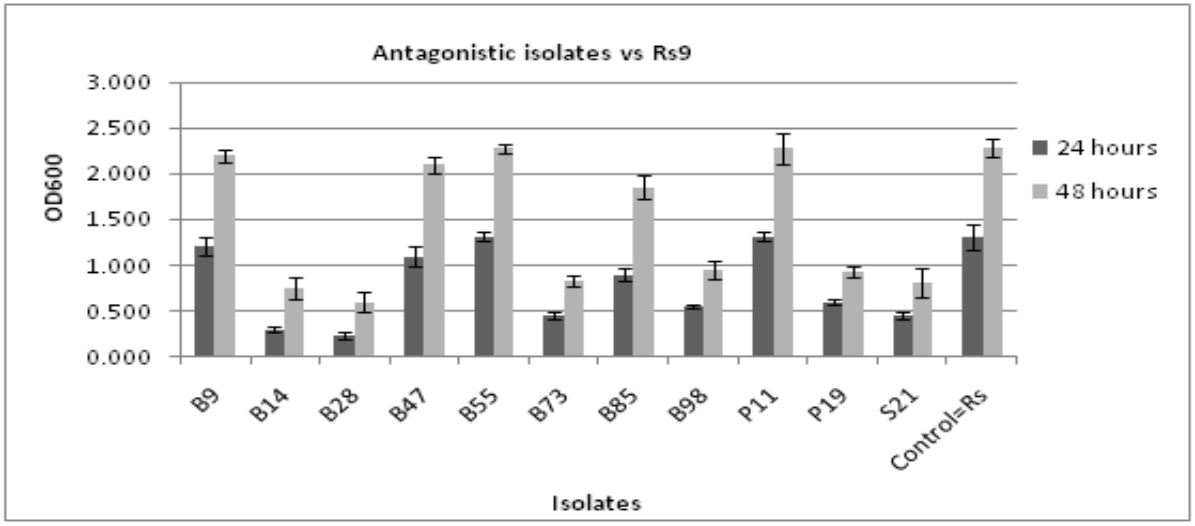

Fig. 1. Effect of culture filtrate of antagonistic rhizobacterial isolates against *R. solanacearum* Rs9.

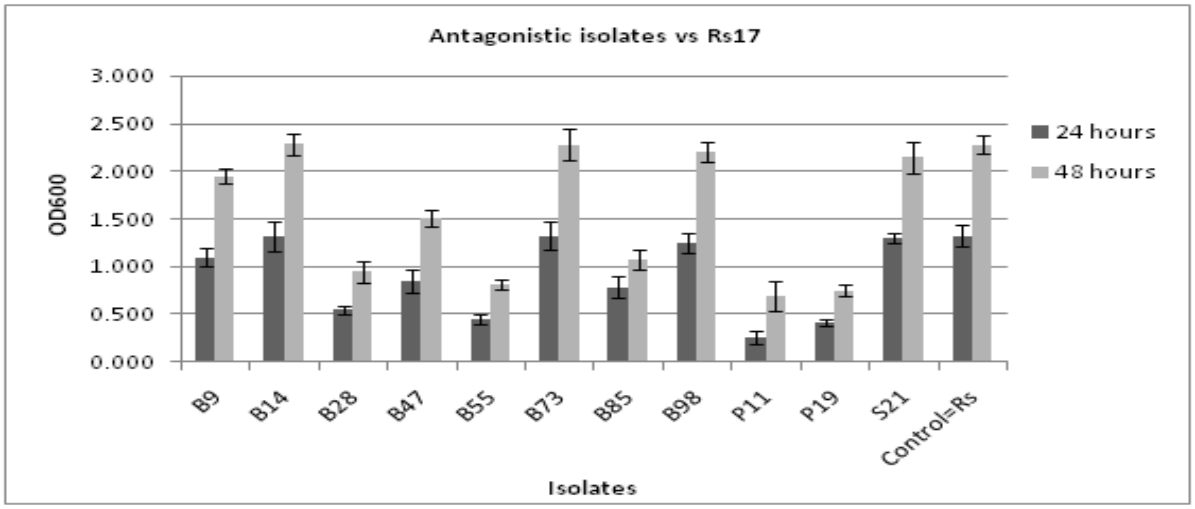

Fig. 2. Effect of culture filtrate of antagonistic rhizobacterial isolates against *R. solanacearum* Rs17.

Subsequently bacteria are the most excessive microorganisms in the rhizosphere, and they impact the plant's physiology to a larger extent, particularly considering their effectiveness in root colonization (Antoun and Kloepper, 2001). It has been reported that among the rhizosphere community, only 2-5 % is beneficial to plants (Antoun and Prévost, 2006). Among the gram positive bacteria in soil, 95 % belong to *Bacillus* spp. while other 5 % comprise of *Arthrobacter* spp. and *Frankia* spp. (Garbeva *et al.*, 2003). While considering gram negative bacteria, *Pseudomonas* is the most ubiquitous genus existing in the rhizosphere (Barriuso *et al.*, 2008).

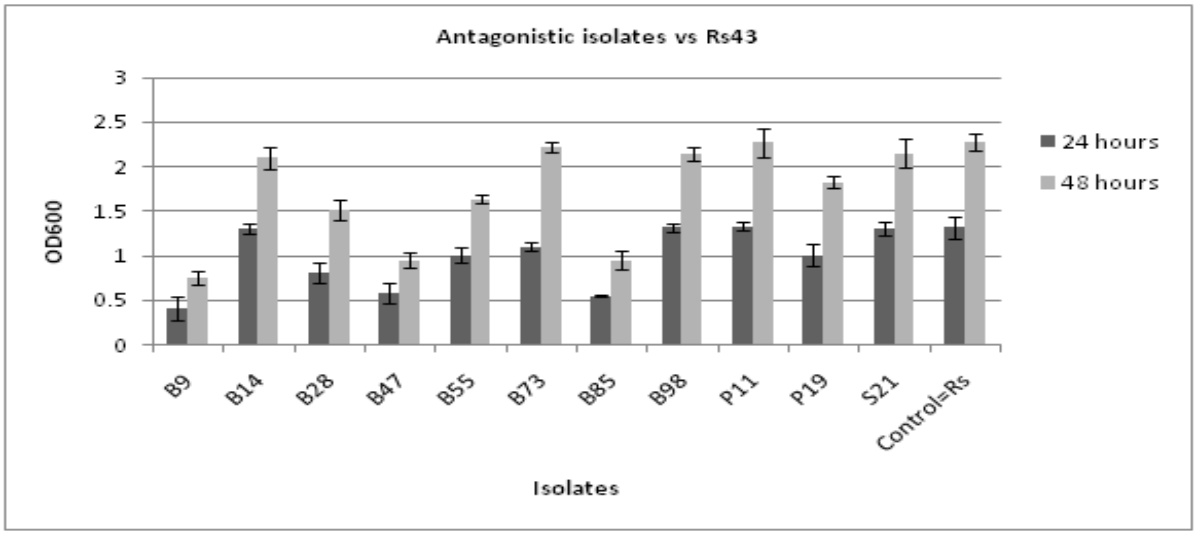

Fig. 3. Effect of culture filtrate of antagonistic rhizobacterial isolates against *R. solanacearum* Rs43.

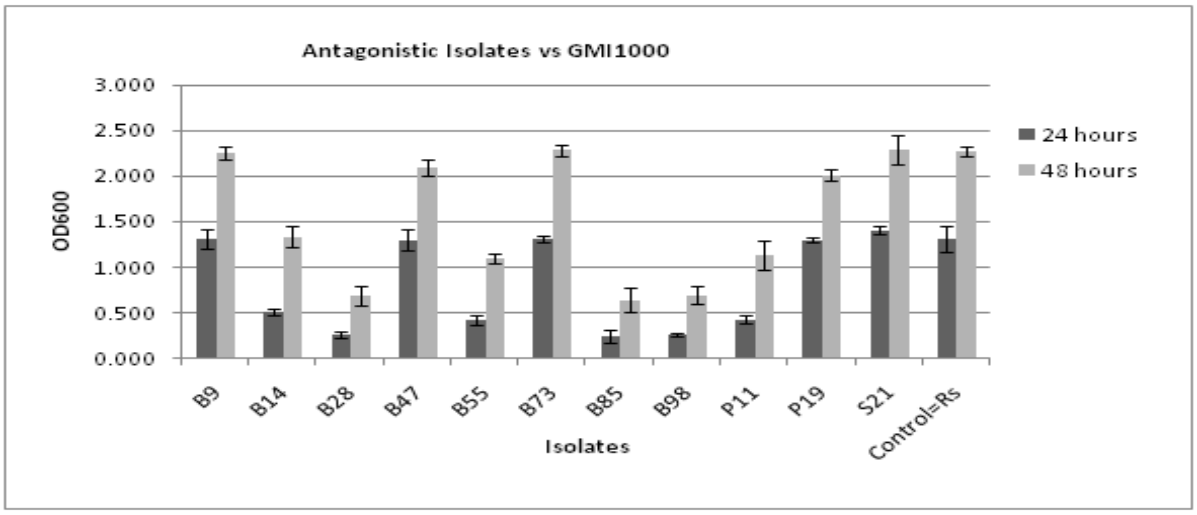

Fig. 4. Effect of culture filtrate of antagonistic rhizobacterial isolates against *R. solanacearum* GMI1000.

We have found *Bacillus* spp. dominating in the rhizosphere of potato crop in surveyed area of the Punjab i.e., 101 *Bacillus* isolates which made 46 % of total isolates obtained. Thirty four percent were belonging to *Pseudomonas* spp. while only 20 % belonging to *Serratia* spp. Although the number of antagonists were quite few but the most efficient ones were selected. Among antagonists *Bacillus* spp. (8) were dominating, followed by *Pseudomonas* spp. (2) and *Serratia* spp. (1) respectively. In terms of percentage *Bacillus* spp. were only 8 % antagonistic to *R. solanacearum,* while among *Pseudomonas* spp. and *Serratia* spp. 2.6 % and 2.2 % respectively. Tariq *et al.* (2010) isolated rhizobacteria from potato rhizosphere in Faisalabad and Naran areas of Pakistan and found *Pseudomonas* spp. and *Bacillus*

spp. dominating. He further observed that major portion of antagonists against *Rhizoctonia solani* were belonging to *Pseudomonas* spp. and *Bacillus* spp. The results of present study are in confirmation with Tariq *et al.* (2010) findings. This shows that rhizosphere harbors several pathogenic and beneficial microorganisms at the same time. The activity of beneficial microorganisms predominates otherwise detrimental organisms would have taken over the control of rhizosphere resulting in unfavorable environment for plant growth.

In our study, out of 221 rhizobacteria only 11 were effectively antagonistic to *R. solanacearum* and have shown variable degree of antagonism in dual culture plate method against different isolates. Only few antagonists antagonized all three virulent isolates of *R. solanacearum*. Different antagonists were studied for antagonism against genetically diverse isolates of *R. solanacearum* and results similar to current study were obtained by Xue *et al.* (2013). He also observed that the same antagonist inhibited different *R. solanacearum* strains differentially. Even the antagonistic isolates with high homology respond differentially to same *R. solanacearum* strain. Our results showed that rhizobacteria from the potato rhizosphere possess the ability to inhibit *R. solanacearum* growth. We further analyzed the cell free culture extracts of antagonists against the virulent strains of *R. solanacearum* and still observed the inhibition of *R. solanacearum* growth in liquid culture medium. It is highly likely that culture extract containing compounds toxic to *R. solanacearum*. In a study by Almoneafy *et al.* (2014) he tested four *Bacillus* strains against *R. solanacearum* in liquid medium and found significant inhibition of pathogen growth. He studied the pathogen cells using transmission electron microscopy and observed that *Bacillus* isolates disrupted *R. solanacearum* cell walls severely, resulted in cell lysis and consequently cytosolic contents were also lost by cells. In *Bacillus* isolates, he studied the expression of antimicrobial genes during interaction with *R. solanacearum* and observed upregulation of *ituC* (coding for iturin) and *srfAA* (coding for surfactin) genes in strains Am1 and D16. It is possible that isolates in this study might also have such an activity which has checked the growth of *R. solanacearum* in liquid media. Also we have seen the production of siderophore which have possibly played role in *R. solanacearum* inhibition. Several reports for *Serratia* being antagonistic to several pathogens are there and it has been used as biocontrol agent (Zhang *et al.*, 2002; Roberts *et al.*, 2007; Ahmed *et al.*, 2008), and it also promote growth of crops (Hameeda *et al.*, 2008; Li *et al.*, 2008). In our study, among 44 *Serratia* isolates only 1 showed significant antagonism against *R. solanacearum* strain. Xue *et al.* (2013) also found few *Serratia* isolates inhibiting *R. solanacearum*. The mechanism involved during *in vitro* inhibition could be as a result of antibiosis, siderophore production or both (Adesina *et al.,* 2007). This suggests that the antagonists are potent enough to be evaluated in field for *in vivo* bioprotection of potato plants from various diseases. The *in vitro* antagonistic activity of rhizobacteria has been reported by several researchers against *R. solanacearum* (Shekhawat *et al.,* 1993). The inhibitory activity of *P. fluorescens* against the pathogen in the current study corroborates with that of Ran *et al.* (2005) and Lemessa and Zeller (2007), where they indicated that isolates of *P. fluorescens* and fluorescent pseudomonads had significantly inhibited the *in vitro* growth of *R. solanacearum*. Notz *et al.* (2001) described that antibiotic compound, 2,4-DAPG, secreted by *P. fluorescens* played a central role in inhibiting phytopathogens. *In vivo* expression technology (IVET) was employed to detect expression of *P. fluorescens* genes upregulated in the rhizosphere (Rainey, 1999) and it was noticed that genes involved in nutrient uptake, stress response and release of compounds were highly expressed (Rainey, 1999).

Conclusion

This study implies that several rhizobacteria have potential to antagonize bacterial wilt pathogen which is posing serious implications on potato cultivation in Pakistan. In addition to biocontrol, these rhizobacteria can be proved helpful in enhancing plant growth through possessing PGP traits. Also, the

production of chitinase was observed by selected antagonistic isolates which can further be investigated against fungal pathogens in potato.

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