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PLANT GROWTH PROMOTING TRAITS OF RHIZOBACTERIA ISOLATED FROM POTATO (*Solanum tuberosum* L.) AND THEIR ANTIFUNGAL ACTIVITY AGAINST *Fusarium oxysporum*

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ABSTRACT

Rhizosphere bacteria are known to augment plant growth and suppress plant diseases. Current study was designed to evaluate the plant growth promoting and disease suppressing traits of indigenous bacteria from the rhizosphere of potato, *Solanum tuberosum.* For this purpose, out of 160 isolates, 20were further investigated to determine their ability for producing hydrogen cyanide (HCN), ammonia (NH_3), indole 3-acetic acid (IAA), lytic enzymes, solubilize phosphate and antagonize *Fusarium oxysporum.* Results revealed that 12 isolates were able to solubilize phosphate thereby creating halo zone between 1.00-2.40 mm, where PRP-6 caused maximum solubilization. Three strains, PRP-6, PRS-17 and PRS-24 were able to produce IAA, but the concentration was significantly different ($p<0.01$) from each other. Majority of the isolates exhibited strong potential (++) for producing NH_3 and HCN. Lytic enzymes production trait was observed in 83% of isolates but with varying potentials (weak, medium, strong). Overall, the percentage growth inhibition of *F. oxysporum* by bacterial isolates ranged from 13-46%, where PRP-6, PRP-13displayed maximum and minimum antagonistic potential respectively. Our results indicate that novel strains from the potato rhizosphere exhibit the plant growth promoting rhizobacteria (PGPR) traits which could be used for the development of potato biofertilizers and as biocontrol agents to antagonize *F. oxysporum.*

Key words: PGPR, Biocontrol, *Solanum tuberosum*, *Fusarium oxysporum*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a member of *Solanaceae* family and as a food crop it has major commercial significance globally. After rice and wheat, it has become the third most important food crop, with surplus energy supplies from starchy tubers, having low fats and rich source of vitamins B, C and potassium. According to the food and agriculture organization (FAO, 2014) of the United Nations, global production of potato was more than 350 million metric tons in 2013 to feed approximately one billion people around the world (Naqqash *et al*. 2016). In Pakistan, this crop was sown on an area of 0.158 million hectares with total production of 28.84 million tons during the cropping year 2013-2014 (GoP, 2015) where KP province ranks second to Punjab for the production of potato. Excessive use of nitrogen and phosphorus for optimum yield of this tuberous crop not only increase its cost of production but also cause many environmental issues (David *et al*. 2002; George and Ed, 2011). Dependency of potato on chemical fertilizers can be minimized through exploration of indigenous rhizosphere bacteria which until recent remain unexplored/underexplored.

Rhizobacteria are the plant associated bacteria living in the immediate vicinity, inside and on surface of roots. These rhizobacteria may directly or indirectly influence the nutritional status of soil (Khosro and Yousef, 2012) and are generally termed as plant growth promoting rhizobacteria (PGPR) imparting numerous benefits to host plants directly or indirectly. Direct roles of PGPR in plant growth include the availability of nitrogen, phosphorous and other mineral nutrients and production of phytohormones while indirect mechanism include the suppression of plant pathogens (Glick, 2012; Pérez-Montaño *et al*. 2014). PGPR belong to well-known genera including but not limited to *Rhizobium*, *Arthrobacter*, *Bacillus* (phosphate solubilizers), *Enterobacter*, *Pseudomonas*, *Azospirillum, Azotobacter* (Nitrogen fixers) which have been widely studied in crops including both legumes and non-legumes (Lucas *et al*. 2009; Bhattacharya, 2012; Pérez-Montaño *et al*. 2014; Majeed *et al*. 2015). In sustainable crop production, plant microbe interaction is of paramount significance (Shoebitz *et al*., 2009).

Soil borne infections caused by fungal pathogens have been reported to be major causes of crop losses annually (Ekundayo *et al*. 2011). Amongst soil

borne pathogens, *Fusarium oxysporum* has become the most devastating pathogen to cause mounting losses in potato crop productivity. *Fusarium* wilt caused by *Fusarium oxysporum* has been reported to cause up to 70% losses in potato crop (Ommati and Sharifi, 2008). Because of increasing cost of chemicals to control these pathogens and environmental concerns, biocontrol is proposed to be the promising option for controlling such pests. Various PGPR strains have yielded valuable results against soil borne pathogens. For instance, *Bacillus* and *Pseudomonas spp* have been successfully applied for biocontrol of soil borne pathogens (Weller *et al*., 2002). Rhizobacteria produce lytic enzymes such as proteases, catalases, glucosidases, chitinases which have well established role in biocontrol of fungal and bacterial pathogens (Asad *et al*., 2014; 2015). Apart from these enzymes, production of secondary metabolites or antibiotics such as hydrogen cyanide (HCN), antifungal compounds, siderophores and fluorescent pigments by PGPR formulate their antipathogenic strategy (Singh *et al*., 2006).

Over the past two decades, an extensive literature has been published highlighting the plant growth promoting and anti-pathogenic traits of PGPR. However, the investigations carried out so far on the topic used commercially available inocula which became ineffective with the passage of time, because the microbes could not adapt successfully to the new environment. To fill this gap, current research was designed to investigate the potential of indigenous population of PGPR in improving the growth of potato and investigating their potential to combat the fungal pathogen, *F. oxysporum*. This objective was addressed by isolating PGPR from the disease suppressive fields of same region with persistent *Fusarium* infections. Native population of PGPR were used in current study because of their abundance and adaptability in the local environment. Overall, this research was aimed to significantly reduce the use of chemical fertilizers and fungicides and enhance crop productivity on sustainable basis.

MATERIALS AND METHODS

Soil samples from the rhizosphere of potato (*Solanum tuberosum*) were collected from the district Mansehra, KP, Pakistan. Entire bunch of potato roots along with adhering soil was excavated and stored in sterilized plastic zipper bags. Samples were transported to the soil biology and biochemistry laboratory of Land Resources Research Institute (LRRI) at National Agricultural Research Centre (NARC) Islamabad and stored at 4°C. From these soil samples, 160 bacterial strains were isolated out of which 20 strains were screened to test their plant growth promoting traits and biocontrol efficacy against *Fusarium oxysporum.* Detailed description of each method used for characterization and evaluation of microbes are detailed in the following paragraphs.

Isolation, purification and morphological identification of bacterial isolates: Microbes were isolated from soil samples through serial dilutions as devised by Johnson and Curl (1972). Then 0.1ml of the final prepared suspension was poured in to the petri plates containing LB agar media and incubated at 28ºC. After 24 hours, bacteria containing plates were placed at 4ºC until thick appearance of bacterial colonies on agar media. Based on morphological appearance full gown bacterial colonies were purified through further inoculation and transferring to fresh LB media containing plates and incubating at 28ºC for 24 hours. Morphological characteristics of bacterial colonies including; color, shape, margin, elevation and opacity were studied by using the light microscope as described by Vincent (1970). Gram staining was conducted for taxonomic classification of microbes (Vincent and Humphrey, 1970).

Bioassays for plant growth promoting traits of rhizobacteria

Indole-3-Acetic Acid (IAA) production: Indole acetic acid (IAA) production capacity of microbial isolates was determined through the method devised by Bric *et al.* (1991). Briefly, isolates were inoculated on tryptone broth in tubes and incubated at 35º±2ºC. After 48 hours, five droplets of Kovacs reagent were poured in to the bacterial colony containing tube. As of protocol, shortly after the addition of reagent, the formation of cherry red ring at the top of medium indicated the indole production by respective microbial strains and vice versa. The concentration of IAA produced by the isolates was quantified by centrifugation of bacterial cultures at 4000 rpm for 20 minutes. After centrifugation, 1ml of supernatant was taken in a test tube and mixed with 2ml Salkowski's reagent. After 20-25 min of reaction, absorbance was recorded at 535nm by using spectrophotometer (Asghar *et al*. 2000).

Phosphate Solubilization Index (SI): Phosphate solubilizing efficacy of bacterial isolates was determined by culturing the isolates on Pikovaskya agar medium. After inoculation, culture plates were incubated at 28ºC for seven days. The appearance of halo zone around the bacterial cultures was an indication of their ability to solubilize phosphate as described by Nautiyal, (1999). Phosphate solubilization index (SI) was calculated according to the method of Edi-Premono *et al*. (1996) detailed in equation 1 below.

$$SI = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}} \quad \text{(Eqn.1)}$$

Ammonia (NH_3) and hydrogen cyanide (HCN)

production: Bacterial isolates were tested for their ability to produce ammonia. As detailed in the method of Cappuccino and Sherman, (1992) bacterial cultures were inoculated in peptone broth already poured in screw caped glass tubes and incubated at 30°C for 2 days. After incubation time was elapsed, 1ml of Nessler's reagent was added to each tube. Appearance of yellow to brownish color was an indication of ammonia production by respective bacterial isolates. Hydrogen cyanide (HCN) production ability of microbes was assessed as according to Lorck, (1948), there by growing bacterial cultures on glycine amended nutrient broth (NB) medium. Sterilized filter paper was soaked in picric acid and affixed on the cover of petri plates followed by incubation at 28ºC for 4 days. Change in color of the filter paper indicated the production of HCN by bacterial isolates. The ability of microbial isolates to produce HCN produced was qualitatively color graded i.e. if filter paper color changed from yellow to light brown, HCN production ability of respective isolates was marked as weak (+). Color changes from yellow to brown and yellow to reddish brown respectively indicated the moderate (++) and strong (+++) ability of microbes HCN production.

Lytic enzymes production: Production of lytic enzymes; catalase, protease and amylase production by bacterial isolates were assayed. Catalase production was determined by placing a 24 hours old bacterial colony on glass slide followed by addition of 30% hydrogen peroxide (H_2O_2). Shortly after the addition of H_2O_2, formation of gas bubbles was the indication of catalase production by respective isolates as detailed by MacFaddin, (2000). ***Protease*** production by microbes was determined by inoculating the bacterial isolates on skimmed milk agar medium (SKM). Inoculated plates were placed in incubator at 35°C for 48 hours. Halozone formation around bacterial colonies evidenced the protease production by respective isolates as observed by Kazempour, (2004). The capability of microbes to hydrolyze starch, i.e. ***amylase*** production was assayed on starch agar plates. Bacterial cultures inoculated on starch containing medium were incubated at 37°C for 24 hours. Culture containing plates were flooded with iodine solution (1%). Clear zone around the bacterial colonies indicated the amylase production ability of respective isolates (Ashwini *et al.* 2011). Similarly, ***pectinase*** production ability of isolates was evaluated by culturing them on pectate agar (PA) medium. The substrate utilized zone around the colony was observed after incubation at 35°C for 24 hours as detailed by Namasivavam *et al.* (2011).

Antipathogenic potential of microbes: Antagonistic potential of bacterial isolates against fungal pathogens was determined through dual culture assay potato dextrose agar (PDA) as devised by Sivan *et al.* (1987). Approximately, 1 mm long mycelial disc of *Fusarium oxysporum* was placed at one corner of petri plate containing PDA. Opposite to the fungal mycelial disc, bacterial colony was placed, and plates were incubated at 28ºC for 7 days. Plates containing only fungal mycelia served as control for this assay and there were three replicates for each treatment. The creation of an inhibition zone around the bacterial colonies indicated the microbial potential to antagonize the fungal pathogens. The percentage growth inhibition of the fungal pathogen was calculated as in equation 2 (Sivan *et al.* 1987) below.

$$PGI = \left[1 - \left(\frac{\text{Fungal growth}}{\text{Control growth}}\right)\right] \times 100 \quad \text{(Eqn.2)}$$

Statistical Analyses: Data were analyzed using statistical analysis program, STATISTIX version 8.1. The analysis results were confirmed by reanalyzing the same data on data analysis tool pack of Microsoft Excel 2016. One-way analysis of variance (ANOVA) was performed in all experiments. The means were compared by least significant difference (LSD) test at p=0.05 as of Steel *et al*., (1997).

RESULTS

Morphological and taxonomic identification: Microscopic observation revealed varying outlook of bacterial isolates. Amongst all 20 tested isolates, no trend was observed regarding colony color, shape, margin and even microbial groups as depicted in table 1. However, few colors dominated over the others, where half of the bacterial colonies exhibited white, off white and pure white colors. Remaining half of the bacterial colonies exhibited one of the five colors including; creamy, lemon, brownish, dark brown and light orange. Isolates were either bacilli or cocci whereby in numerical terms, 13 of bacterial isolates were cocci (Micrococcus/ Streptococcus/ Diplococcus/ Staphylococcus) while remaining 7 were *Bacilli* (Streptobacillus/ Diplobacillus). Similarly, gram staining revealed that 50% of the tested isolates were gram positive while remaining half did not retain the crystal violet stain (gram negative). Other attributes (form, margins, elevation and opacity) of bacterial colonies varied from one isolate to another.

Indole 3-acetic acid (IAA) and phosphate solubilization index (SI): Bacterial isolates were tested for their ability to produce growth hormone IAA. Data in table 2 indicated that only three isolates produced this growth hormone while remaining did not exhibit this trait. Out of those growth hormones producing strains, PRS-24 produced highest concentration (16.17µg ml^{-1}) of IAA. Other two strains, PRP-6 and PRS-17respectively produced 14.06 and 11.66 µg ml^{-1} of this hormone. The amount of IAA produced by each of these three strains were statistically different from each other ($p<0.01$). Compared with 3 strains capable to produce IAA, 12

strains exhibited the trait to solubilize tri-calcium phosphate. Microbial isolate, PRP-6 solubilized maximum phosphate thereby resulting in the creation of the widest halo zone (2.4mm) which was statistically similar to the halo zone produced by the strain PER-12 (2.2mm) but different from all other phosphate solubilizers. The smallest zone was produced by the strain, PRP-13 (1.0mm) followed by PRS-16, PRS-17 strains which produced halo zone of 1.08 and 1.33mm respectively.

Ammonia (NH_3) and hydrogen cyanide (HCN) production: Data in table 3 highlights that 16 out of 20 strains were able to produce ammonia. Amongst ammonia producers, majority of the isolates including; PRS-5, PRP-7, PRP-9, PER-10, PRS-16, PER-20, PER-21, PER-22, PRS-23 produced the greater concentration (++) of ammonia compared with their counterparts; PRP-4, PRP-6, PRS-11, PER-12, PRS-17, PER-18 and PRS-24 which produced smaller amount (+) of this gas. As shown in table 3, half of the microbial isolates produced HCN. Because HCN production was tested qualitatively, microbial strains, PER-12, PRS-17 and PER-21 exhibited the strong ability (+++) to produce HCN followed by that produced by PER-22, PRS-23 and PRS-24 (++). The lowest amount (+) of this antibiotic was produced by microbial isolates, PRS-5, PRP-6, PRP-7 and PRP-9. Remaining isolates lacked the ability to produce HCN as is evident from the data in table 3.

Lytic enzymes production: Data presented in table 3 highlights the lytic enzyme production ability of experimental strains. As is noticeable from the data, majority of the strains produced catalase, protease, amylase and pectinase enzymes. However, their enzyme production capacity varied from one strain to another. The isolates exhibited the strong ability (+++) to produce pectinase followed by amylase and protease. Surprisingly, strong ability was observed in only three strains for each of amylase and protease enzymes whereas, remaining strains exhibited either medium or weak potential to produce these enzymes. Similar trend was observed in case of catalase production where majority of the isolates exhibited weak potential to produce this enzyme.

Antipathogenic potential: Microbial isolates were tested *in vitro* for gauging their potential to antagonize fungal pathogen, *Fusarium oxysporum* where half of the bacterial population was noticed to antagonize this pathogen (Table 4; Figures 1, 2). As observed in other experiments involving, HCN, ammonia, IAA and lytic enzymes production, antagonistic potential of isolates also did not show any symmetry but varied among isolates. Overall, the percentage growth inhibition ranged 13-47%. Three strains, PRP-6, PRP-4 and PRS-23 proved to be more efficient antagonists thereby inhibiting the pathogen invasion by 46.33, 42.9 and 41.9 % respectively. Moreover, the inhibition zones created by all these three antagonists were statistically different from each other. Lowest antagonistic potential was noticed in treatments involving strain; PRP-13which reduced the fungal invasions by 13% followed by PRS-5 and PRP-9 which exhibited statistically similar potential to antagonize the pathogen.

Table 1. Morphological characteristics of bacterial isolates used in current study.

Microbial isolate	Colony color	Form	Elevation	Margin	Opacity	Gram reaction	Shape/Group
PRP-4	White	Circular	Flat	Erose	Translucent	+ve	Coccus/ *Streptococcus*
PRS-5	White	Circular	Convex	Entire	Translucent	+ve	Coccus/ *Streptococcus*
PRP-6	Creamy	Punctiform	Flat	Entire	Translucent	-ve	Bacillus/ *Streptobacillus*
PRP-7	Brownish	Irregular	Raised	Undulate	Translucent	-ve	Coccus/*Diplococcus*
PER-8	White	Circular	Raised	Curled	Opaque	+ve	Bacillus/ *Streptobacillus*
PRP-9	Pure white	Circular	Raised	Entire	Translucent	-ve	Bacillus/ *Streptobacillus*
PER-10	Off white	Circular	Flat	Entire	Opaque	+ve	Coccus/*Staphylococcus*
PRS-11	White	Circular	Raised	Undulate	Opaque	-ve	Bacillus/*Diplobacillus*
PER-12	Brownish	Circular	Raised	Entire	Transparent	-ve	Coccus/*Micrococcus*
PRP-13	Lemon	Circular	Pulvinate	Entire	Transparent	+ve	Coccus/ *Staphylococcus*
PER-14	Dark brownish	Punctiform	Convex	Erose	Translucent	+ve	Coccus/ *Diplococcus*
PRP-15	Milky white	Circular	Raised	Entire	Opaque	+ve	Coccus/*Streptococcus*
PRS-16	Light Orange	Punctiform	Raised	Undulate	Transparent	+ve	Coccus/ *Micrococcus*
PRS-17	Off white	Circular	Pulvinate	Entire	Opaque	-ve	Coccus/ *Micrococcus*
PER-18	Lemon	Irregular	Raised	Curled	Opaque	+ve	Bacillus/*Diplobacillus*
PER-20	Creamy	Punctiform	Umbonate	Entire	Opaque	+ve	Bacillus/ *Streptobacillus*
PER-21	Off white	Circular	Flat	Undulate	Opaque	-ve	Coccus/ *Micrococcus*
PER-22	Pure white	Circular	Raised	Curled	Opaque	-ve	Bacillus/ *Streptobacillus*
PRS-23	Milky white	Punctiform	Raised	Entire	Translucent	-ve	Coccus/ *Staphylococcus*
PRS-24	Off white	Circular	Pulvinate	Curled	Opaque	-ve	Coccus/ *Diplococcus*

Table 2. IAA and phosphate solubilization ability of isolated strains

Microbial isolates	IAA Conc. (µg ml^{-1})	Phosphate Solubilization Index (SI)
PRP-4	0.00a	1.20b
PRS-5	0.00a	0.00a
PRP-6	14.06c	2.40c
PRP-7	0.00a	1.71d
PER-8	0.00a	0.00a
PRP-9	0.00a	2.20ce
PER-10	0.00a	1.50df
PRS-11	0.00a	0.00a
PER-12	0.00a	2.00ge
PRP-13	0.00a	1.00bh
PER-14	0.00a	0.00a
PRP-15	0.00a	0.00a
PRS-16	0.00a	1.08bi
PRS-17	11.66b	1.33bj
PER-18	0.00a	0.00a
PER-20	0.00a	0.00a
PER-21	0.00a	1.40 bfk
PER-22	0.00a	1.25bl
PRS-23	0.00a	2.14me
PRS-24	16.17d	0.00 a
LSD	1.728	0.213

Values followed by different letters are statistically different from each other (P=0.05)

Table 3. Evaluation of bacterial isolates for Ammonia and lytic enzyme production.

Bacterial Isolate	Ammonia	Hydrogen Cyanide	Lytic enzymes			
			Catalase	Protease	Amylase	Pectinase
PRP-4	+	-	+	+	++	++
PRS-5	++	+	_	+	+++	+++
PRP-6	+	+	+	+++	++	+++
PRP-7	++	+	++	+	_	+++
PER-8	_	-	++	++	++	++
PRP-9	++	+	+	+	++	+++
PER-10	++	-	+	_	_	_
PRS-11	+	-	+	+++	+	_
PER-12	+	+++	+	++	+	_
PRP-13	_	-	+	_	_	++
PER-14	_	-	+	_	+++	++
PRP-15	_	-	+	_	++	++
PRS-16	++	-	+	_	_	++
PRS-17	+	+++	++	++	++	++
PER-18	+	-	+	++	+++	++
PER-20	++	-	++	+	++	+++
PER-21	++	+++	++	++	+	++
PER-22	++	++	++	++	+	+++
PRS-23	++	++	++	++	++	+++
PRS-24	+	++	++	++	_	+++

Table 4. Antagonistic potential (in percentage) of bacterial isolates against fungal pathogen, *Fusarium oxysporum.*

Treatment	Pathogen Growth (mm)	Bacterial Zone (mm)	Percentage Growth Inhibition %
Control	8.00 a	0.00 i	0.00 h
PRP-4	4.56i	2.03b	42.90 b
PRS-5	5.56cd	1.53d	30.33f
PRP-6	4.33j	2.16a	46.33a
PRP-7	8.00 a	0.00 i	0.00 h
PER-8	8.00 a	0.00 i	0.00 h
PRP-9	5.50 d	1.46e	30.66f
PER-10	8.00 a	0.00 i	0.00 h
PRS-11	8.00 a	0.00 i	0.00 h
PER-12	5.60 c	1.26g	30.00 f
PRP-13	6.96b	0.53h	13.23g
PER-14	8.00 a	0.00 i	0.00 h
PRP-15	8.00 a	0.00 i	0.00 h
PRS-16	8.00 a	0.00 i	0.00 h
PRS-17	5.26g	1.76c	34.13d
PER-18	8.00 a	0.00 i	0.00 h
PER-20	8.00 a	0.00 i	0.00 h
PER-21	5.43e	1.46e	32.00 e
PER-22	5.33f	1.73c	33.46d
PRS-23	4.63h	1.36f	41.9c
PRS-24	4.56i	0.00 i	0.00 h
LSD (0.05)	0.06	0.06	0.76

Values followed by different letters are statistically different from each other (P=0.05)

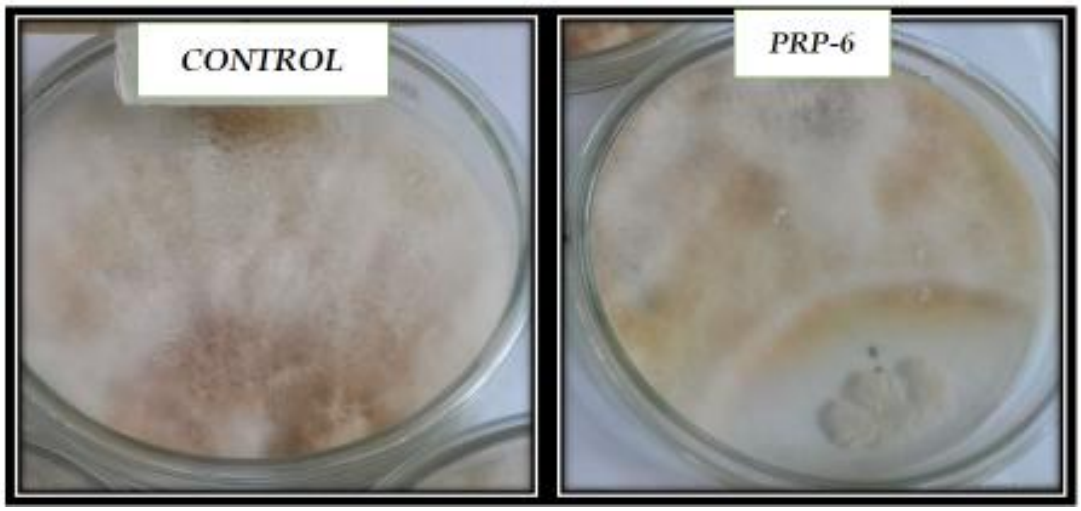

Figure 1. Antagonistic potential of bacterial isolate PRP-6 against fungal pathogen, *F. oxysporum*. In control treatment, plates were infected only with mycelia of *F. oxysporum*, while in PRP-6 treatment, pathogen mycelia containing plate was inoculated with bacterial isolate PRP-6 which significantly inhibited the fungal growth (46.33%). Fungal growth diameter; control=8.00mm; with bacterial isolate=4.33mm (P<0.05).

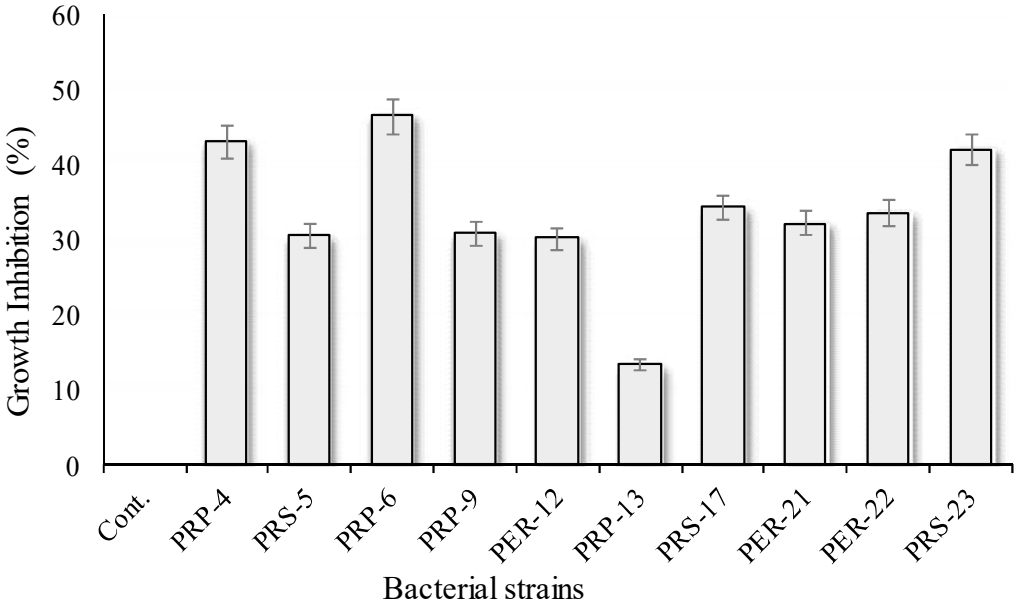

Figure 2. Growth inhibition ability (%) of potato rhizosphere microbial isolates against *Fusarium oxysporum*. All strains exhibited significant potential to inhibit the pathogen growth. Data are means of three replicates ± SE.

DISCUSSION

Current investigation was carried out to evaluate the plant growth promoting (PGP) traits of rhizosphere bacteria isolated from the root zone of potato (*Solanum tuberosum*) and determining their biocontrol efficacy against fungal pathogen, *Fusarium oxysporum*. Plant growth promoting rhizobacteria (PGPR) residing in the rhizosphere, rhizoplane and endo rhizosphere play important role in the plant growth and development (Glick *et al*., 1999; Gerhardt *et al*., 2009). Out of 160 microbes, 20 isolates were morphologically and taxonomically identified followed by testing their ability to exhibit any or multiple of; Auxins (IAA), ammonia, HCN, phosphate solubilization, lytic enzymes production and antagonism traits. A great deal of variability in the biochemical and morphological traits was observed in isolates (table 1) which may be attributed to the diverse soil types (Kim *et al*. 2011).

In our study, 60 and 50 % of isolates produced NH_3 and HCN respectively. These two metabolites are notable characteristics of PGPR, influencing plant growth through nitrogen fixation and enhancing the antagonistic potential of PGPR. *Rhizobia* and *Azospirillum* formulate an important group of PGPR and have been reported to produce ammonia in legumes and non-legumes (Malik *et al*. 2002; Manasa *et al*. 2017). Ammonia has very active role in nitrogen fixation through symbiotic relationship with legumes (Geetha *et al.* 2014; Jha and Saraf, 2015). Similarly, inoculation of cotton and sugarcane with *Azospirillum* significantly increased the N-content in respective plants (Fayez and Daw, 1987; Muthukumarasamy *et al*. 1999) suggesting that not only *Rhizobia* but other forms of PGPR also produce ammonia. HCN is part of powerful antifungal compounds produced by PGPR to trigger the biological control of pathogens. Interestingly, HCN production trait has been widely reported in *Pseudomonas* and *Bacillus spp* (Ahmad *et al.* 2008). Empirical evidences revealed that PGPR would use HCN like antibiotics to combat the dangerous species of microbes (Antoun and Prevost, 2000) but in the recent past its anti-pathogenic role has been identified (Kremer and Souissi, 2001; Devi *et al*. 2007).

Indole acetic acid (IAA) production capability of bacterial isolates varied significantly (table 2). This is not surprising, as all strains were isolated from diverse environments, hence exhibited different potential to produce hormone (Khakipour *et al.* 2008; Dawwam *et al.* 2013; Ghodsalavi *et al.* 2013). IAA is an important secondary metabolite having remarkable function in plant growth and development to produce more adventitious roots for nutrient uptake (Salisbury, 1994; Gutiérrez Mañero *et al*. 1996; Jha and Saraf, 2015). Majority of the tested isolates were adept to solubilize phosphate (table 2) which is in line with our previous research (Yasmin *et al*. 2009; Afzal *et al*. 2010; Alia *et al*. 2013). Infact, phosphate solubilizing PGPR release various low molecular weight organic acids which acidify and solubilize phosphate complexes, releasing orthophosphate to be taken up by plants (Oteino *et al*. 2015).

Microbial isolates produced lytic enzymes including: catalase, protease, amylase and pectinase

(table 3) which have extensive potential to antagonize fungal pathogens. Enzyme production by PGPR have been reported in many researches and various roles have been attributed to these enzymes including their key role in protecting the cell from oxidative damage by reactive oxygen species (Brantlee *et al*. 2011; Akazawa and Nishimura, 2011; Duarah *et al*. 2011). *Bacillus* and *Pseudomonas* bacteria are well researched to produce this catalase enzyme (Hallmann and Berg, 2006; Kamboh *et al*. 2009; Zahid *et al*. 2015) and in our case all *Bacilli* exhibited the trait to produce catalase. Lytic enzymes have also been reported in fungal species involved in biocontrol (Asad *et al*. 2014; 2015), to disintegrate pathogen cell wall consisting of beta-glucan and chitin (Ait- Lahsen *et al.*2001). PGPR defend plants through secretion of antibiotics like siderophores, pyocyanine, HCN and production of pathogen cell wall degrading enzymes (Mauch *et al*. 1988; Glick, 1995; Bisen *et al*. 2016). In current trial, the influence of lytic enzymes on disease reduction was evident from the reduced growth of fungal pathogen, *Fusarium oxysporum* (table 4; fig. 1). PGPR strains formed strong inhibition zone against pathogen, which could be attributed to the production of lytic enzymes and HCN like antibiotics or a combination of these metabolites. Resultantly, growth of *F. oxysporum* was inhibited up to 47% as compared to control. Our results stand in par with previous investigations where *P. fluorescence* isolated from the rhizosphere soil of *Solanaceae* crops secreted secondary metabolites to confront the growth of fungal pathogens (Ramyasmruthi *et al.* 2012).

Conclusion: Rhizosphere of potato contained microorganisms which exhibited PGPR like traits, and majority of the strains expressed more than one trait. It was concluded that indigenous microbial population from potato rhizosphere could be successfully used for the development of potato biofertilizers. Amongst all tested isolates, PRS-24, was potent to produce growth hormone IAA. However, PRP-6 proved to be the most efficient strain to solubilize phosphate, produce HCN, NH_3, and lytic enzymes and effectively antagonized the soil borne pathogen, t*F. oxysporum,* a causal agent for potato wilt disease. Developing the biofertilizers based on these two strains may ensure the healthy and disease-free crop of potato.

Conflict of interest: Authors declare no conflict of interest while publishing this paper.

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