Pak. J. Agri. Sci., Vol. 58(1), 61-67; 2021
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI:10.21162/PAKJAS/21.1024
http://www.pakjas.com.pk

CHARACTERIZATION OF RHIZOBACTERIA FOR GROWTH PROMOTING TRAITS AND THEIR POTENTIAL TO INCREASE POTATO YIELD

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Plant growth promoting rhizobacteria (PGPR) are associated with rhizosphere as well as non-rhizosphere. PGPR are involved in plant growth promotion through different direct and indirect mechanisms. The excessive and imbalanced use of chemical fertilizers is a burning issue. The scientists are emphasizing on reducing the use of chemical fertilizers and moving towards biological approaches. The current study includes isolation, screening, and characterization of bacteria isolated from potato rhizosphere. The bacterial isolates were characterized for siderophore production, iron solubilization, phosphate solubilization, auxin production, catalase and chitinase activity. All the bacterial isolates were found positive for phosphorous solubilization, indole acetic acid (IAA), siderophore and catalase activity. Further, bacterial isolates were tested in a growth chamber for their growth promoting potential in potato. Out of eleven bacterial isolates, two bacterial isolates (O-13 and K-10) significantly improved the agronomic and physiological parameters (SPAD, chlorophyll a, b and carotenoids) under axenic condition. It was concluded that bacterial isolates can be used to improve growth and yield of potato. However, there is a dire need to explore the character involved in growth promotion of selected rhizobacteria under field conditions to verify their response in growth promotion and to reduce the use of chemical fertilizers. The current experiment leads towards interactive approach of microbial inoculation and chemical fertilizers, ultimately reducing the use of chemical fertilizers.
Keywords: PGPR, Bio-fertilizer, Potato, Growth promotion.

INTRODUCTION

Potato has been an important food crop for growers and consumers not only in Pakistan but all over the globe. Potato is listed among the most important crops because of its higher production rate and nutritious value. Globally, potato is playing a vital role in food security to overcome the hunger (Devaux et al., 2014). In Pakistan, among the major food crops, potato is the one of the most important crops after wheat, maize and rice(Rauf et al., 2007). Potato has the potential to earn significant amount of foreign exchange (Rauf *et al.,* 2007). According to Pakistan Economic Survey (2017), potato was grown on an area of 179.3 million hectare, with production around 3849.5 thousand tons. Although, Pakistan is among the largest potato producing countries, but still per hectare yield is around 19.34 tons, which is very much lower as compared to leading potato growing countries (Placide *et al.,* 2019). Potato is an exhaustive-tuberous, high-yielding and short duration crop. The excessive and imbalanced use of chemical fertilizers is of major concern with potato plant, no-doubt improves the plant growth and nutrient contents, but its residues enter the potato tuber creating an alarming condition along with environmental constraints (Ju et al., 2007).

In the twenty-first century, agricultural sector throughout the world faced challenges in sustainability of agro-ecosystem. In consonance with United Nations, the world's population is expected to be above 9 billion by 2050 (Wood, 2001). Along with this, demand of food is also being increased (Gouel and Guimbard, 2018). To fulfill this demand, agricultural sector will have to raise the production by 70% to meet the demand (FAO, 2014).

Rhizobacteria are naturally occurring beneficial soil microorganisms present in rhizosphere that act as a biocontrol agent and can improve plant growth under various environmental conditions through attenuating the biotic and abiotic stresses (Saravanakumar, 2012; Paungfoo-Lonhienne et al., 2019). Microbial based inoculation is a novel, cost-effective and environmentally friendly technique to mitigate the micronutrient malnutrition (Agrawal et al., 2018). PGPR improve the plant growth, development, physiological process and yield through different direct and indirect mechanisms (Mehmood et al., 2018). The application of soil microorganisms is an efficacious technique to minimize the excessive and non-judicious use of pesticides and chemical fertilizers (Rana et al., 2012). The most important role of PGPR is to enhance the nutrient use efficiency and crop yield (Mushtaq et al., 2020; Jou et al., 2012; Kohler et al., 2008; Arshad et al., 2008).

Rhizobacteria can improve plant growth and development by producing plant growth regulators and have an effective role in improving plant nutrition through facilitating nutrient solubilization and uptake from soil (Mushtaq et al., 2020; Saleem et al., 2018). PGPR are non-pathogenic bacteria that

perform a prominent role in the development of crop-plants under normal and stress environment (Gusain et al., 2019). Soil microorganism performs a variety of soil activities like decomposition of organic matter, weathering of soil, retention of nutrients, and exudation of soluble salts, siderophore production, nutrient cycling and mineralization, plant nutrition, mineral solubilization and most importantly play their part in photosynthesis (Syed et al., 2019). Biofortification using microbes is the most economical, effective and emerging approach being steadily introduced in agricultural to overcome micronutrient malnutrition (Kaur et al., 2020).

The aim of the experiment was to isolate, screen and microbial characterization of bacterial isolates to investigate their impact on growth of potato under controlled conditions to minimize the use of chemical fertilizers to lead towards integrated microbial-chemical approach to improve plant growth, development and physiological processes.

MATERIALS AND METHODS

***Source of rhizobacteria*:** Rhizobacteria were collected and isolated from potato rhizosphere grown in Punjab, Pakistan. Rhizospheric soil samples were carried to the laboratory for further analysis in sampling ice box to maintain the minimum microbial activity.

***Isolation of rhizobacteria from soil samples*:** Isolation from soil samples was carried out through dilution plate technique. Ten gram of soil sample was taken, dissolved in 95 mL of distilled water and shaked for 5-10 minutes. Further, one ml soil sample was added to test tube, already containing 9 mL of autoclaved distilled water to acquire 10^{-2} dilution and the process was repeated up to 10^{-8} dilution. At the end, 100 µL of each dilution was spread onto LB agar plates through spreader and placed in incubator for 24-48 hours.

Microbial Characterization

***Iron solubilizing-assay*:** According to Nishio and Ishida (1989), the iron solubilizing activity of bacterial isolates was determined on specific media having insoluble source of iron. Each single bacterial isolate was streaked on LB medium agar plates having 1g of $FePO_4.4H_2O$ and 20 g of glucose and kept in incubator at 28±2 ºC. After 3-4 weeks, the bacteria which solubilize the granules of iron were marked as positive for iron solubilization.

***Siderophore production-assay*:** The universal method of Sckwyn and Neilands (1987) was followed to find out the qualitative siderophore. Siderophore specific MM9 agar media which is low in iron was prepared andspot inoculation of respective bacterial strains was made on five different places on the plates and kept in the incubator at 28º±2 C for 48 hrs.

***Chitinase activity*:** According to Chernin et al. (1998), Chitinase activity was estimated with some alteration. Colloidal chitin was added at the rate of 0.2% w/v into chitin specific media, followed by pouring into sterilized plates. DF-minimal salt media was autoclaved. After solidification of media, at five equidistant places bacterial isolates were inoculated. At 28 ±1ºC plates were inoculated for 72 to 96 hours until around the plates clearing zone was developed.

***Phosphate solubilization assay*:** Capability to solubilize inorganic phosphate was assessed from the bacterial isolates. In this essay, agar medium having tri-calcium phosphate as an inorganic phosphate was employed (Goldstein, 1986). After culturing the bacterial isolates, a loop full of each isolate was implanted on the agar petri-plates and placed in incubator for seven days at 28±1 °C. Around the colonies, clearing zone was developed after 6-7 days which was an indicator for phosphate solubilization.

***Indol-3-acetic acid production*:** The procedure of Sarwar et al. (1992) was adapted to find out bacterial auxin production. Luria Bertani (LB) media@25 mL was autoclaved, cooled and 1 g L^{-1}L-tryptophan was added to the media and inoculated with bacterial strains. After inoculation, incubation was done at 28 ± 1 °C and filtered through filter paper. Then 3 mL filtrate was collected from the filtered sample and 2 ml Salkowski's reagent was added. For comparison un-inoculated control with LB broth and L-tryptophan alone was prepared. Sample was run on spectrophotometer at 535 nm. Intensity of the color was determined by using the standard curve.

***Catalase activity*:** Catalase (peroxidase) is an enzyme that breaks down the hydrogen peroxide to water and oxygen. For determination of catalase production, a loop full of respective bacteria was placed on a slide and 1-3 drops of hydrogen peroxide (H_2O_2) were added. At the same time, the development of rapid and sustained bubbling indicates as catalase positive (Graham and Parker, 1964).

***Growth chamber trial*:** A glass trial was conducted under axenic condition to screen out the bacterial isolates based on growth promotion in growth chamber (KK-750 TOP$^+$ FIT P). Top performing 11-isolates based on microbial characters were selected for growth promotion trial. Inoculum of respective bacterial strains were prepared in 250 mL conical flasks and placed in the shaking incubator at 28 ± 2℃ for 3 days. Potato tubers were dipped in selective bacterial inoculum prior to sowing.

***Determination of growth parameters*:** Agronomic attributes such as shoot length (SL), shoot fresh weight (SFW), shoot dry weight (SDW), root length (RL), root fresh weigh (RFW) and root dry weight (RDW) was recorded at harvesting. Plant samples were placed in an oven at 70℃ till constant weight to get the dry weight.

***Determination of chlorophyll a, b and carotenoids*:** Fresh potato leaf sample (0.5 g) was thoroughly mixed with 80% acetone (v/v) and filtered through filter paper. The filtrate sample was run on spectrophotometer at 663, 645 and 480 nm, respectively for a, b and carotenoids (Arnon, 1949).

RESULTS

***Iron solubilizing assay*:** Iron is one of the major micro-nutrients and deficiency of iron is prevailing throughout the World mostly in developing countries leading towards anemia. From potato rhizosphere, out of 178 bacterial isolates, 53 isolates were found to be positive for solubilizing iron. Iron in the ferric (Fe^{+3}) form is not taken up by the plant. The microbes release specific organic acid which influences the pH of rhizosphere soil to convert the ferric form of iron (Fe^{+3}) to plant available ferrous form (Fe^{+2}), as pH is the major factor affecting the iron availability (Table 1).

***Production of siderophore*:** Siderophore improves the iron nutrition through iron chelation in the rhizosphere. PGPR secrets specialized iron chelating low molecular compounds known as siderophore which enhance the availability of iron to plants (Khalid et al., 2015; Arora et al., 2013). These iron chelating siderophores reduce the availability of iron to pathogens through making iron chelation (Munees and Mohammad, 2009). Siderophore was marked by the production of transparent halo-zone. Out of 53 isolates, 11 isolates were found capable of producing siderophore. Siderophore produced from rhizosphere isolates can promote the plant growth through improving the nutrient use efficiency (Table 1).

***Indole acetic acid production (IAA*):** Auxin is involved in cell division, cell differentiation and elongation and most importantly in gene regulation. IAA is a natural phytohormone which shows all activities of auxin and affects plant physiological activities. The potential of bacterial isolates to produce auxin was recorded through pink color after adding Salkowski reagent. In current experiment, all the bacterial isolates were capable of producing IAA. The maximum IAA was recorded with K-10 (15.61 ug mL^{-1}), followed by O-13 (19.35 ug mL^{-1}) (Table 1).

***Phosphorous solubilization*:** Phosphorous is one of the principal macronutrients required for the proper plant growth and development. Pakistani soils have the problem of phosphorous fixation due to alkaline conditions. The respective bacterial isolates were examined for their potential to phosphorous solubilization. All the bacterial isolates were capable of solubilizing phosphorous (Table 1).

***Catalase production*:** Catalase/peroxidase is an enzyme that brings about breakdown of hydrogen peroxide to oxygen and water. A loop full of bacterial colony was placed at five equidistance places on a glass slide and 1-3 drops of H_2O_2 were added. The development of sustained and rapid bubbling indicates as catalase positive (Graham and Parker, 1964). The results of catalase activity are explained in table. Except the two bacterial isolates D-14 and D-26, all the remaining isolates were found capable of producing catalase (Table 1).

***Chitinase test*:** All the bacterial isolates were tested against chitinase activity. A loop full of bacterial isolates were placed at five different places on agar petri-plates with specific source of chitin (0.05% chitin w/v) and placed in incubator at 28±1°C. The production of halo zone is a positive sign for chitinase activity. All the bacterial isolates were found negative with chitinase activity (Table 1)

***Growth chamber trial*:** For growth promotion trial, the pre-isolated 11-iron solubilizing and siderophore producing bacterial isolates were used. After 45 days, growth and physiological parameters were studied. The experiment was planned under completely randomized design (CRD).

***Agronomic parameters*:** Inoculation with iron solubilizing and siderophore producing bacteria significantly improved the agronomic parameters of potato (Table 2). The shoot and root attributes of potato plant manifested a positive response towards inoculation with selective iron solubilizing and siderophore producing bacterial isolates. Maximum shoot length of potato was observed in strain O-13 (28.167 cm) and it was found statistically significant over all microbial isolates except K-10 (25.83 cm) and D-19 (22.67 cm). Inoculation with bacterial isolates significantly improved the shoot dry weight of potato. Potato plants have fibrous root system and it needs to be extended more into the soil to improve nutrient uptake efficiency. The root attributes of potato were also

Table 1. Microbial characterization of rhizobacteria isolated from potato rhizosphere.

Sr. No.	Isolates	Iron Solubilization	Siderophore production	P-Solubilization	IAA (ug mL^{-1})	Catalase activity	Chitinase activity
1	C-17	+	++	++	8.23 e	++	_
2	C-22	++	++	++	8.33 e	++	_
3	D-4	+	++	++	8.34 e	++	_
4	D-7	++	++	++	10.38 e	++	_
5	D-14	++	++	++	10.68 de	++	_
6	D-19	+	++	++	11.37 c-e	++	_
7	D-26	++	++	++	14.08 b-d	++	_
8	K-7	++	++	++	14.52 bc	++	_
9	K-10	++	++	+++	17.50 ab	++	_
10	O-1	++	++	++	15.70 b	++	_
11	O-13	++	++	++	19.39 a	++	_

Mean sharing same letter/letters don't differ significantly ($p \leq 0.5$)

Table 2. Effect of bacterial inoculation on potato agronomic parameters.

Strains	SL (cm)	SFW (g)	SDW (g)	RL (cm)	RFW (g)	RDW (g)
Control	7.50 g	5.48 e	2.25 d	4.85 e	3.35 f	1.34 f
C-17	11.67 ef	8.65 cd	3.53 b-d	7.50 cd	4.17 ef	1.67 ef
C-22	14.33 d-f	10.67 b-d	4.36 bc	9.00 bc	5.33 de	2.13 de
D-4	14.83 de	8.17 c-e	3.34 cd	7.67 cd	4.08 f	1.63 f
D-7	16.67 de	9.33 b-d	3.81 bc	9.00 bc	5.59 d	2.24 d
D-14	17.17 c-e	7.82 de	3.19 cd	9.83 bc	6.05 cd	2.42 cd
D-19	22.67 a-c	8.33 c-e	3.40 cd	9.00 bc	5.56 d	2.23 d
D-26	19.00 cd	11.83 b	4.83 b	11.33 b	7.37 b	2.95 b
O-1	16.17 de	11.00 bc	4.49 bc	8.33 c	7.05 bc	2.82 bc
O-13	28.17 a	18.18 a	7.43 a	15.67 a	10.16 a	4.06 a
K-7	22.33 bc	8.83 b-d	3.61 bc	11.17 b	7.67 b	3.07 b
K-10	25.83 ab	20.50 a	8.36 a	17.20 a	11.21 a	4.48 a

Mean sharing same letter/letters don't differ significantly ($p \leq 0.5$)

improved through inoculation. The root fresh weight (RFW) and root dry weight (RDW) was enhanced significantly up to two-fold through inoculation with O-13 and K-10. Overall, potato growth was significantly improved through inoculation as compared with uninoculated control (Table 2).

***Physiological parameters*:** Inoculation with iron solubilizing and siderophore producing bacteria not only improved the agronomic attributes but also improved the physiological attributes of potato crop (Table 3).

Table 3. Effect of bacterial inoculation on physiological parameters of potato.

Strains	SPAD	Chl a (mg kg^{-1})	Chl b (mg kg^{-1})	Carotenoids (mg kg^{-1})
Control	21.83g	7.33f	4.20d	3.08h
C-17	35.73c-e	12.33de	6.83bd	4.33gh
C-22	31.57ef	14.00cd	7.27bc	5.52e-h
D-4	37.33c	9.33ef	5.63cd	6.83d-g
D-7	34.17c-e	13.93cd	6.93b-d	5.03f-h
D-14	29.93f	16.37bc	8.82b	9.33b-d
D-19	32.17d-f	13.83cd	6.47b-d	7.43d-f
D-26	34.87c-e	9.57ef	7.50bc	10.33bc
O-1	36.67c	10.17ef	6.47b-d	8.17c-e
O-13	47.03a	21.33a	12.67a	13.48a
K-7	36.17cd	11.17de	8.57b	6.48e-g
K-10	41.77b	17.90b	14.83a	11.50ab

Mean sharing same letter (s) don't differ significantly at $p \leq 0.5$

Soil plant analysis development (SPAD) meter was used to measure the SPAD. All the bacterial isolates showed positive response in terms of increasing SPAD. The maximum SPAD were observed in strain O-13 and K-10, 44.86 SPAD and 41.76 SPAD, respectively. Inoculation with respective bacterial isolates showed statistically significant results over uninoculated control. The minimum SPAD were observed in uninoculated control (21.83 SPAD). Inoculation significantly improved the chlorophyll a, chlorophyll b and carotenoids of potato plant up to one fold as compared with uninoculated control. The maximum chlorophyll a (21.33 mg kg^{-1}) and b (12.67 mg kg^{-1}) were recorded through inoculating the plants with O-13 (Table 3). Applications of iron solubilizing and sideophore producing bacteria significantly improved the carotenoid contents of potato. The inoculation with O-13 significantly improved the carotenoids contents as compared with uninoculated control. However, inoculation with C-17, C-22 and D-7 was statistically non-significant over uninoculated control. Maximum carotenoids were recorded in O-13 (13.48 mg kg^{-1}) followed by K-10 (11.5 mg kg^{-1}) and D-26 (10.33 mg kg^{-1}). This showed the significant effect of rhizobacteria on physiological attributes of potato.

DISCUSSION

Plant growth promoting rhizobacteria play a vital and prominent role in biogeochemical cycling of nitrogen, phosphorous, potassium and other important nutrients to maintain the sustainable agriculture (Caldwell, 2005). The microbes through different direct or indirect mechanism like hormonal production such as auxin, gibberellins and cytokinin or increase in nutrient concentration improved the plant growth, development, physiological processes and yield, leading toward more promising, efficient, cost effective and sustainable approach (Mushtaq *et al.,* 2020). This is true reflection of the efficacy of the microbes in promoting plant growth, biomass, yield and increased nutrient uptake (Prasanna et al., 2015). Plant-microbe interactions are the principal factors determining the soil fertility, plant health and productivity. Bacterial inoculants can contribute to enhance the agronomic attributes through mitigating the environmental pollution and production costs (Souza et al., 2015). Therefore, it can be concluded that growth promoting traits of microbes and their root colonization lead towards crop growth and development (Kabiraj et al., 2020). These associations not only affect growth, development and yield by improving nutrient uptake mechanism, but also develop a

Table 4. Relationship (Pearson correlation coefficient, r) of different variables of potato under axenic conditions.

	Chl "a"	Chl "b"	Carotenoids	SPAD	SL	SFW	SDW	RL	RFW
Chl b	0.7806	-	-	-	-	-	-	-	-
Carotenoids	0.6449	0.7193	-	-	-	-	-	-	-
SPAD	0.5929	0.6575	0.7161	-	-	-	-	-	-
SL	0.6811	0.7567	0.7709	0.7153	-	-	-	-	-
SFW	0.6903	0.7892	0.7667	0.7685	0.7125	-	-	-	-
SDW	0.6917	0.7962	0.7623	0.7671	0.7139	0.9900	-	-	-
RL	0.7360	0.8579	0.7936	0.7360	0.8523	0.9058	0.9056	-	-
RFW	0.6593	0.8396	0.8285	0.7342	0.8481	0.8921	0.8927	0.9366	-
RDW	0.6400	0.8500	0.8400	0.7500	0.8600	0.9000	0.9134	0.9455	1.0000

stable core rhizosphere microbiome (Hirsch and Mauchline, 2012).

The growth promotion of wheat, maize and rice through PGPR have been reported by many scientists, however little information is available on the screening and use of rhizobacteria isolated from rhizosphere of potato (Aloo *et al.,* 2020). In current study, all the bacterial isolates were found capable of solubilizing phosphorous, which indicated that numerous numbers of phosphorous solubilizing bacteria are present in the soil (Reyes *et al.,* 2006). An established phytohormone IAA played a vital role as plant growth regulator (Zahir et al., 1997). Under axenic conditions inoculation with bacterial isolates significantly improved the agronomic and physiological parameters of potato. Zahir *et al.* (1997) inoculated the potato tuber with *Azotobacterial* and found significant increase in plant growth. The interactive effect of iron chelating factor (EDTA and EDDHA), mycorrhizal colonization and rhizobacterial strains had found to improve fresh and dry tuber weight (Baradar *et al.,*2015). Vosatka and Gryndler (1999) stated that combination of *Pseudomonas putida* and AMF increased the physiological and agronomic characters of potato. Pearson correlation was checked between agronomic and physiological parameters and according to the results (Table 4), it was found that agronomic and physiological parameters are strongly correlated with each other under axenic condition.

In current experiment improvement in agronomic attributes and physiological parameters were found significant in inoculated potato plants as compared with uninoculated control, which provides a baseline information for selection of rhizobacteria to be used as PGPR for improving the growth of potato. Further investigation is in progress to develop a correlation between different plant growth promoting traits tested in laboratory and their effect on plant growth, development and yield in pot and field conditions.

***Conclusion*:** The use of PGPR could be a sound option to increase the yield of potato. PGPR may serve as a source of microbial base fertilizers, which can improve plant growth and development, also leads towards the reduced use of chemical fertilizers. The use of natural microorganism to reduce the excessive application of chemical fertilizers and pesticides could be a sound, cost-effective and environmentally friendly approach to increase the physiological and agronomic parameters of potato. PGPR facilitate the plant through improving nutrient uptake such as solubilization of phosphorous and iron, siderophore production, and stimulate the hormonal production like IAA.

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[Received 25 Aug 2020; Accepted 15 Oct 2020; Published (online) 11 Jan 2021]