Research Article

PGP Characterization of Rhizobacteria Associated with Apple Gourd (*Praecitrullus fistulosus* L.)

Taqi Raza[1*], Sergio de Los Santos Villalobos[2], Muhammad Shehzad[3], Shakeel Imran[4] and Derly José Henriques da Silva[5]

[1]Land Resources Research Institute, National Agriculture Research Center, Islamabad, Pakistan; [2]Instituto Tecnológico de Sonora, 5 de febrero 818 Sur, C.P. 85000, Col. Centro, Cd. Obregón, Sonora, México; [3]Government college university Lahore, Lahore, Pakistan; [4]University of Agriculture Faisalabad, UAF Sub-Campus Burewala 61010, Pakistan; [5]Breeding and Genetic Resource of Vegetable - Public University of Viçosa, Brazil.

Abstract | Plant growth-promoting rhizobacteria (PGPR) are beneficial group of microorganisms which improve plant and soil health by enhancing nutrient availability and protecting plant against stresses. The current study was carried out to characterization of rhizobacteria associated with Apple Guard (*Praecitrullus fistulous* L.). Morphological, qualitative and quantitative tests were performed to characterize the PGPR abilities of isolated rhizobacteria. Lab study indicated that isolated bacterial strains have morphologically different colonies, shapes, and colors. The isolated strains mostly belonging to *Coccus, Bacillus, and Spirillum*. Nearly, 63% belonging to *Coccus*, 23% to *Bacillus*, and 14% to *Spirillum*. Only 18% of strains gave the gram positive staining results and 14% strains showed the hydrogen cyanide (HCN) production abilities by slightly changing the color on media. While, in the case of enzyme production test, almost 90%, of isolated strains confirmed the amylase and pectinase production activities and 36% confirms the protease activities. Similarly, 82% and 45% of isolates confirmed the solubilization of zinc (Zn) and phosphorus (P) respectively. Almost, the entire isolated strains were given positive results for ammonia (NH4), nitrogen fixation, and indole acetic acid (IAA). Furthermore, for exopolysaccharide (EPS) test, 73% of isolated strains confirmed the EPS production. The conclusion revealed that almost all isolated bacterial strains exposed the growth-promoting abilities and may be used as bio-fertilizer to enhance crop production.

Received | July 10, 2020; **Accepted** | October 08, 2020; **Published** | December 19, 2020
***Correspondence** | Taqi Raza, Land Resources Research Institute, National Agriculture Research Center, Islamabad, Pakistan; **Email:** taqiraza85@gmail.com
Citation | Raza, T., S.L.S. Villalobos, M. Shehzad, S. Imran and D.J.H. Silva. 2020. PGPR characterization of rhizobacteria associated with apple gourd (*Praecitrullus fistulosus* L.). *Pakistan Journal of Agricultural Research,* 33(4): 926-939.
DOI | http://dx.doi.org/10.17582/journal.pjar/2020/33.4.926.939
Keywords | Morphological, Qualitative and quantitative characterization, Apple guard and Bio-fertilizer

Introduction

Microbial communities are an important component involved in the organic matter transformation, nutrient cycling, formation and aeration of soil aggregates, carbon sequestration, xenobiotic bioremediation, plant/pathogen growth promotion and regulation. Similarly, these communities also develop significant tolerance to plants against the abiotic stress (Santos *et al*., 2016), being responsible for carrying out 80 to 90% of biological processes in soil (Nannipieri and Badalucco, 2003). Most of beneficial or detrimental plant and microorganisms interactions occurs in close rhizosphere, in other words in a micro-ecological zone which is direct proximity with bunch of roots (Lopez-Raez *et al*., 2017; Valenzuela-Aragon *et al*., 2019). These microorganism, especially bacteria, showed aptitude to stimulate plant basic development, improve health, and also restoration of soil fertility, thus named Plant Growth-Promoting Rhizobacteria

(PGPR) (Montoya *et al*., 2020; Padilla *et al*., 2020). Most of times, beneficial interaction of PGPR improves the growth along development through two mechanisms; direct or/by indirect (Pathak *et al*., 2020). The influence of PGPR either direct or indirect depends on the release of metabolites which stimulate the growth and development of plant. Thus, several mechanisms have been postulated to explain beneficial role of PGPR on the host plant, such as: the ability to produce phyto-hormones (Yasmi *et al*., 2020; Valenzuela-Ruiz *et al*., 2019); enhancing a symbiotic N_2 fixation (El-Akhdar and Ghazi, 2019; Bechtaoui *et al*., 2020), solubilizing the inorganic form of phosphate, mineralization of organic form of phosphate and likewise many other important basic nutrients (Razakhani *et al*., 2019). Similarly, siderophores production, enzymes production, and synthesis of antibiotics (Villarreal-Delgado *et al*., 2018; Bajracharya, 2019). At present, most of bacterial strains have been reported associated with genus *Bacillus, Pseudomonas*, *Azotobacter*, *Enterobacter and Azospirillum*, and this genus also called as PGPR (Valenzuela-Aragon *et al*., 2019; El-Sayed and Hagab, 2020). Vide number of research works have conveyed, *Bacillus* spp strains are more affective as they are prevailing in soil. These strains are also well notorious in case of production siderophores and antimicrobial activities (Villarreal-Delgado *et al*., 2018). *Bacillus* strains, in addition have the ability to produce biosurfactants, as a specific mechanism that involved in biological control agent against phyto-pathogens through quorum sensing phenomenon. This strains also have one more important role which is initiation of resistance in plant system (Villa-Rodríguez *et al*., 2019). However, significant number strains which also consider as PGPR do not show the positive expected effect in agriculture might be due to inability to i) colonization in plant tissues, or ii) bio-synthesize metabolites involved in plant growth regulation; both due to the plant genotype, soil type, climatric conditions, other many agricultural practices, and native microbial communities (Bhatt and Maheshwari, 2020). Inoculation of soil, seed or plant seedling with PGPR may have number of positive and significant impacts on plant growth and its development, like improvement in seed and seedling germination, seedling vigor and health, nutrient availability and acquisition, chlorophyll contents, nodulation in leguminous crops and ultimately enhance the crop growth and yield attributes (Lata and Gond, 2019; Singh *et al*., 2019).

Apple gourd (*Praecitrullus fistulosus*) is a summer vegetable a rich source of carbohydrates, fiber, thiamine, riboflavin as well as energy. In view of health point, Apple gourd regulated the cholesterol level, control the blood pressure, reduce the chances of heart attack and reduce the chances of cancer (Tyagi *et al*., 2017). PGPR associated with vegetables play role of key to achieve sustainability in agro-industry (Gouda *et al*., 2018). Thus, application of plant associated PGPR especially with vegetables and leguminous crops has been gained success and proven as environment friendly approach to enhance the crop production through direct or indirect mechanisms. In literature, number of bacterial species described which work as PGPR and proved successful in application in the field of agriculture which are playing a significant role in improving crop yield (Backer *et al*., 2018). Objectively, current research study was conducted to assess cultivable bacterial communities associated with rhizosphere, rhizoplane, and endosphere of Apple Gourd, and also to identify plant growth-promoting traits in these strains for further study in bio fertilizer production.

Materials and Methods

Study site and sample collection

This study was conducted at University of Agriculture Faisalabad sub-campus, Burewala, Pakistan. For sampling, fully matured at fruiting stage, Apple Gourd plants samples were collected from vegetable research area during summer, 2018-19. For sampling purpose Apple Gourd plants with adhered soil (rhizosphere) were uprooted and save in polythene bag, and preserved at room temperature. About 1 gram rhizosphere soil was taken from each sample and further used it for experiment in lab.

Bacteria isolation

Bacterial isolation was carried out by dependent culture techniques, where 1.0 g of each sample was placed in a 25mL Erlenmeyer flask consist of 9mL sterile at 121°C with pressure of 15 psi for duration of 20 minutes in distilled water. Samples were homogenized in a rotatory shaker at 150 rpm for duration of 1 hour only. Serial dilutions (1:10) were prepared until 10^{-6}. Then, 100 µL of each serial dilution was pouring on Petri dishes containing LB agar medium having pH 7. Inoculated Petri plates were placed in an incubator to accomplish their incubation at 37°C for 2 days. Bacterial population in each sample was estimate by Colony Forming Units

(CFU) technique (Villa-Rodríguez *et al*., 2016). Bacterial colonies having different macroscopic and microscopic (Gram staining) morphology, growth pattern, were picked and purified according to the procedure described above.

Metabolic characterization of bacterial strains

Amylase activity: Amylase activity by bacterial strains was determined by agar plate method enriched with starch according to Capuccino and Sherman (2002). Bacteria strains were streaked on agar containing plates enriched with starch and incubated in incubator for almost 1-2 days. The ability to produce amylase by studied strains was confirmed after application of iodine solution on plates through dropper and plates were kept for ten minutes in iodine solution and the discarded he solution from plates. Amylase producing bacteria were showed clear zone in the plates when plates enriched with starch and placed in incubator for ten minutes at 150rpm and temperature 4°C (Hols *et al*., 1994). The diameter of halo zone (cm) was measured.

Protease activity: Protease production activities of cultured strains were identified by skim milk agar medium as described by the method of Smibert and Krieg (1994). The per litter skim media consist of agar, casein (digested pancreatic), yeast extract and glucose 15g, 5g, 3g and 1g respectively, with 1% solution of skim milk. Isolated bacteria strains were streaked on milk media containing plates. After two hours the protease activity of strains was confirmed through formation of evident growth zone around colonies.

Pectinase activity: This enzymatic activity of strains were measured using a method proposed two Indians, Raju and Divakar (2013). All the bacterial-strains were culture on VM-ethanol enriched media and growing culture washed in 0.9% NaCl and their optical density adjusted at 578nm. About 10 µl suspension of bacteria was spotted PSAM (pectinase screening agar media) which amended with 1 gram per litter pectin. These plated were placed in incubator for 2 days at room temperature. Plats were rained with 50mM solution of concentrated Iodine. Pectinase activity of strains was confirmed evident halo zone formation around spotted colonies of bacteria after removal of Iodine solution from plates.

Production of hydrogen cyanide (HCN)

All studied bacterial strains were tested to assay their ability to produce hydrogen cyanide (HCN) by fowling the Castric method (Castric, 1975). Thus, 4.5 g/L glycine was added to nutrient broth medium and strains were consciously streaked on that medium; then, a 2% solution of sodium carbonate mixed in 0.45% picric-acid, Whatman filter paper (size No. 2) dipped in it and placed the paper on upper lid of plate. A changing in paper color from orange to red confirmed the production of HCN by bacterial strains.

Exo-polysaccharide production (EPS)

EPS production by strains was determined through the method of spot plate using RCV-sucrose media which was amended with Weavers medium (Amellal *et al*., 1998).

The EPS medium consists of KH_2PO_4 (0.2g), K_2HPO_4 (0.8g), $MgSO_4.7H_2O$ (0.2g), $CaSO_4.2H_2O$ (0.1g), $FeCl_3$ (2.0g) and traces of $Na_2MoO_4.2H_2O$. This medium amended with Yeast Extract (0.5g) and Sucrose (2.0g) and maintained the medium pH 7. Bacterial strains were streaked on sugar media which was ten times diluted with another TSA (tryptic soy agar). ESP bacteria strains produce slimy type of growth on the agar plates. Well growth EPS producing bacterial strains were further purified by streaking on plates using TSA medium. Furthermore, theses purified bacterial strains once again were grown on the LB (liquid broth) and continuously agitated on shaker at 30°C for overnight.

ZN- solubilization

The Zn solubilization activity of strains was determined throught Bunt-Rovira Agar medium (Bunt and Rovira, 1955). All the strains were carefully streaked on LB medium and kept for overnight for proper growth. Around 5µl suspension of each separate bacterial strain with normalized 0.5 optical density was placed on plates containing Bunt-Rovira Agar. These inoculated plates were instantly incubated for a period of 36–96 hours at 30°C. Formation of halo zone around colonies confirmed the ability of strains to solubilize Zn. The diameter of these halo zones were measured to quantify Zn solubilization.

Phosphate solubilization (PSB)

This activity was detected in the Pikovskaya Agar media (Johri *et al*., 1999). All studied bacterial strains were streaked on Pikovskaya agar medium after all placed for time of 1 to 5 days in orbital shaker at 37°C. P-solubilizing activity by strains was confirmed

through clear zone formation around bacterial colonies.

Nitrogen fixation test

For this trait, a nitrogen free media (Malate medium) emended with bromothymol blue was prepared (Okon *et al*., 1977) in Erlenmeyer flasks (250ml). Bacterial colonies through sterilized lop from inoculated culture plates were added into broth contain flasks and incubated at 10rpm for 1 day at 37°C. The nitrogen fixers produced a blue halo on the culture plates. The color halo was measured by calculating the diameter of color zone.

Ammonia production

The ammonia production ability of studied bacterial strains was assessed in peptone containing water. Peptone water was prepared by adding 10g peptone in 1 litter distilled water and 7.4 pH of this water maintained. Fresh culture of bacterial strains incubated at temperature (37 ± 2°C) for duration of 48 to 78 hours in test tubes. After all, every cultural tube was amended with regent of Nessler (0.5 ml), and color changing from brown to yellow was recorded which confirmed positive test in case ammonia of produc[illegible] (Cappuccino and Sherman, 1992).

Indole compound production

Production of indole compound[illegible] was assured using the sp[illegible]hotome[illegible]ic with modified method given b[illegible]ic *et al*., [illegible]991. In addition, concentration of indol[illegible] prod[illegible]tion was quantitatively essayed through m[illegible]thod [illegible]opos[illegible] by scientists Loper and Scoroth, [illegible]86 [illegible]sing [illegible]ptophan at 58 µg/ml. Strains were g[illegible] LB Broth media for 48 to 72 hours at 3000 rp[illegible] for 25 minutes at 28±2°C. Then, 2 ml supernatant was amended in 4 ml Salkowski reagent with orthophosphoric acid almost 3 drops. Indole production was confirmed by the changing of pink color of broth and absorbance was taken with UV/ visible spectrophotometer at 535 nm. Hi- media with range 10-100 µg/ml used as standard to measure the indole production by strains using standard curve method.

Statistical analysis

One way analysis was carried out by ANOVA (analysis of variance) test to calculate significant difference among variables. At the same time, statistic 8.1 software was used to compare the results using Tukey–Kramer test having P value less than 0.05.

Results and Discussion

Bacterial population and morphological diversity

The morphological characterization of isolates obtained from Apple gourd rhizosphere showed that most of the bacterial strains were coccus or bacillus. Similarly, color of strains was varied from pink to purple. Likewise shape, colonies of isolates also varied among round, spherical and filamentous irregular shapes. Out of 22 strains, 4 strains (AGRS4, 7, AGRP3 and AGRO3) strains were clearly gave gram positive, and leftover all were gave the Gram negative rests. Complete description of morphological characteristic of isolated strains is given in (Table 1).

Table 1: *Morphological [illegible] of isolated from apple gourd.*

No.	ID	[illegible]ource	[illegible] staining	Observation	Shape
1	AGR[illegible]	Rh[illegible]sph[illegible]	Pink	Negative	Coccus
2	[illegible]GRS3	Rhizosphere	Pink	Negative	Coccus
[illegible]	AGRS4	Rhizosphere	Purple	Positive	Bacillus
4	AG[illegible]S5	Rhizosphere	Pink	Negative	Coccus
5	[illegible]GRS6	Rhizosphere	Pink	Negative	Coccus
[illegible]	AGRS7	Rhizosphere	Purple	Positive	Bacillus
7	AGRS9	Rhizosphere	Pink	Negative	Coccus
8	AGRS10	Rhizosphere	Pink	Negative	Bacillus
9	AGRS11	Rhizosphere	Pink	Negative	Coccus
10	AGRP1	Rhizoplane	Pink	Negative	Coccus
11	AGRP2	Rhizoplane	Pink	Negative	Coccus
12	AGRP3(a)	Rhizoplane	Pink	Negative	Spirillum
13	AGRP3(b)	Rhizoplane	Purple	Positive	Spirillum
14	AGRP4	Rhizoplane	Pink	Negative	Spirillum
15	AGRP5	Rhizoplane	Pink	Negative	Coccus
16	AGRO1	Endosphere	Pink	Negative	Coccus
17	AGRO2	Endosphere	Purple	Positive	Coccus
18	AGRO3	Endosphere	Pink	Negative	Coccus
19	AGRO4	Endosphere	Pink	Negative	Bacillus
20	AGRO5	Endosphere	Pink	Negative	Coccus
21	AGRO6	Endosphere	Pink	Negative	Coccus
22	AGRO7	Endosphere	Pink	Negative	Bacillus

Enzymes production activities

During amylase production activity test, most of isolates showed the ability to produce amylase. Out of 22 strains, 20 strains (AGRS1- 11, AGRP1, 2, 3 and AGRO1 -7) developed clear zone around the bacterial colonies (Figure 2), which showed positive results

while only 2 strains (AGRP3, 4) did not confirmed the positive results (Table 2). While during protease production activity test, only 8 isolates (AGRS1, 3, 4, 9, and AGRO2, 4, 5, 7) showed clear zone around the colonies (Figure 2), which confirmed the positive test for protease production of isolates. However, rest strains did not form clear zone around the bacterial colonies in protease production test (Table 3). In case of pectinase test, 20 strains (AGRS1-11, AGRP1-5 and AGRO1-7) showed positive results through clear zone formation around colonies (Figure 3) and only 02 (AGRP 4 and 5) isolates showed negative results (Table 4).

Figure 1: *Amylase production by isolates associated with Apple Gourd.*

Figure 2: *Protease production by isolates associated with Apple Gourd.*

Qualitative tests

Out of 22 isolates, only 3 isolated strains (AGRS1, 10, and AGRO1) showed slightly change in color which represent positive for HCN production while all others (AGRS2-9 and 11, AGRP1-7 and AGRO2-7) no changes of the color in plates which represented negative results for HCN production (Figure 4, Table 5). While, in case of EPS test, 16 isolated strains (AGRS1-10, AGRP1, 3 and AGRO1, 4, 5, 6, and 7) showed positive results while rest strains are negative strains (Figure 5, Table 6). 10 strains (AGRS7, 9, 10, AGRP1, 3 and AGRO1-7) showed positive results while rest strains showed negative results. Similarly, positive strains developed clear zone around the colonies while negative did not form zone around colonies during Zn-solubilizing test (Figure 6, Table 7). out of 22, 18 strains (AGRS1, 4-11, AGRP1,3 and AGRO1-7) showed positive results by formation of zone around the colonies and rest strains showed negative results during phosphate solubilizing bacteria test (Figure 7, Table 8). When Nitrogen fixation test was carried out surprising results were obtained. All bacterial strains (AGRS1-11, AGRP1, 3-7 and AGRO1-7) except strain (AGRP2) showed positive results in NH test (Table 9). While, in case of NH_4 production test, strains (AGRS1-11, AGRP1-7 and AGRO1-7) confirmed the NH_4 production activates of strains except strains (AGRS1-11, AGRP1-7 and AGRO1-7) showed in Figure 8, Table 10.

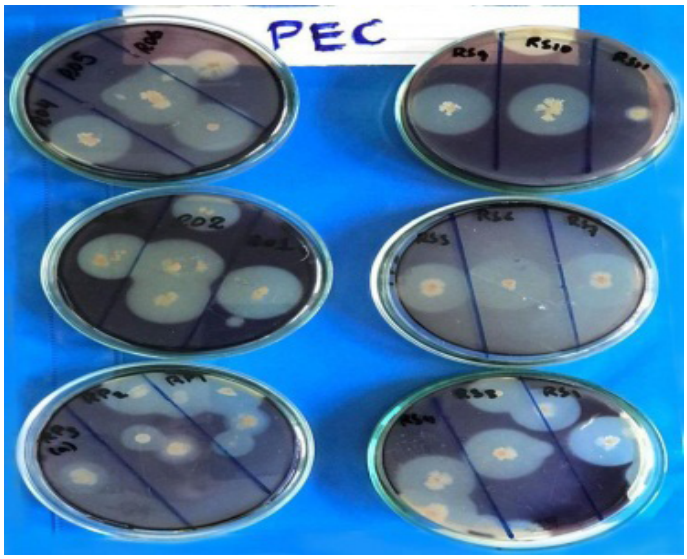

Figure 3: *Protease production by isolates associated with Apple Gourd.*

Quantitative test

Production indole acetic-acid and solubilization of phosphate was estimated by quantitative studies of isolated strains. All isolated strains (AGRS1-11, AGRP1-7 and AGRO1-7) showed positive results in indole acetic acid production test (Figure 9, Table 11). While only strains AGRS11, AGRP3 and AGRP6

Table 2: *Amylase production activities of isolates from apple gourd.*

No.	ID	Results	Z+C	C	SI	SE%	No.	ID	Results	Z+C	C	SI	SE%
1	AGRS1	+++	3.4	0.3	11.33	240%	11	AGRP2	+	1.8	1.5	1.20	80%
2	AGRS3	+++	3	0.3	10.00	200%	12	AGRP3 (a)	+++	2.9	0.45	6.44	190%
3	AGRS4	+++	3.3	0.35	9.43	230%	13	AGRP (b)	+	1.8	1.53	1.18	80%
4	AGRS5	+++	3.1	0.3	10.33	210%	14	AGRO1	+++	3.4	0.5	6.80	240%
5	AGRS6	+++	3.5	0.65	5.38	250%	15	AGRO2	+++	3.4	0.35	9.71	240%
6	AGRS7	+++	3.3	0.45	7.33	230%	16	AGRO3	+	2	0.25	8.00	100%
7	AGRS9	++	3	0.35	8.57	200%	17	AGRO4	+++	3.5	0.35	10	250%
8	AGRS10	++	3.2	0.35	9.14	220%	18	AGRO5	++	3.2	0.6	5.33	220%
9	AGRS11	+	1.1	0.5	2.20	10%	19	AGRO6	++	3	0.2	15	200%
10	AGRP1	+	2	1.65	1.21	100%	20	AGRO7	++	3.2	0.45	7.11	220%

Whereas; Z+C= Zone+Colony diameter; C: Colony diameter; SI: Solubilization intensity; SE: Solubilization efficiency; SE%= Z+C/C SI= Z-C/C ×100.

Table 3: *Protease production activities of isolates from apple gourd.*

No.	ID	Results	Z+C	C	SI	SE%
1	AGRS1	+++	2.1	0.45	4.67	110%
2	AGRS3	++	1.55	0.4	3.88	55%
3	AGRS4	++	1.7	0.45	3.78	70%
4	AGRS9	++	1.4	0.5	2.80	40%
5	AGRO2	++	1.6	0.6	2.67	60%
6	AGRO4	+	1.35	0.4	3.38	35%
7	AGRO5	+	1.4	0.4	3.50	40%
8	AGRO7	+	1.05	0.25	4.20	5%

Table 4: *Pectinase production activities of isolates from apple gourd.*

No.	ID	Results	Z+C	C	SI	SE%
1	AGRS1	+++	2.65	0.4	6.63	165%
2	AGRS3	+++	2.93	0.35	8.37	193%
3	AGRS4	+++	2.5	0.35	7.14	150%
4	AGRS5	+	2.5	0.35	7.29	155%
5	AGRS6	+++	2.85	0.5	5.70	185%
6	AGRS7	+++	3	0.45	6.67	200%
7	AGRS9	+++	2.95	0.3	9.83	195%
8	AGRS10	+++	3	0.25	12.00	200%
9	AGRS11	+	1	0.3	3.33	0%
10	AGRP1	++	1.56	0.35	4.46	56%
11	AGRP2	++	1.55	0.25	6.20	55%
12	AGRP3 (a)	++	1.8	0.25	7.20	80%
13	AGRP3 (b)	++	1.7	0.4	4.25	70%
14	AGRP4	++	1.6	0.35	4.57	60%
15	AGRP5	++	1.45	0.35	4.14	45%
16	AGRO1	+++	2.95	0.1	29.50	195%
17	AGRO2	+++	2.7	0.36	7.50	170%
18	AGRO3	+++	2.5	0.25	10.00	150%
19	AGRO4	+++	2.7	0.35	7.71	170%
20	AGRO5	+++	2.9	0.33	8.79	190%
21	AGRO6	++	1.65	0.35	4.71	65%
22	AGRO7	+++	2.2	0.35	6.29	120%

Table 5: *HCN production activities of isolates from apple gourd.*

No.	ID	Results	No.	ID	Results
1	AGRS1	+ve (slightly)	11	AGRP2	-ve
2	AGRS3	-ve	12	AGRP3(a)	-ve
3	AGRS4	-ve	13	AGRP3 (b)	-ve
4	AGRS5	-ve	14	AGRP4	-ve
5	AGRS6	-ve	15	AGRP5	-ve
6	AGRS7	-ve	16	AGRO1	+ve (slightly)
7	AGRS9	-ve	17	AGRO2	-ve
8	AGRS10	+ve (slightly)	18	AGRO3	-ve
9	AGRS11	-ve	19	AGRO4	-ve
10	AGRP1	-ve	20	AGRO5	-ve
21	AGRO6	-ve	22	AGRO7	-ve

Table 6: *EPS production activities of isolates from apple gourd.*

No.	ID	Results	No.	ID	Results
1	AGRS1	++	9	AGRP1	++
2	AGRS3	+	10	AGRP3 (a)	
3	AGRS4	+	11	AGRP (b)	+
4	AGRS5	+++	12	AGRO1	+
5	AGRS6	+++	13	AGRO4	++
6	AGRS7	+++	14	AGRO5	++
7	AGRS9	++	15	AGRO6	+++
8	AGRS10	+	16	AGRO7	+++

gave the high absorbance of phosphorus (Table 12) which strongly confirm the P solubilization activity of these isolates. Apple Gourd (*Praecitrullus fistulosus* L.) is cultivated as a vegetable worldwide and widely in Asian countries especially in Indo-Pak regions. But probably, origin is north as well as western areas of India, where wild types of apple gourd might

be still found. Apple Gourd is also cultivated as a vegetable in different Asian countries but more prominent in India, Pakistan and Afghan locations (Syed *et al.*, 2014). PGPR are microorganisms which can promote plant growth through production of Amonia, HCN, antibiotics, phyto-hormones, dominance over dangerous organism, solubilization and uptake of macro and micro nutrients, aggregate and colonization, and formation of root microbes niches (Velmourougane *et al.*, 2017). Several research studies are available that highlight the benefits as well as the screening method of microbial strains having plant growth-promoting trait from various vegetables especially potato, tomato, cucumber, chili, bitter melon but research studies for isolation and characterization of PGPR group from Apple Gourd with rhizosphere soil and their evaluation as bio-fertilizer are particularly limited.

Table 7: *Zn solubilization activities of isolates from apple gourd.*

No.	ID	Results	Z+C	C	SI	SE%
1	AGRS7	++	0.56	0.2	2.80	180%
2	AGRS9	+++	0.4	0.13	3.08	208%
3	AGRS10	+++	0.63	0.2	3.15	215
4	AGRP1	+	0.55	0.4	1.38	38%
5	AGRP3 (a)	+	0.51	0.45	1	13%
6	AGRO1	+++	0.75	0.25	3.00	200%
7	AGRO4	++	0.7	25		
8	AGRO5	+	0.5	35	1.5	57%
9	AGRO6	+++	0.5	0.	5.00	400%
10	AGRO7	++	0.7	0.25	2.80	180%

The rhizosph c b cteria ommunities were soil specific and ourd rhizosphere have all the right physic hemical properties which were basic for the batter growth of growth promoting bacteria like soil pH, moisture percentage, total nitrogen, organic carbon and some other (Singh *et al.*, 2017). The dominant PGPR in Apple Gourd rhizospheric soil were belonging to *Bacillus* sp. which confirmed that the PGPR of *Coccus and Bacillus* are effective PGPR and their positive role have already been recognized (Jamal *et al.*, 2018). Similarly, solubilization of phosphate was more frequently found in *Coccus* and *Bacillus* but lower in *Spirillum* sp. and was more evident when clear zone of phosphate solubilization formated around colonies on inoculated plates. Rhizospheric soil is rich with genera of P solubilizing bacteria than nonrhizospheric soil (Kumar *et al.*, 2012).

Table 8: *P solubilization activities of isolates from apple gourd.*

No.	ID	Results	Z+C	C	SI	SE %
1	RS1	++	1.8	0.2	9.00	80
2	RS4	+	1.55	0.15	10.33	55
3	RS5	+++	2.25	0.4	5.63	125
4	RS6	+++	2.4	0.3	8.00	140
5	RS7	+++	2.25	0.4	5.63	125
6	RS9	++	1.6	0.5	3.20	60
7	RS10	++	1.9	0.3	6.33	90
8	RS11	+	1.1	0.5	2.20	10
9	RP1	++	1.7	0.35	4.86	70
10	RP3 (a)	+	0.55	0.2	2.75	175
11	RP3 (b)	+	1.23	0.4	3.08	23
12	RO1	++	1.7	0.35	4.86	70
13	RO2	+	1.46	0.35	4.17	46
14	RO3	+	.33	.35	3.80	33
15	RO4	++		35	4.57	60
16	RO5	+	0.9	.3	3.00	200
17	RO6	+	2.15	0.55	3.91	115
18	RO7	+	.3	0.35	3.71	30

Table 9: *Nitrogen fixation activities of isolates from apple gourd.*

No.	ID	Results	No.	ID	Results
1	RS1	+	12	RP3 (a)	+++
2	RS3	+	13	RP3 (b)	+
3	RS4	++	14	RP4	+++
4	RS5	+	15	RP5	+
5	RS6	++	16	RO1	+
6	RS7	++	17	RO2	+
7	RS9	++	18	RO3	+
8	RS10	+	19	RO4	+
9	RS11	++	20	RO5	+
10	RP1	++	21	RO6	+
11	RP2	++	22	RO7	++

Table 10: *Ammonia production of isolates from apple gourd.*

No.	ID	Results	No.	ID	Results
1	AGRS1	++	12	AGRP3 (a)	+++
2	AGRS3	+	13	AGRP3 (b)	+++
3	AGRS4	+++	14	AGRP4	+++
4	AGRS5	+++	15	AGRP5	+++
5	AGRS6	+++	16	AGRO1	+++
6	AGRS7	++	17	AGRO2	++
7	AGRS9	+++	18	AGRO3	+++
8	AGRS10	+++	19	AGRO4	+++
9	AGRS11	+++	20	AGRO5	+++
10	AGRP1	++	21	AGRO6	+++
11	AGRP2	++	22	AGRO7	+++

Table 11: *Indole acetic acid production of isolates from apple gourd.*

No.	ID	Y (PPm)	No.	ID	Y (PPm)
1	RS1	3.77	12	RP3 (a)	0.94
2	RS3	3.95	13	RP3 (b)	7.72
3	RS4	0.55	14	RP4	5.05
4	RS5	2.48	15	RP5	0.18
5	RS6	4.96	16	RO1	51.46
6	RS7	7.53	17	RO2	0.83
7	RS9	0.74	18	RO3	3.49
8	RS10	6.52	19	RO4	3.40
9	RS11	4.41	20	RO5	5.88
10	RP1	5.97	21	RO6	5.24
11	RP2	5.15	22	RO7	5.15

Table 12: *Quantitative screening of isolates from apple gourd for P solubilization test.*

No.	ID	Y (PPm)	No.	ID	Y (PPm)
1	RS1	1.29	12	RP3 (a)	46.72
2	RS3	1.44	13	RP3 (b)	27.84
3	RS4	2.29	14	RP4	5.84
4	RS5	4.20	15	RP5	6.80
5	RS6	1.67	16	RO1	11.33
6	RS7	13.18	17	RO2	10.24
7	RS9	9.12	18	RO3	17.10
8	RS10	6.53	19	RO4	8.[illegible]
9	RS11	18.62	20	[illegible]05	12
10	RP1	2.36	21	RO[illegible]	1.20
11	RP2	10.53	22	RO7	14.09

Figure 4: *HCN production by isolates associated with Apple Gourd.*

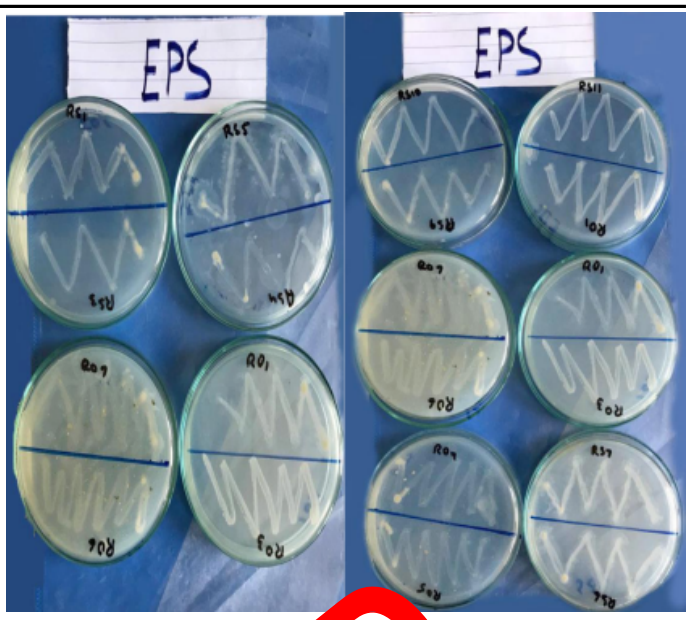

Figure 5: *EPS production by isolates associated with Apple Gourd.*

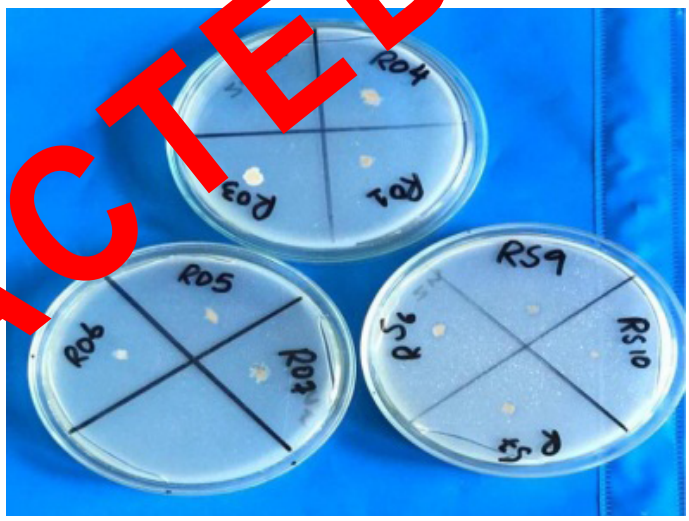

Figure 6: *Zinc solubilization by isolates associated with Apple Gourd.*

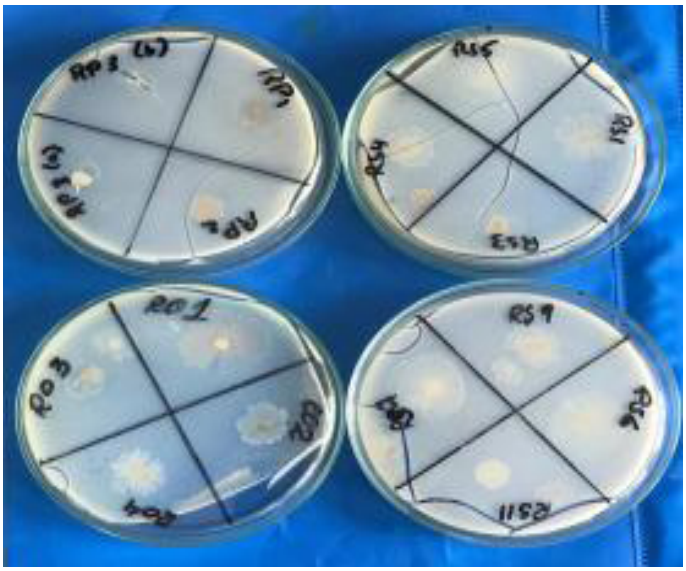

Figure 7: *Phosphorus solubilization by isolates associated with Apple Gourd.*

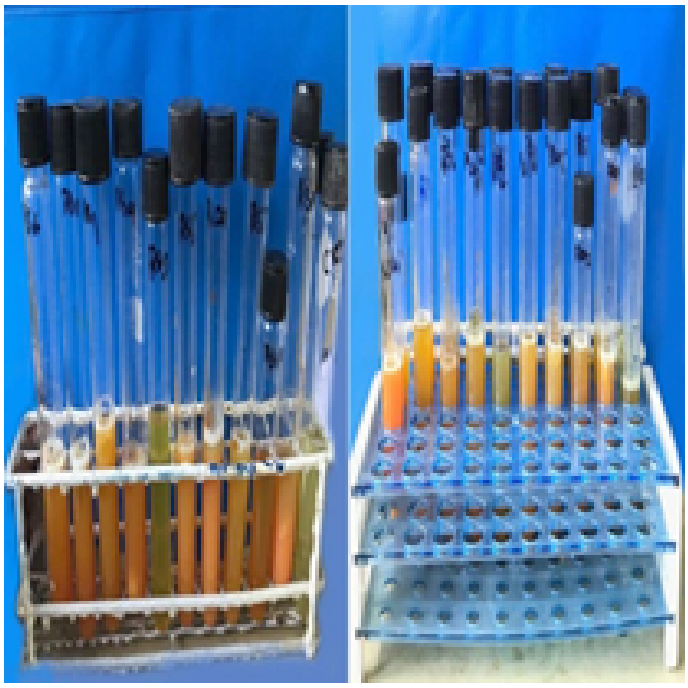

Figure 8: *Ammonia production by isolates associated with Apple Gourd.*

Figure 9: *Indole acetic acid by isolates associated with Apple Gourd.*

Indole Acetic-Acid production is a basic function of PGPR which recognized as an essential phytohormones. In addition, it also involved in the signal regulation of molecule which further involved in the functioning of plant development (Singh *et al.*, 2017). Production of NH4 and IAA by the PGPR is varies from one species to another species as well as from one microbial strains to another microbial strains which observed by multiple experiments. It also influenced by the growth stage, availability of substrate and condition of culture (Kumar *et al.*, 2019). Production of soluble phosphate and zinc (Shakeel *et al.*, 2015), and IAA are the basic characteristics of most of PGPR, and other associated microbial communities in rhizosphere (Paungfoo-Lonhienne *et al.*, 2019). Likewise, another basic which also consider a key trait of PGPR is NH4 production by specific strains of rhizospheric bacteria which influence the growth of plant. Likewise, ammonia production, siderophore and exo polysaccharide production is the common characteristics of PGPR which were also confirmed by the current experiment. Both are directly involved in the stimulation and biosynthesis of ani-microbial chemicals through improving the availability of basic nutrients to bacteria (Paungfoo-Lonhienne *et al.*, 2019). Production of HCN in rhizosphere by the mi anism play evident role to control the harm l pat gens which is also common trait of GPR. The bilities of isolated strains of *Bacill* and others produce HCN were found pos ve w ch a ditionally confirmed that the HC uction pecific by strains and triat of PG and int duce different kind resistance in the nt (Kaur and Sharma, 2013; Askar *et al.*, 2020). zyme roduction is the one of the most important and mon characteristic of rhizospheric bacteria nd it is concluded that enzymatic activities such as production of amylase catalase and pectinase by bacteria induce resistance in plant against different type of stresses such as chemical, mechanical and environmental (Kumar *et al.*, 2012; Askar *et al.*, 2020). Protease and amylase producing microbes not only decompose the organic matter, plant growth promotion and nutrient mineralization but also acts as bio control agents, on protein and cellulose cell well bearing pathogens such as phytopthora and phytham species (Sathya *et al.*, 2017). Nitrogen fixation is most basic, common and key characteristic of rhizospheric bacteria. Currently, the nitrogen fixation study showed that all the strains of *Coccus*, *Bacillus* and *Spirillum* sp. fixed the nitrogen. Many studies concluded that the nitrogen fixation ability of PGPR improved the development and yield attribute in crops (Mogal *et al.*, 2020).

PGPR inoculation to crops at primary development stage increase the production of plant biomass by increasing the root and shoot growth. Those PGPR are mostly used in crop having special characteristics such as make the availability of natural products as well as work as alternative of chemicals based fertilizers, having ability ability to fix atmospheric nitrogen,

primarily this was assume to increase crop production by adding nitrogen into soil (Hadide *et al*., 2019). Plant nutrition is closely associated with the activity of PGPR; including potassium (K) and zinc (Zn) solubilizing bacteria. These bacteria are interestingly used and apply as biofertilizer in many countries that where in soil are deficient in available potassium and zinc. Most of soils containing K sources in silicate form of minerals viz., illite, feldspar, vermiculite, mica, smectite etc. Total K pools in soil are present in extremely complex forms having availability increases through bacterial solublization through release of acid produced during solublization process and will become easily plant available K (Rawat *et al*., 2016).

The carried out study proven that isolated bacterial strains from Rhizosphere, Rhizoplane and roots of Apple Gourd had morphologically different colonies, shape, color, elevation and margin. Some strains were gram positive while some were gram negative. All bacterial starins had different morphological characters, so this study revealed that there are no limits for bacterial morphology and it depends from the where samples were collected and also depend upon the environment, where they are surviving. PGPRs are able to produce IAA that results totally match with findings of Das research team (Das *et al*., 2019). Further study shows that all isolated strains are highly able to produce ammonia and indole acetic acid which is enough character of plant growth promotion strongly supported by findings Ahmed group (Ahmed *et al*., 2020). Plant growth-promoting rhizobacteria also improve the extent and quality of plant growth in the Apple Gourd by the process of P solubilization in area of plant root and soil interaction area (rhizosphere). Inorganic P is solubilized differently through organic acids excreting of microorganisms that dissolve phosphatic component such as minerals. Similarly, microorganisms by another way increase the availability P to plants through the mineralization of organic P in soil. Morever, through thr process of solubilization of precipitated phosphate. This all mechanism is considering as the basic mode of action carried by the plant growth-promoting rhizobacteria that ultimately enhanced the availability of basic nutrients to the host plants (Bhatt *et al*., 2020). In current lab study, all strains were also characterized and screened to identified the enzyme production activity like amylase, protease, pectinase that also involved in the inhibition of un-control fungal growth, and various kind of stress.

Conclusions and Recommendations

The isolated bacterial strains from apple gourd have growth promoting abilities and can be used as bio fertilizer.

Acknowledgment

This work was part of master study research program conducted at University of Agriculture Faisalabad Sub-campus, Burewala, Pakistan. Authors sincerely thanks to supervisor Dr. Shakeel Imaran for supervising this research study. Authors also very thankful to Muhammad Shehzad from Govt. College University Lahore, Pakistan for his assistance in soil analyses and provision of instruments for characterization of bacterial strains. Authors also grateful to international coordination for thesis evaluation, write up and providing financial assistance to carried out the study in true manner.

Novelty Statement

This manuscript is completely novelty since microbes represent a promising sustainable strategy like growth promoting abilities for the plant to achieve global food security.

Author's Contribution

Taqi Raza: Designed the study, wrote the protocol of study and conducted experiment under the supervision of Dr. Shakeel Imran.
Sergio de los Santos-Villalobos: Involved in writing, editing, and revising the manuscript.
Muhammad Shehzad: Managed the literature searches and provided some chemicals characterization of bacterial strains.
Shakeel Imran: Supervised the research work and assist in statistical analysis as well as in paper write up.
Derly José Henriques da Silva: Involved in improving the quality of paper and provide the financial support for research work as well as for publication.

Conflict of interest

The authors have declared no conflict of interest.

References

Ahmed, A., T. Sultan, G. Qadir, O. Afzal, M. Ahmed, S.S. Shah, M. Asif, S. Ali and M.Z.

Mehmood. 2020. Impact assessment of plant growth promoting rhizobacteria on growth and nutrient uptake of maize (*Zea Mays*). Pak. J. Agric. Sci., 33(2): 234-246. https://doi.org/10.17582/journal.pjar/2020/33.2.234.246

Amellal, N., G. Burtin, F. Bartoli and T. Heulin. 1998. Colonization of wheat roots by an exopolysaccharide producingpantoea agglomerans strain and its effect on rhizosphere soil aggregation. Appl. Environ. Microbiol., 64(10): 3740-3747. https://doi.org/10.1128/AEM.64.10.3740-3747.1998

Askar, A., Y. Shoujun, M.W. Abbasi, M. Azeem, M. Hamayun, M. Rauf, M. Shah, M. Tariq, R. Dong and H. Gul. 2020. Isolation and evaluation of halotolerant rhizobacteria from *Xanthium strumarium L.* As plant growth promoting rhizobacteria. Pak. J. Bot., 52(3): 1105-1113. https://doi.org/10.30848/PJB2020-3(30)

Backer, R., J.S. Rokem, G. Ilangumaran, J. Lamont, D. Praslickova, E. Ricci, S. Subramanian and D.L. Smith. 2018. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front. Plant Sci., 9: 1473. https://doi.org/10.3389/fpls.2018.01473

Bajracharya, A.M., 2019. Plant growth promoting rhizobacteria (PGPR): Biofertiliser and Biocontrol agent-Review article. J. Balkumari Coll., 8: 42-45. https://doi.org/10.3126/jbkc.v8i0.29304

Bechtaoui, N., A. Raklami, L. Benidire, A.I. Tahiri, M. Göttfert and K. Oufdou. 2020. Effects of PGPR co-inoculation on growth, phosphorus nutrition and phosphatase/phytase activities of faba bean under different phosphorus availability conditions. Pol. J. Environ. Stud., 29(2): 1557-1565. https://doi.org/10.15244/pjoes/110345

Bhatt, K. and D.K. Maheshwari. 2020. Zinc solubilizing bacteria (*Bacillus megaterium*) with multifarious plant growth promoting activities alleviates growth in *Capsicum annuum* L. 3 Biotech., 10(2): 36. https://doi.org/10.1007/s13205-019-2033-9

Bric, J.M., R.M. Bostock and S.E. Silverstone. 1991. Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl. Environ. Microbiol., 57(2): 535-538.

Bunt, J. and A. Rovira. 1955. Microbiological studies of some subantarctic soils. J. Soil Sci., 6(1): 119-128. https://doi.org/10.1111/j.1365-2389.1955.tb00836.x

Cappuccino, J.G. and N. Sherman. 2002. Microbiol. A laboratory manual. Pearson Education, Inc. San Francisco, CA.

Cappuccino, J. and N. Sherman. 1992. Biochemical activities of microorganisms. In: Microbiology, A laboratory manual. The Benjamin/Cummings Publishing Co. California, USA. pp. 188-247.

Castric, P.A., 1975. Hydrogen cyanide, a secondary metabolite of pseudomonas aeruginosa. Can. J. Microbiol., 21(5): 613-618. https://doi.org/10.1139/m75-088

Das, S., T.R. Nurunnabi, R. Parveen, A.N. Mou, M.E. Islam, K.M. Islam and S.M. Rahman. 2019. Isolation and characterization of indole acetic acid producing bacteria from rhizosphere soil and their effect on seed germination. Int. J. Curr. Microbiol. App. Sci., 8(3): 1237-1245. https://doi.org/10.20546/ijcmas.2019.803.146

El-Akhdar, I. and A. Ghazi. 2019. Prospection of plant growth-promoting rhizobacterium enterobacter cloacae and N2-fixing rhizobium leguminosarum bv. viciae Effect on Faba Bean Evaluation in Sustainable Agriculture. J. Adv. Biol. Biotechnol., pp. 1-11. https://doi.org/10.9734/jabb/2019/v22i430130

El-Sayed, S.Y. and R.H. Hagab. 2020. Effect of organic acids and plant growth promoting rhizobacteria (PGPR) on biochemical content and productivity of wheat under saline soil conditions. Middle East J. 9(2): 227-242.

Garg, N., 2017. Genetic diversity in round gourd [praecitrullus fistulosus (stocks) pangalo] accessions introduced from usda for various qualitative and quantitative traits. J. Crop Improv., 31(6):801-815. https://doi.org/10.1080/15427528.2017.1370626

Gouda, S., R.G. Kerry, G. Das, S. Paramithiotis, H.-S. Shin and J.K. Patra. 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol. Res., 206: 131-140. https://doi.org/10.1016/j.micres.2017.08.016

Hadide, S.T.E., L.A. Moussa and H.M. El-Gharbawy. 2019. Effect of biofertilizers rates on yield, physicochemical and technological characteristics of wheat. Middle East J. Agric. Res., 8(3): 928-936.

Hols, P., T. Ferain, D. Garmyn, N. Bernard and J.

Delcour. 1994. Use of homologous expression-secretion signals and vector-free stable chromosomal integration in engineering of lactobacillus plantarum for alpha-amylase and levanase expression. Appl. Environ. Microbiol., 60(5): 1401-1413. https://doi.org/10.1128/AEM.60.5.1401-1413.1994

Jamal, Q., Y.S. Lee, S. Munir, M.S. Malik and K.Y. Kim. 2018. The combined use of five bacteria including Bacillus amyloliquefaciens Y1 as biofertilizer in compost improved low bush blueberry growth, rhizosphere bacteria and enzyme at various pH. Pak. J. Agric. Sci., 55(2): 409-416. https://doi.org/10.21162/PAKJAS/18.6359

Johri, J.K., S. Surange and C.S. Nautiyal. 1999. Occurrence of salt, pH, and temperature-tolerant, phosphate-solubilizing bacteria in alkaline soils. Curr. Microbiol., 39(2): 89-93. https://doi.org/10.1007/s002849900424

Kannan, T.R. and C. Kanagaraj. 2019. Molecular characteristic of α-AMYLASE enzymes producing from Bacillus lichenformis (JQ946317) using solid state fermentation. Biocatal. Agric. Biotechnol., 20: 101240. https://doi.org/10.1016/j.bcab.2019.101240

Kaur, N. and P. Sharma. 2013. Screening and characterization of native pseudomonas sp. As plant growth promoting rhizobacteria in chickpea (*Cicer arietinum* L.) rhizosphere. Afr. J. Microbiol. Res., 7(16): 1465-1474. https://doi.org/10.5897/AJMR12.362

Kumar, A., A. panwar and G. joshi. 2019. Efficiency of a culture medium developed from Parthenium leaves for isolation of plant growth-promoting bacteria. Plant Cell Biotech. Mol. Biol., pp. 1439-1446.

Kumar, A., A. Kumar, S. Devi, S. Patil, C. Payal and S. Negi. 2012. Isolation, screening and characterization of bacteria from rhizospheric soils for different plant growth promotion (pgp) activities: An *in vitro* study. Res. Sci. Technol. Educ., 4(1): 1-5.

Lata, R. and S.K. Gond. 2019. Plant growth-promoting microbes for abiotic stress tolerance in plants. In: Role of plant growth promoting microorganisms in sustainable agriculture and nanotechnology. Elsevier. pp. 89-105. https://doi.org/10.1016/B978-0-12-817004-5.00006-3

Lopez-Raez, A. June, K. Shirasu and E. Foo. 2017. Strigolactones in plant interactions with beneficial and detrimental organisms: The Yin and Yang. Trends Plant Sci., 22(6): 527-537. https://doi.org/10.1016/j.tplants.2017.03.011

Loper, J.E. and M.N. Schroth. 1986. Influence of bacterial sources on indole-3 acetic acid on root elongation of sugarbeet. Phytopathology, 76: 386–389.

Mogal, C., S. Jha, H. Suthar, V. Parekh and B.K. Rajkumar. 2020. Efficiency of Plant Growth Promoting Rhizobacteria (PGPR) consortia for modulation of phytohormone and better nutrient acquisition. Plantae Sci., 3(4): 20-29. https://doi.org/10.32439/ps.v3i4.20-29

Montoya, R.I.R., L.A.C. Encinas, F.I.P. Cota and S. de los Santos Villalobos. 2020. Mejorando rasgos biométricos de plántulas de trigo con la inoculación de un consorcio nativo de bacillus. Rev. Mexicana Scienc. Agric., 11(1): 229-235. https://doi.org/10.29312/remexca.v11i1.2162

Nannipieri, P. and L. Badalucco, 2003. Biological processes. In: DK Benbi, and R. Niedere (eds.). Handbook of processes and modeling in the soil plant system. Haworth, Binghamton, New York. pp. 57-82.

Okon, Y., S.L. Albrecht and R. Burris. 1977. Methods for growing spirillum lipoferum and for counting it in pure culture and in association with plants. Appl. Environ. Microbiol., 33(1): 85-88. https://doi.org/10.1128/AEM.33.1.85-88.1977

Padilla, J.R., L.A.C. Encinas, R.I.R. Montoya and S. de los Santos Villalobos. 2020. Growth promotion on wheat (*Triticum turgidum* L. Subsp. Durum) by co-inoculation of native bacillus strains isolated from the yaqui valley, mexico. Nova Sci., 12(24): 1-27.

Pathak, D., V.P. Singh, J. Sharma and A. Sheera. 2020. Plant growth promoting rhizobacteria (PGPR): A biological tool for improving plant health. Biotica Res. Today, 2(7): 593-595.

Raju, E.V.N. and G. Divakar. 2013. Production of pectinase by using Bacillus circulans isolated from dump yards of vegetable wastes. Int. J. Pharm. Sci. Res., 4(7): 2615.

Rawat, J., P. Sanwal and J. Saxena. 2016. Potassium and its role in sustainable agriculture. In: Potassium solubilizing microorganisms for sustainable agriculture. Springer, New Delhi. pp. 235-253. https://doi.org/10.1007/978-81-322-2776-2_17

Rezakhani, L., B. Motesharezadeh, M.M. Tehrani, H. Etesami and H.M. Hosseini. 2019. Phosphate–solubilizing bacteria and silicon synergistically augment phosphorus (P) uptake by wheat (*Triticum aestivum* L.) plant fertilized with soluble or insoluble P source. Ecotoxicol. Environ. Saf., 173: 504-513. https://doi.org/10.1016/j.ecoenv.2019.02.060

Saharan, B. and V. Nehra. 2011. Plant growth promoting rhizobacteria: A critical review. Life Sci. Med. Res., 21(1): 30.

Santos, V., A. Valenzuela, S. Herrera, R. Villa, V. Ibarra and C. Parra. 2016. A new collection of native soil microorganisms with potential agro-biotechnological uses: Colmena. 3rd Biotechnology Summit 2016, Ciudad Obregón, Sonora, Mexico, 24-28 October 2016: 365-370.

Sathya, A., R. Vijayabharathi and S. Gopalakrishnan. 2017. Plant growth-promoting actinobacteria: A new strategy for enhancing sustainable production and protection of grain legumes. 3 Biotech., 7(2): 102. https://doi.org/10.1007/s13205-017-0736-3

Shakeel, M., A. Rais, M.N. Hassan and F.Y. Hafeez. 2015. Root associated bacillus sp. Improves growth, yield and zinc translocation for basmati rice (oryza sativa) varieties. Front. Microbiol., 6: 1286. https://doi.org/10.3389/fmicb.2015.01286

Smibert. R.M. and N.R. Krieg. 1994. Phenotypic characterization. In: Method for general and molecular bacteriology. Am. Soc. Microbiol., 34: 607–654.

Singh, D., P. Ghosh, J. Kumar and A. Kumar. 2019. Plant growth-promoting rhizobacteria (PGPRs): Functions and benefits. In: Microbial interventions in agriculture and environment. Springer. pp. 205-227. https://doi.org/10.1007/978-981-13-8383-0_7

Singh, R., D. Pandey, A. Kumar and M. Singh. 2017. Pgpr isolates from the rhizosphere of vegetable crop momordica charantia: Characterization and application as biofertilizer. Int J. Curr. Microbiol. App. Sci., 6(3): 1789-1802. https://doi.org/10.20546/ijcmas.2017.603.205

Syed, J.H., A. Alamdar, A. Mohammad, K. Ahad, Z. Shabir, H. Ahmed, S.M. Ali, S.G.A.S. Sani, H. Bokhari and K.D. Gallagher. 2014. Pesticide residues in fruits and vegetables from pakistan: A review of the occurrence and associated human health risks. Environ. Sci. Pollut. Res., 21(23): 13367-13393. https://doi.org/10.1007/s11356-014-3117-z

Tyagi, N., G.N. Sharma and B. Shrivastava. 2017. Phytochemical and pharmacological profile of praecitrullus fistulosus: An overview. J. Ind. Herb. Drugs, 2(3): 29-35.

Valenzuela-Aragon, B., F.I. Parra-Cota, G. Santoyo, G.L. Arellano-Wattenbarger and S. de los Santos-Villalobos. 2019. Plant-assisted selection: A promising alternative for in vivo identification of wheat (*Triticum turgidum* L. Subsp. Durum) growth promoting bacteria. Plant Soil, 435(1-2): 367-384. https://doi.org/10.1007/s11104-018-03901-1

Valenzuela-Ruiz, V., R. Robles-Montoya, F. Parra-Cota, G. Santoyo, M. Orozco-Mosqueda, R. Rodríguez-Ramírez and S. de los Santos-Villalobos. 2019. Draft genome sequence of bacillus paralicheniformis trq65, a biological control agent and plant growth promoting bacterium isolated from wheat (*Triticum turgidum* subsp. Durum) rhizosphere in the yaqui valley, mexico. 3 Biotech., 9 (1): 436-442. https://doi.org/10.1007/s13205-019-1972-5

Velmourougane, K., R. Prasanna, S. Singh, G. Chawla, A. Kumar and A.K. Saxena. 2017. Modulating rhizosphere colonisation, plant growth, soil nutrient availability and plant defense enzyme activity through Trichoderma viride-Azotobacter chroococcum biofilm inoculation in chickpea. Plant Soil, 421(1-2): 157-174. https://doi.org/10.1007/s11104-017-3445-0

Paungfoo-Lonhienne, C., M. Redding, C. Pratt and W. Wang. 2019. Plant growth promoting rhizobacteria increase the efficiency of fertilisers while reducing nitrogen loss. J. Environ. Manage., 233: 337-341. https://doi.org/10.1016/j.jenvman.2018.12.052

Villa-Rodríguez, E., F. Parra-Cota, E. Castro-Longoria, J. López-Cervantes and S. de los Santos-Villalobos. 2019. Bacillus subtilis te3: A promising biological control agent against bipolaris sorokiniana, the causal agent of spot blotch in wheat (*Triticum turgidum* L. Subsp. Durum). Biol. Contr., 132: 135-143. https://doi.org/10.1016/j.biocontrol.2019.02.012

Villarreal-Delgado, M.F., E.D. Villa-Rodríguez, L.A. Cira-Chávez, M.I. Estrada-Alvarado, F.I. Parra-Cota and S. de los Santos-Villalobos. 2018. The genus bacillus as a biological control

agent and its implications in the agricultural biosecurity. Rev. Mex. Fitopatol., 36(1): 95-130. https://doi.org/10.18781/R.MEX.FIT.1706-5

Yasmin, F., R.Othman and M.N.H. Maziz. 2020. Yield and nutrient content of sweet potato in response of plant growth-promoting rhizobacteria (PGPR) Inoculation and N Fertilization. Jordan J. Biol. Sci., 13(1): 117-122.