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ISOLATION AND CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (FLUORESCENT PSEUDOMONADS) FROM ERBIL SOIL, KURDISTAN REGION-IRAQ AND USE TO IMPROVE GROWTH AND YIELD OF *ZEA MAYS*

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria which can enhance growth parameters and yield of host plants and can be used as biofertilizers. Fluorescent pseudomonads are considered to be one of the most promising groups of plant growth promoting rhizobacteria involved in promoting of plant growth. The current study was aimed to isolate and identify plant growth promoting fluorescent pseudomonads from Erbil soil, and evaluate the plant growth promoting bioagents of the isolated strains then selecting the most efficient isolates and use to improve Zea mays growth and yield. For this purpose, random sampling from the rhizosphere area was performed. Fluorescent Pseudomonads were isolated by culturing in enriched and selective King B medium and were identified based on morphological and biochemical assays. A total of 14 strains of fluorescent Pseudomonads were isolated and 8 isolates identified as Pseudomonas fluorescens and 6 isolates belonged to Pseudomonas putida. Plant growth promoting traits of the isolates were also studied such as phosphate solubilizing activity, IAA, siderophore, and HCN production. All isolates exhibited high potential of phosphate solubilisation, IAA, HCN and siderophore production, except (Ppu9) isolate and (Pfl6, Pfl12, and Ppu13) isolates showed negative results in HCN and siderophore production, respectively. The most efficient isolates (Pfl3, Pfl10, Ppu8, and Ppu11) were selected for pot experiment and used to inoculate Zea mays seeds before sowing. After harvesting, data were

collected on shoot and root length, shoot and root dry weight, number of grain per cob, grain yield per plant and grain yield per hectare. Results showed that all isolates had significantly ($p \leq 0.05$) increased crop growth and productivity. The highest growth and yield were found in combined inoculated plants with Pfl3+Ppu8 followed by Pfl3+Ppu11treated plants, which increased significantly over than single inoculated and non-inoculated plants. The results revealed that plant growth promoting fluorescent pseudomonads can be used as biofertilizer to improve plant growth.

Key words*: PGPR, Fluorescent Pseudomonads, rhizosphere, Zea mays.*

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1. INTRODUCTION

Rhizosphere, the layer of soil which influenced by plant root, is known to play an essential role in plant growth and development and in the rhizospheric soil; the density of rhizospheric microorganisms is much higher rather than the surrounding soil because different secondary metabolites secreted by plant roots are used as a source of nutrient by these organisms [1]. Rhizobacteria aggressively colonize roots of plants, able to multiply and colonize on the root system at all stages of plant growth, survive in the presence of a competing micro-flora [2]. Some of the rhizobacteria positively influence on plant growth and health which were referred to Plant Growth Promoting Rhizobacteria (PGPR). These rhizobacteria are abundant in rhizosheric soil, and these maintained the ecological balance in niche needed for their survival. PGPR played a pivotal role in both promoting plant growth and controlling plant disease. The mechanisms of growth promotion by these PGPRs are complex and appear to comprise both changes in the microbial balance in the rhizosphere and alterations in host plant physiology [3]. Several mechanisms have been postulated to explain how PGPR promote plant growth. These include: secreting of plant growth regulators or phytohormones such as indole acetic acid (IAA), cytokinins, gibberellins, GAs, Kinetins besides ACC (1-Aminocycloprapane-1-carboxylic acid) deaminase [4], fixing atmospheric nitrogen, solubilizing insoluble phosphates [3]. Induced systemic resistance (ISR), competition for nutrients, parasitism, and production of metabolites (hydrogen cyanide, siderophores) suppressive to deleterious rhizobacteria are some of the mechanism that benefits plant growth. Biological control of diseases by plant growth promoting

rhizobacteria was well-established phenomenon [5, 6]. The antibiotics, HCN, H2S, and siderophore have been shown to play a major role in the suppression of several plant pathogens [7, 8].

Among rhizobacteria: Fluorescent Pseudomonads are considered to be one of the most promising groups of plant growth promoting rhizobacteria involved in promoting of plant growth [9, 10]. They produce secondary metabolites such as phytohormones [11], volatile compound, hydrogen cyanide (HCN) and siderophores [7]. Plant growth-promoting ability of these bacteria is mainly due to the production of indole-3-acetic acid (IAA) [12], siderophores and antibiotics [11, 13].

Pseudomonas is an aerobic gram negative, rod-shaped, non-spore forming, fast growing, competitive root colonizing bacteria, and commonly found in the rhizosphere of various plants, the largest of the plant growth promoter bacterial groups that includes both fluorescent and non-fluorescent species [13]. The most important fluorescent species are *Ps. aeruginosa, Ps. fluorescens, Ps. putida* and the plant pathogen species is *Ps. syringae* [14]. Several species of rRNA group I pseudomonads have the ability to produce and excrete, under iron limitation condition, soluble yellow green pigments that fluorescence under UV light [15], named pyoverdines (PVDs) or pseudobactins, which act as siderophores for these rhizobacteria [16]. These molecules are associated with bio control of fungal pathogens in the biosphere [17]. Fluorescent pseudomonads have frequently been considered as effective biological control substances against soil-borne plant pathogens due to their quick and aggressive colonization of plant roots system. Other mechanisms include competition for nutrients in the rhizosphere at preferred colonization sites and production of secondary metabolites, such as antibiotics, siderophores and hydrogen cyanide [11]. The abundance of literature on genus Pseudomonas is due to their elevated metabolic versatility capable of utilizing a wide range of simple and complex organic compounds and holding an important position in biosphere ecology [14]. The role of Pseudomonas species to solubilize fixed phosphorus to available phosphorus has also been observed [18].

The main objective of this study was isolation, identification, and selection of the most active and beneficial ecofriendly strains of fluorescent pseudomonads from Erbil soil, which have a

broad spectrum of plant-promoting capabilities. The efficiency of this group on the *Zea mays* growth promotion was also elucidated in pot experiments.

2. MATERIALS AND METHODS

2.1.Soil sampling

Rhizospheric soil samples were collected from different geographical areas in Erbil governorate/Kurdistan region- Iraq (Minara, Ankawa, Grdarasha, Tobzawa, Bnaslawa, Shaqlawa, Salahaddin, Soran, Harir, Choman, Maxmur, Koya, Barzan, Kalak, Shawes, Grdhazaban) in June 2016, samples were composed of roots of different plants, rhizospheric soil and bulk soil samples (about 1 kg) were collected along the interiors, after removing the superficial soil layer. The soils were brought to Microbiology laboratory, Department of Plant Protection, College of Agriculture, Salahaddin University.

2.2.Isolation and extraction of fluorescent pseudomonads

Plant root samples were shaken vigorously to remove loosely adhering soil. To isolate rhizospheric bacteria (fluorescent pseudomonads), roots were shaken in 20 ml sterile phosphate buffered saline (PBS). Roots were then washed three times in sterile PBS and shaken vigorously in 10 ml sterile PBS containing 0.025% Tween 20 (PBS-T) to remove bacteria from the rhizoplane. To isolate fluorescent pseudomonads, PBS and PBS-T extracts from root samples tenfold diluted and plated on King B medium. To isolate fluorescent pseudomonads from rhizospher and bulk soil samples, 1g of soil sample was suspended in 99 ml of sterile distilled water. Samples were serially diluted and 0.1 ml of sample was spread on King's B medium plates. After incubation at 28oC for 48 h, the plates were exposed to UV light at 365 nm for few seconds and the colonies exhibiting the fluorescence were picked up and streaked on to the slants for maintenance, purified on King's B medium plates [20].

To quantify fluorescent pseudomonads from root and soil samples, tenfold dilution of PBS extracts from root and rhizospheric soils were plated on trypticase-soya agar (TSA) and on King B medium, respectively. After incubation at 28 °C for 48 h, counts were made of total colonies on TSA and fluorescent colonies under UV. light on King B medium. Results were expressed as colony forming units per gram (cfu.g−1). Fluorescent colonies with different morphological characteristics on King B medium were sub cultured twice before identification and bacterial

population was estimated by most probable numbers (PMN) count according to method described by [19].

2.3.Biochemical and physiological characterization of fluorescent Pseudomonas isolates

Fluorescent bacteria were identified to species on the basis of phenotypic level, biochemical and physiological test. Characterization of pseudomonads were carried out by subjecting the bacterial isolate to morphological characterization (Colony morphology, size, color, shape, gram's nature , and growth pattern were recorded after 24 h of growth on LB agar plates at 28 ± 2˚C as described by [4], and biochemical characterization (hydrolysis of starch, gelatin, urea, catalase, and oxidase, arginine dihydrolase, glucose fermentation and levan production, utilization of trehalose and tryptophane, and nitrate reduction, growth at 4˚C and 41˚C [21], and by using API 20NE strips, as recommended by the manufacturer (bioMérieux, Marcy l'Etoile, France).

2.4. Plant growth promotion (PGP) traits

2.4.1. Detection of cyanide production

Hydrogen cyanide (HCN) production by all isolated fluorescent pseudomonads strains was assessed by the propagation of these isolates in 15 ml of King's broth medium in test tube containing sterilized filter paper strip (Whatman No. 1) saturated with cyanide reagent (2% sodium carbonate + 0.5% picric acid), inoculated with loop of the tested isolate and incubated at 30 °C for 1–2 days. Positive results were recorded when the paper strip turned from yellow to orange- brown color according to [25].

2.4.2. Determination of Indole Acetic Acid

To measure the IAA production of bacteria, a loopful of the bacterial isolates were inoculated in 100 ml King's B broth added by 0.1mg/ml tryptophan and incubated at 28 ± 2 °C. After 4 days of incubation, the cultures were centrifuged for 10 min at 13,000 rpm in 4˚C. The IAA in the supernatant was, then, detected colorimetrically. One mL of the supernatant was reacted with 2.0 mL Salkowski's reagent. Pink to red color transformation indicated positive reaction [22].

2.4.3. HPLC analysis of microbial hormones (IAA)

The amount of IAA produced in liquid culture by bacterial strains was determined quantitatively by High performance liquid chromatography (HPLC). The most efficient bacterial isolates were

grown in King's B broth supplemented with 1 mg.ml-1 tryptophan for indols production and incubated at 28 °C under shaking culture (150 rpm) for 5 days. Extraction, purification and quantification of IAA were applied according to the method described by [23] using HPLC (Hewlett Packard series 1050) equipped with variable UV detector and BDS-HYPESIL C18 column (Dim 250 × 4.6 mm for Particle size (µ)). The growth hormones were identified on the basis of retention time of phytohormone standards (commercially grade, Sigma Chemical USA Company).

2.4.4. Analysis of phosphate solubilizing activity

Phosphate solubilizing activity of all isolated bacterial strain was assayed using plate screening method and broth culture method. All the suspected colonies were screened for phosphate solubilization on Pikovskayas (PVK) agar medium (10g glucose, 5g Ca(PO4)2, 0.5g(NH4)2SO4, 0.2g NaCl, 0.1g MgSO4.7H2O, 0.2g KCl, 0.5g yeast extract, 0.002g MnSO4.H2O and 0.002g FeSO4.7H2O). Spot inoculation at the center of PVK plate was done and incubated at 30°C. Diameter of clear halo was measured successively after 24hr, up to 7 days. Phosphate solubilization efficiency (fluorescent Pseudomonads) of each isolates was evaluated according to the following equation [24]:

PSE= solubilization diameter × 100

Growth diameter

2.4.5. Siderophore production

Siderophore production was determined by Chrome Azurol S (CAS) assay. The CAS agar medium was prepared according to procedure given by [26]. The bacterial culture was streaked on the CAS plate. Change of medium color to orange or presence of light orange halo surrounding the bacterial growth indicates siderophore production by the bacterial isolates.

2.5. Inoculant preparation

For preparation of bacterial inoculants, bacterial strains were grown in King's broth medium for 48 h at 28 ± 2 °C on rotary shaker (150 rpm) and cell counts were determined on King's broth medium by MPN technique. Inoculation was carried out by seed treatment with bacterial culture containing about 108 cells.ml-1 which prepared by spectrophotometer accounting method.

2.6.Pot experiment:

Effect of bacterial inoculation on plant growth was examined on Zea mays in a pot experiment. For pot experiment the most active *Pseudomonas* strains were selected according to their results of plant growth promoting traits and used as inoculum for seed inoculation. Sterilized Seeds of Zea mays were obtained from the Agriculture Research center, Ministry of Agriculture, Kurdistan region, Iraq. For seed treatments, seeds were soaked in the bacterial suspension *[P. fluorescens* (Pfl3, Pfl10) and *P. putida* (Ppu8, Ppu11)] of 108cells/ml amended with sucrose (0.2%) to facilitate the adherence of the bacteria to the seeds, 50 seeds of maize were treated with 1 ml bacterial suspension (seeds and bacterial suspension were shaken together in sterilized plastic bag for 5 min), and some seeds were soaked in distilled water (which served as the control). Then, Maize seeds were sown (7seeds.pot-1) during January 2017 into pots (25 cm in diameter) containing equal amounts of homogeneous soil (9kg soil.pot-1). The soil characteristics were as follows: silty clay loam in texture, sand 10%; silt 52%; clay 38%; pH, 7.9; EC 0.41 dS m–1and organic matter 2.02%. Each treatment contained four pots. There were 9 treatments: (non-inoculated control, *P. fluorescens* 3 treatment (Pfl3), *P. fluorescens* 10 treatment (Pfl10), *P.putida* 8 treatment (Ppu8), *P.putida* 11 treatment (Ppu11) *P. fluorescens* 3+ *P.putida* 8(Pfl3+Ppu8) treatment., *P. fluorescens* 10 + *P.putida* 11 (Pfl10+Ppu11) treatment, *P. fluorescens* 3+ *P.putida* 11(Pfl3+Ppu11) treatment, and *P. fluorescens* 10+ *P.putida* 8(Pfl10+Ppu8) treatment. The experiment was set up in randomized complete design (CRD) using four replicates per treatment. Plants were harvested 120 days after transplantation and data was recorded for shoot and root length, grain yield, and shoot and root dry weight. The shoots and roots were dried at 70 °C for 24hr to determine the dry weight.

2.7. Statistical Analysis

The data were subjected to analysis of variance using SPSS (Version 17). A completely randomized design was used for all experiments, with 4 replications for each treatment. The data presented are from representative experiments that were repeated at least twice with similar results. Treatments were compared via ANOVA using the least significant differences test (LSD) at 5% ($P \leq 0.05$) probability level [27].

3. RESULTS

3.1. Isolation and identification of fluorescent pseudomonads

The isolation and characterization of fluorescent pseudomonads from different places of Erbil city (Minara, Ankawa, Grdarasha, Tobzawa, Bnaslawa, Shaqlawa, Salahaddin, Soran, Harir, Choman, Maxmur, Koya, Barzan, Kalak, Shawes, Grdhazaban) were undertaken in this study. A total of 14 fluorescent pseudomonads isolates were obtained from both rhizospheric portion as well as root interior and bulk soil. Isolated fluorescent pseudomonads strain were always more abundant in rhizospheric soils than root interior and bulk soil. When fluorescent pseudomonads were detected, their numbers varied from 6.0 × 103 to 2.0 × 106 cell.g−1 in soil samples.

All isolates produced fluorescent pigment on King B medium, negative to gram nature, rod shaped, motile, aerobic, non-spore former and all fluorescent pseudomonas Isolates were biochemically characterized for their ability to produce catalase, gelatinase, arginine dihydrolase, oxidase, starch and urea hydrolysis, and ability to utilize trehalose , levan production from sucrose, nitrate reduction, and growth at 4C and 41C (table 1). Results of morphological, physiological and biochemical characterization indicated that eight isolates (Pfl1, Pfl2, Pfl3, Pfl5, Pfl6, Pfl7, Pfl10, and Pfl12) belong to the genus *Pseudomonas fluorescens* and six isolates (Ppu4, Ppu8, Ppu9, Ppu11, Ppu13, and Ppu14) referred to the genus *Pseudomonas putida*. According to methods described in Bergey's Manual of Determinative Bacteriology [28]. Further the isolates were also evaluated for the different traits typically associated with the PGP such as production of hydrogen cyanide, IAA, siderophore, and inorganic phosphate solubilization.

Table 1. Some morphological and Biochemical properties of fluorescent pseudomonads isolates.

Isolate cod	Pfl1	Pfl2	Pfl3	Ppu4	Pfl5	Pfl6	Pfl7	Ppu8	Ppu9	Pfl10	Ppu11	Pfl12	Ppu13	Ppu14
Gram nature	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Fluorescent pigment	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility test	motile	motile	motile	motile	motile	motile	motile	motile	motile	motile	motile	motile	motile	motile
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+	+	+	-	+	+	+	-	-	+	-	+	-	-
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Urea hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Levan formation	+	+	+	-	+	+	+	-	-	+	-	+	-	-
Trhalose utilization	+	+	+	-	+	+	+	-	-	+	-	+	-	-
Arginin dihydrolase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 4°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 41 °C	+	+	+	-	+	+	+	-	-	+	-	+	-	-

3.2. Plant Growth Promoting Traits of isolated fluorescent pseudomonads:

3.2.1. Production of hydrogen cyanide (HCN)

All of the 8 tested *Pseudomonas fluorescens* isolates (Pfl1, Pfl2, Pfl3, Pfl5, Pfl6, Pfl7, Pfl10, and Pfl12) showed high ability in hydrogen cyanide production which indicated by the discoloration of the filter paper from orange to brown after incubation period (table 2). Weak HCN production was observed by Ppu8, Ppu9, Ppu13, and Ppu14 isolates of *Pseudomonas putida*, while Ppu4 and Ppu11isolates of *Pseudomonas putida* showed moderate HCN production. Hydrogen cyanide is produced by many rhizobacteria and is postulated to play a role in biological control of plant pathogens [8]. Although cyanide acts as a general metabolic inhibitor, host plants are not harmfully affected by rhizobacterial production of hydrogen cyanide and host specific rhizobacteria can act as biological control agents [29].

3.2.2. Indole-3-acetic acid production

All 14 tested Pseudomonas isolates produced IAA in vitro in tryptophan supplemented LB medium, indicating that they have the capability to convert tryptophan into IAA (table 2). The production of IAA was manifested by the color change of the broth culture from pink to red. Quantity of IAA produced by isolates was estimated and IAA concentrations range was varied from 40.93 to 12.43µg.ml-1 at 5 days of incubation. Statistical analysis showed that there was significant difference among isolates in IAA production. The highest amount of IAA was produced by isolate Pfl3 followed by Pbu8 and Pfl10 over others strains. The lowest amounts of IAA were produced by strain Pfl7. It has been recorded that the amount of IAA compounds produced in vitro depends on the particular bacterial genus, species, strain, or the conditions of the culture media such as aeration and pH [30]. The variation among PGPR strain to produce IAA found in the present study had also been reported earlier [31]. This difference due to the

various metaboilic pathways, position of the genes involved, and the presence of enzymes to convert active free IAA into conjugated forms [32]. A high amount of IAA production was reported in different strains of bacteria with the members of the genera *Pseudomonas spp*., *Bacillus spp., Rhizobium,* and *Mesorhizobium spp*. by other workers [24].

Table.2 Plant Growth Promoting Traits of Isolates

Isolate code	HCN Production	Siderphore Production	IAA Production	Quantitative IAA production ($\mu g.ml^{-1}$)	Phosphate solubilization (clear zone (%)
Pfl1	+	+	+	14.13^{i}	47.6^{f}
Pfl2	+	+	+	17.97^{h}	54.4^{e}
Pfl3	+++	++	+++	40.93^{a}	85.2^{a}
Ppu4	+	+	++	22.15^{ef}	38.3^{h}
Pfl5	+	++	+	19.32^{fgh}	55.4^{e}
Pfl6	+	-	+	25.54^{e}	37.1^{h}
Pfl7	++	+	+	12.43^{i}	40.0^{h}
Ppu8	±	++	++	37.58^{b}	75.9^{b}
Ppu9	-	+	+	14.58^{i}	70.1^{c}
Pfl10	++	+	+	29.76^{d}	55.5^{e}
Ppu11	+	+	+	20.31^{fg}	65.6^{d}
Pfl12	++	-	+	21.98^{f}	48.4^{f}
Ppu13	±	-	+	18.43^{gh}	73.3^{bc}
Ppu14	±	+	+	33.87^{c}	57.4^{e}
LSD P≤0.01				3.4	5

3.2.3. Siderophores production

The fluorescent Pseudomonas isolates are known to produce many secondary metabolites, such as siderophores which are antagonistic properties against many phytopathogens. Among 14 isolates, six isolates (Pfl1, Pfl2, Pfl3, Pfl5, Pfl7, and Pfl10) , which belonged to the genus *Pseudomonas fluorescens* and five isolates (Ppu4, Ppu8, Ppu9, Ppu11, and Ppu14) of Pseudomonas putida were positive for siderophores production which was indicated by production of yellow/orange-colored zone surrounding the bacterial colony, and other 3 isolates (Pfl6, Pfl12, Ppu13) were negative for siderophores production (Table 2). Results showed that there were differ among isolates in siderophores production ability. Suryakala et al [7] showed that the siderophores which produced by *Pseudomonas fluorescens* were antagonistic to pathogenic fungi.

3.2.4. Phosphate solubilizing activities

All isolated fluorescent Pseudomonads strains were tested for P-solubilizing activity on solid PVK agar media . Production of clear halo zone on agar plates indicating P-solubilizing activity. The results (Table 2) showed that all isolates were able to solubilize P on PVK media plates. The highest P-solubilizing activity (85.2%) was found in Pfl3 treatment followed by Ppu8 (75.9%),This may be attributed to the efficiency of Pseudomonas strains (Pfl3 and Ppu8) to produce higher quantity of acid which led to increase the available phosphorus compared with other tested bacterial strain. This result is in agreement with Castro et al. [18] who observed the role of *Pseudomonas species* to solubilize fixed phosphorus to available phosphorus. While isolate Pfl6 recorded minimum solubilization index (37.1%), which differed significantly from other isolates. ability of isolates to solubilize inorganic phosphate due to the production of organic acids such as gluconic acid and ketogluconic acid, also possess some genes which encoded for several enzymes that have also been shown to be involved in making insoluble phosphorous compounds available for cell growth such asphosphatases enzymes [6].

3.3. Evaluation of Plant Growth Promotion

3.3.1. Plant Growth Parameters

The performance of the selected bacterial isolates (Pfl3, Pfl10, Ppu8, and Ppu11) exerted a significant influence on *Zea mays* growth parameters and yield at pot experiment (Table 3). Comparisons were made among inoculated treatments and a non-inoculated control. All treatments showed significant effects on various growth parameters (shoot height, root length, shoot and root dry weight, and yield) compared to non- inoculated *Zea mays* plant. Growth was found to be further enhanced when seeds inoculated with selected bacterial isolates. Generally, combined inoculation increased *Zea mays* growth and yield higher than single-inoculation.

The inoculated plants with different inoculums alone or incombination showed root length ranging from (48.8. cm) up to (79.2cm). All the isolates which used as inoculant exhibited significant increase in root length of *Zea mays* over control, the maximum increase (79.2cm) was shown by co-inoculation of Pfl3+Ppu8, while the plants treated by Ppu11 alone was reported with the lowest increase of root length.

Table 3. Effects of PGPR (Fluorescent pseudomonads: Pseudomonas fluorescens and Pseudomonas putida) on growth and yield of Zea mays

Treatments	Root length (cm)	shoot length (cm)	Shoot dry wt. (g.plant^{-1})	Root dry wt.(g.plant^{-1})	No.of grains.cob^{-1}	Grain yield.plant^{-1} (g)	Grain yield (t/ha)
Pfl3	61.0^{e}	73.5^{f}	12.8^{e}	4.9^{c}	259^{e}	175.8^{d}	6.79^{cde}
Pfl10	59.5^{e}	70.0^{g}	11.7^{e}	4.3^{cd}	264^{e}	176.6^{d}	6.70^{de}
Ppu8	52.3^{f}	66.6^{f}	10.3^{f}	3.8^{de}	239^{f}	171.4^{e}	6.54^{e}
Ppu11	48.8^{f}	62.0^{g}	8.3^{g}	3.5^{e}	234^{f}	170.9^{e}	6.52^{e}
Pfl3+Ppu8	79.2^{a}	93.6^{a}	20.6^{a}	6.8^{a}	340^{a}	209.8^{a}	7.50^{a}
Pfl10+Ppu11	69.8^{c}	86.4^{c}	16.0^{b}	5.6^{be}	299^{c}	192.5^{b}	7.00^{c}
Pfl3+Ppu11	73.5^{b}	89.0^{b}	17.7^{c}	6.1^{ab}	323^{b}	204.7^{b}	7.34^{b}
Pfl0+Ppu8	65.4^{d}	80.4^{d}	14.5^{d}	5.0^{ce}	285^{d}	180.6^{c}	6.81^{cd}
Noninoculated control	40.3^{g}	55.3^{h}	9.8^{h}	2.1^{f}	201^{g}	136. 5^{f}	4.6^{f}
LSD P≤0.05	2.1	2.6	1.25	0.80	9.4	3.6	0.3

The relative increase in shoot length due to bacterial isolates ranged between 62.0 cm to 93.6cm, over the un-inoculated control. The highest increase in shoot length recorded in plants which co-inoculated by Pfl3+Ppu8. While the corresponding increase in the shoot and root biomass ranged between 8–20 g.plant-1 and 3.5–6.8 g.plant-1, respectively. Similarly, co-inoculated plants with (Pfl3+Ppu8) inoculum exhibited maximum increase in shoot and root biomass followed by treated plant with (Pfl3+Ppu11) and control (un-inoculated) plants showed the lowest shoot and root biomass.

All treatments (Pfl3, Pfl10, Ppu8, Ppu11, Pfl3+Ppu, Pfl10+Ppu11, Pfl3+Ppu11, Pfl0+Ppu8) resulted in the production of significantly higher grain number per cob and grain yield per plant than un-inoculated control. High increase in grain number per cob and grain yield per plant were observed in co-inoculated plants compairing to single inoculated plants and un-inoculated plants. The highest grain yield per plant and grain number per cob were founded in combined inoculation of plants with (Pfl3+Ppu8) inoculums, 209.8 g. plant-1 and 340 grain. cob-1, respectively. The minimum increase in grain yield per plant and grain number per cob were recorded by Ppu 11 inoculum, 170.9 g. plant-1 and 334 grain. cob-1, respectively.

4. DISCUSSION

As an initial step in the collection, isolation, characterization, identification and exploitation of plant growth promoting bioagents of fluorescent pseudomonads isolates were done from different plant rhizospheres in Erbil city, and some more additional isolates were also collected from bulk soil and root interior for the comparison of nature and performance among isolates. A total of 14 fluorescent pseudomonads strains of which, 8 were *pseudomonas fluorescens* and 6 *pseudomonas putida* were isolated and identified, and all isolates showed efficiency in plant growth promoting bioagents production such as IAA, siderphores, HCN, and they had high ability in inorganic phosphate-solubilization, these results in agreement with [18, 29, 8, 2], who detected the plant growth promoting bioagents of fluorescent pseudomonads such as siderphores, IAA, Gibberellins, HCN and observed the role of *Pseudomonas* species to solubilize fixed phosphorus to available phosphorus through production of different organic acids such as fumaric, lactic, malic, ketobutyric, succinic, and tartaric and phosphatase enzyme[11], which chelate the cations bound to phosphate, thereby converting it into available forms. The growth of phosphate-solubilizing bacteria (PSB) often causes soil acidification, which leads to phosphorus solubilization [18]. The difference among isolates in their efficiency in plant growth promoting bioagents production may be attributed to the various biosynthetic pathways, regulatory sequences, location of the genes involved [32].

Results in the tables indicated that inoculation with plant growth promoting fluorescent pseudomonads (*Pseudomonas fluorescens* and *Pseudomonas putida*) significantly increased *Zea mays* growth and yield as compared to control. In the present study, observed abundance of IAA, siderphores, HCN, and ability in inorganic phosphate-solubilization did result in significant root and shoot growth promotion in *Zea mays*, whereas isolates with moderate IAA, siderphores, HCN production and phosphate solubilization showed considerable growth promotion. Probably, increment of plant Growth by inoculation with plant growth promoting fluorescent pseudomonads *(Pseudomonas fluorescens* and *Pseudomonas putida*) due to the production of growth stimulating phytohormones [33], siderophore production [26] solubilization of unavailable forms of nutrients is one of the essential criteria in facilitating the transport of the most of nutrients mobilization of phosphate [34], induction of plant systemic resistances to

pathogens [35], production of volatile compounds, hydrocyanic acid (HCN), enzymes antibiotic production and[36], inhibition of plant ethylene synthesis [37] .

5. CONCLUSIONS AND RECOMMENDATIONS

The current study was conducted to isolate and identify plant growth promoting fluorescent pseudomonads from Erbil soil. The results revealed that rhizosphere soil could be a good source of potential plant growth-promoting fluorescent pseudomonads. Several strain of *pseudomonas fluorescens* and *Pseudomonas putida* were isolated and proved to be promising plant growth-promoters based on the series of screening conducted. Growth-promoting activities of these bacteria include phosphate solubilization, siderphore, HCN, and IAA production, . These characteristics were known to positively affect *Zea mays* growth and yield by various direct and indirect mechanisms. Furthermore, isolated and identified bacterial species could be useful in the formulation of new inoculant in agriculture and use for bio fertilizer preparation. However, further evaluation of the identified isolates under field conditions is recommended to uncover their efficacy as effective microbial inoculant.

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