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A Unique Microbial Fingerprint: Isolation And Characterization Of PGPBs Bacteria From Weeds Associated With Cucumber Crop In Iraq

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Abstract
Plant growth-promoting bacteria as bioioculauts (PGPBs) are abundant around plant roots including some species of Pseudomonas which have an effect on plant growth by stimulating systemic resistance and inhibiting the growth of pathogens. In addition, they protect the plant from environmental and toxic chemical stresses. Therefore, the current study aimed to detect new isolates of PGPBs bacteria colonizing the roots of weeds associated with cucumber crop cultivated in greenhouses. PGPBs samples were collected from the rhizosphere soil of weeds associated with cucumber crop in Diwaniyah province from ten different regions. The results of phenotypic and biochemical diagnosis supported by molecular diagnosis included the registration of three new isolates of Pseudomonas putida, Pseudomonas fluorescens and Pseudomonas chlororaphis, which were deposited in NCBI under the accession numbers (PP582375, PP582376 and PP582377 respectively for the first time in Iraq.
Keywords: *PGPBs bacteria, rhizosphere soil, cucumber, weeds, molecular diagnosis.*

INTRODUCTION

Plant growth-promoting bacteria (PGPBs) that colonize plant roots are considered bioifirtilizers as they are characterized by many properties, including nitrogen fixation and phosphate solubilization, in addition to enhancing plant growth through the production of plant hormones. They are also used as bio-inoculants, which contributes to increasing agricultural efficiency and reducing the use of chemical fertilizers as well as play a role in reducing stress on the plant (Glic et al., 2012). It facilitates beneficial interactions between bacteria and plants and enhances plant defenses against pathogens, thus increasing agricultural yields (Saadani et al., 2016; Al-Fallooji et al., 2024). *Pseudomonas* is a Gram-negative bacterium belonging to the family Pseudomonateceae as a common and important species found in a wide range of environments including soil. It has multiple mechanisms to stimulate plant growth and is considered part of the group of PGPBs. This bacteria has the ability to use many organic compounds as energy sources because it has a great ability to concentrate around the roots of plants. The presence of these types of bacteria in the root zone increases the health of the plant and enhances its growth as well as soil fertility through a wide range of mechanisms such as nitrogen fixation, production of plant hormones and dissolving phosphate. They also contribute to being biological pollinators that increase production and reduce environmental pollution resulting from the use of chemical fertilizers, as these interactions between the plant and bacteria are a symbiotic process due to the presence of these species near the roots where they can benefit from root secretions (Compant et al., 2010). PGPBs bacteria are also characterized by the formation of biofilms that contain multiple layers of sugars, which play an effective role in increasing the plant's tolerance to biotic and abiotic stresses.

One of the most important challenges that hinder crop cultivation is weeds due to their ability to compete for basic growth requirements (Al-Gburi et al., 2024; Al-Gburi and Al-Gburi 2024). Weeds are characterized as being resistant to harsh environmental conditions and can adapt to high and low temperatures, even freezing and grow in all types of soil (Al-Gburi, 2021; Al-Badri et al., 2022). Due to the excessive use of weedicides, new resistant weeds have been emerged (Shama et al., 2021; Al-Gburi and Mohammed, 2019). Thus, many studies have indicated the ability of PGPBs to biodegrade various pollutants, including weedicides by enzymes or oxidation-reduction processes, all of which fall within the metabolic pathways of the PGPB bacteria which leads to the possibility of colonization of weed roots by

types of *Pseudomonas* bacteria. Therefore, the current study aimed to detect new isolates of PGPBs bacteria colonizing weed roots associated with cucumber crops in greenhouses.

MATERIALS AND METHODS

Isolation of PGPBs

Bacteria were isolated from ten agricultural areas in Diwaniyah province, which are famous for growing cucumber in greenhouses. The bacteria were isolated from the rhizosphere soil of the weeds accompanying the cucumber crop, which is characterized by dense growth, which amounted to (104) samples. Then, a series of dilutions were prepared by adding 1 gram of the selected soil samples to 9 ml of sterile distilled water in test tubes. Serial dilutions were carried out from 1 to 7 by transferring 1 ml of the soil suspension to test tubes containing 9 ml of sterile distilled water for each sample. 1 ml was taken from the seventh dilution prepared above then placed in the Petri plates contained the solid medium Nutrient Agar. Plates were moved with a light rotating motion and incubated at a temperature of 25 + 2°C for three days. After that, the purification process was carried out on a new nutrient medium several times in succession to obtain pure colonies. The isolates were preserved for the purpose of studying their properties and diagnosis.

Diagnosis of Pseudomonas

The bacteria were diagnosed morphologically by growing them on the specialized King,s B medium for 48 hours at 25 + 2°C, then observing the nature of the bacterial growth on the medium (Meera and Ahmed, 2012). Then it was diagnosed microscopically by staining it with Gram stain and examined under a light microscope using an oil lens (100X), where the reaction of the cells to the stain, the shape of the cells and their clusters were recorded (Ijaz et al., 2023)

Biochemical tests to diagnose Pseudomonas

The characteristics of the bacterial species *P. putida*, *P. Fluorescens* and *P. Chlororaphis* were studied through several biochemical tests that were carried out as follows: Catalase test, Oxidase test, Hydrolysis of gelatin, Hydrolysis of starch, Indol test, Motility and Hydrolysis of arginine. The bacteria also gave fluorescence under ultraviolet rays (Iqbal et al., 2015).

Molecular diagnosis of Pseudomonas using PCR technology

DNA was extracted from six selected samples confirmed by biochemical identification of *Pseudomonas* using the Favour prep TM Genomic DNA Mini kit provided by Inteon, Korea. The 16S rRNA gene was amplified using the forward primer 16s RNA-27 and the reverse primer 16s RNA-1492 (Hu et al., 2022). The gene amplification products, along with the forward and reverse primers were then sent to the Korean company Macrogen for the purpose of identifying bacterial species.

RESULTS AND DISCUSSION

Morphological and biochemical diagnosis of *P. putida*, *P. fluorescens*, and *P. chlororaphis*

Through morphological examination, the results showed that the bacteria growing on the King'sB medium belonged to the genus *Pseudomonas*, as the colonies growing on the medium were in the form of yellow to green, round, slightly convex colonies with a sticky, mucous texture, and gave a green-yellowish sheen when exposed to ultraviolet rays at a wavelength of 365 nanometers Figure (1) (Margaryan et al., 2016).

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Figure (1): Shows the identified types of bacteria

P. Fluorescens *P. Chlororaphis* *P. putida*

Results of the biochemical tests presented in Table (1) showed three types of *Pseudomonas* bacteria consistent with the results of previous studies (Girard et al., 2021). The bacteria are negative for the Gram test, while the Oxidase test shows that the colony changes color to purple within 5-10 seconds due to the oxidation of the reagent used by the cytochrome present in the bacterial cell which forms a purple substance. The bacteria showed positive result in the Hydrolysis of Gelatin test, as the gelatin in the test tubes treated with bacteria is transformed from a solid form to a liquid due to the gelatinase enzyme produced by the bacteria which works to decompose the gelatin due to the loss of amino acid components as it loses its ability to solidify even in the refrigerator, while the test tubes remained solid in the control treatment (Dela and Torres, 2012). The bacteria gave positive result in the Indol test and a red ring appears on the surface. A positive result in the hydrolysis of arginine is also given when the color of the medium changes to purple after 14 days of incubation, indicating that the bacteria have exploited the amino acid. In starch decomposition, the result is positive as transparent areas appear around the colonies, indicating the bacteria's ability to decompose starch (Singh et al., 2017). Moreover, in the motility test, the result is also positive as bacterial growth appears outside the stabbing area indicating the bacteria's ability to move (Muratoglu et al., 2011).

Table (1): Demonstrates bio-chemical tests for studied bacteria

Tests	***P. chlororaphis*** **9 x 10^7 CFU.g^{-1}**	***P. fluorescens*** **8.2 x 10^7 CFU.g^{-1}**	***P. putida*** **8 x 10^7 CFU.g^{-1}**
Gram stain	-	-	-
Catalase test	+	+	+
Oxidase test	+	+	+
Hydrolysis of Gelatin	-	+	-
Indol test	-	-	-
Hydrolysis of Arginine	+	+	+
Motility	+	+	+
Hydrolysis of starch	-	+	-
Fluorescence under	+	+	+

UV			

*** Positive (+) Negative (-)**

Molecular diagnosis of *Pseudomonas* bacteria using PCR technology

The results of the analysis of the nitrogenous base sequence of *Pseudomonas* species in Table (2) compared with the data available at the National Center for Biotechnology Information (NCBI) revealed three isolates of different species *P. chlororaphis*, *P. fluorescens*, and *P. putida*, which were recorded as new isolates for the first time in Iraq. Molecular identification of organisms based on bacterial DNA is considered the decisive factor in identifying the identity of the microscopic organism (Fakruddin and Mannan, 2013; Silva et al., 2023). However, the presence of new isolates that are genetically different from their counterparts depends on the ability of *Pseudomonas* species to evolve and withstand the surrounding biological or chemical stress (Silby et al., 2011; Bertani et al., 2021; Toman and Al-Ghuburi, 2023). On the other hand, this development may be the result of a compatibility based on the symbiosis of life with the jungles through stimulating growth, resisting biotic and abiotic stresses, and removing chemical toxins from the jungles, in return for providing micro-mineral elements and organic acids to *Pseudomonas* bacteria (Bagul et al., 2023; Bianco, 2024; Al-Ghuburi et al., 2025).

Table (2): Represents bacterial isolates registered in NCBI

Accession number	Name	Strain	Isolation source	Accession number
PP580375	*Pseudomonas fluorscens*	A-B1	*Convolvulus arvensis*	PP916213
PP580376	*Pseudomonas putida*	M-B2	*Imperata cylindrica*	PP916217
PP580377	*Pseudomonas chlororaphis*	N-B3	*Cyperus rotundus*	PP916214

CONCLUSION

The results of laboratory experiments on weeds accompanying cucumber crop resulted in recording three isolates of bacteria including *P. putida*, *P. fluorescens* and *P. chlororaphis* belonging to the Pseudomonateceae family and they were deposited in NCBI under the accession numbers (PP582375, PP582376 and PP582377) for the first time in Iraq.

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