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Isolation and identification of *Pseudomonas putida* from soils of plant roots and determine the ability to produce hydrolases Enzymes

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Abstract

Two *Pseudomonas putida* isolated from soils of plants roots. The bacterial isolates were identified by morphological tests. Biochemical reactions the result confirmed that they belong to *p.putida*. The bacterial isolates were produced hydrolases enzymes such as pectinase, protease and phosphates (Phosphate solubilization) by these isolates were screened. All *P. putida* isolates were able to produce these types of enzymes.

Keywords: *Pseudomonas putida*, hydrolases enzymes, pectinase, Protease, Phosphatase

عزل وتشخيص بكتريا *Pseudomonas putida* من ترب الجذور النباتية واختبار قابليتها على أنتاج الانزيمات الحالة

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الخلاصة

تم الحصول على عزلتين لبكتريا *Pseudomonas putida* من ترب جذور النبات وشخصت هذه العزلات اعتماداً على الصفات المظهرية واختبارات الكيمياء الحيوية، اظهرت النتائج انها تعود الى *P.putida* . اظهرت جميع العزلات القابلية على انتاج الانواع الثلاثة من الانزيمات الحالة(البيكتينيز, البروتيز والفوسفيتيز).

Introduction

Psendomonas putida and *P. fluorescences* influence plant growth though versatile mechanisms, they include increase nutrient uptake, enhance stress resistance, vitamin production, siderophores and biocontrol, and solubilize phosphor [1]. Plant hormone production and nutrient mobilization, hydrolytic enzyme production generally accepted mechanisms for plant growth promotion bacteria (PGPB) [2]. PGPB also produce many secondary metabolites and hydrolytic enzymes, which act as antifungal cell wall [3].

Soil microorganisms, like *Pseudomonas* Hydrolases are hydrolytic enzymes, biochemical catalysts that utilize water to split chemical bonds, usually divided a greater molecule onto two minimum molecules. An examples of general hydrolytic enzymes involve proteinase, glycosidase, nucleosidase, esterase, lipase [3].

Soil microorganisms like *Azotobacter* sp, *Azospirillum* sp. and *Enterobacter* SP. have shown to encourage plant upgrowth by promoting the outbreak of secondary roots.

Bio - Fertilizer is a substance which contains living microorganisms that provide to seed, plant surface or soil.

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Bio – fertilizer adds nutrients through the natural process, nitrogen fixation, hydrolytic enzymes production, solubilizing phosphorus and stimulating plant growth promoting substances. Bio – fertilizer can be expected to diminish the use of chemical, fertilizer as well as pesticides. The microorganisms in Bio – Fertilizers restore the soil natural nutrient cycle and construct soil organic substances[4].The aim of this study is to obtain of *pseudomonas* putida from of plant roots and determine the ability to produce hydrolases.

Material and methods

1-Samples Collection

Thirteen soil samples were assembled from four different regions in Baghdad city(AL- Gadriya, Abu-Ghraib, Mahmodia and Al-Khadra). The samples were collected randomly from rhizospheric soils (5-10 cm in depth). Using sterile containers and transported to the laboratory and preserved at 4˚c until using.

2- Isolation of *Pseudomonas*

For soil samples, suspension was prepared by adding 1g (dry weight) of each soil sample in 10 ml of sterile distilled water, and mixed well. Flask containing 100 ml of selective liquid mineral salt medium, consisted of 1g KH_2PO_4 1g K_2HPO_4, 1g NH_4NO_3, 1g $(NH_4)_2SO_4$, 0,2g $MgSO4_4.7H_2O$, 0.5g NaCL, 0.5 g ($FeSO_47H_2O$) all these components were dissolved in 900ml of distilled water and 1 ml from trace element solution consisted of 0.23 g $ZnSO_4$. $7H_2O$, 1.8g $MnSO_4.5H_2O$, 1g NH_4NO_3 0.1g $CuSO_4$. $5H_2O$ all these components were dissolved in 100 ml of distilled water was added [5].

The culture was incubated in a shaker incubator at 120rpm at 35c for seven days, then in order to get separated colonies, a loop full of broth culture mentioned above was transferred to inoculate the nutrient agar (dispensed in sterile petri dishes) using ABC streaking method.

Then incubated at 35c for seven days. the process was repeated several times to get pure culture. [6].

The isolated bacteria was purified by inoculating them on plates containing nutrients medium. The bacteria were purified by repeated inoculation. After ensuring purity the cultures were sub- cultured on nutrient slants and allowed to grow for a period of 24 hrs and subsequently stored at 4c˚ as stock culture were transferred on fresh nutrients slants at regular intervals of 3 months.

3- Identification of *Pseudomonas*

Pseudomonas isolates were identified by morphological features microscopic examination, and biochemical tests.

4- Determination of the ability of bacteria to produce hydrolytic enzymes.

4.1 production of protease

Milk agar plates consisted of (10%) skimmed milk and (2%) sterilized agar. The two component were prepared separately, mixed together, pH was adjusted to5,7 and poured in (petri dishes), [7], were inoculated with 24 hrs and incubated at 32c˚ for 24hrs. A positive result was observed as the appearance of bacterial growth, and appearance of clear zone around colonies.

4.2 Production of Pectinase2

Pectin agar plates consisted of 0.1g pectin, 0.5g yeast extract, 0,01g MgSO4.7H2O, 0.6 KH2PO4, 0,5 g NaCl, 0.1g K2HPO4, 2 g Agar, 100ml D.W, pH was adjusted to 7.5 and autoclaved [8].. A positive result was observed as the appearance of bacterial growth.

4.3 Production of phosphatase

The medium consisted of 10g Glucose, 5g Tribasic phosphate, 0.5g $(NH_4)2SO_4$, 0.2g KCl, 0.1g $MgSO_4.7H_2O$, Trace of $MnSO_4$ and $FeSO_4$ 0.5g Yeast extract, 15 agar, 1L Dw.

pH was adjusted to 7 and autoclaved [9]. The bacterial culture was inoculated with 24hr of bacterial culture and incubated at 32c˚ for 3-5 days. The appearance of bacterial growth indicates positive result.

Result and Discussion

Isolation and identification of *Pseudomonas*

Two isolates of bacteria was obtained from thirteen samples of soil, after culturing on liquid mineral salts selective medium, the growth characteristics of these isolates on *Pseudomonas* medium indicated that the isolates were classified as member of genus *pseudomonas* [5]. These isolates were further identified by morphological and biochemical test. The results showed that they were identified as strains of *pseudomonas Putida* as in Figure-1 and Table-1.

Table 1-Biochemical tests of *P.Putida*

Organism	*Indol test*	*Gas from nitrate*	*Growth at 42 c*	*Of Maltose*	*Arginine dihyrolyase*	*Motality test*	*Pigments production*	*Of manitol*	*Gelatin liquification*
p.putide	-	-	-	**v**	+	+	-	**v**	-

+ positive result
- Negative result
V variable result

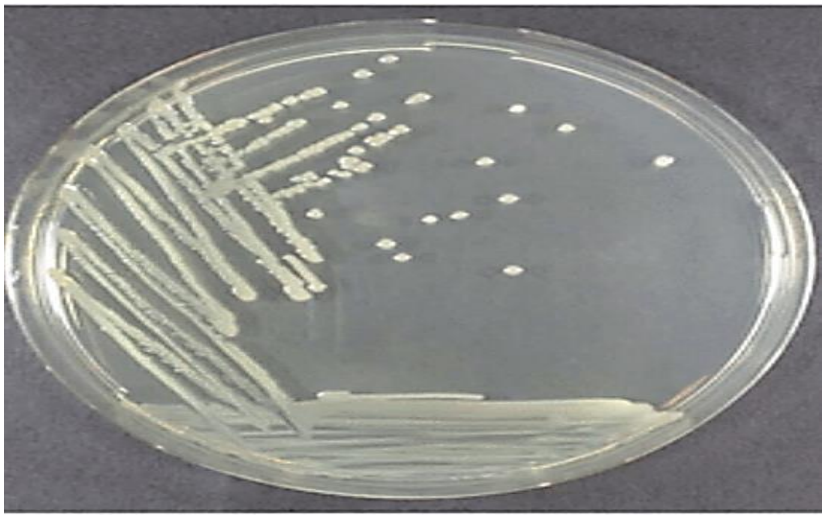

Figure 1-*pseudomonas putide* on nutrient agar.

The ability of P.*putida* isolates the produce hydrolytic enzymes

All *P.putida* isolates were able to produce hydrolytic enzyme pectinase, phosphatase and protease Figure-2. Pectin is a complex poly saccharide has an important role in plant morphogenesis and displaying rapid growth [10].

All bacteria isolates were able to produce protease with complete hydrolysis, protease break down damaged proteins to recycle their amino acids for other uses to help regulate plant growth, development and defense [11].

Extracellular enzymes target macromolecules such as carbohydras (Cellulase), lignin (Oxidase), organic phosphate(Phosphatase), Amino sugar polymerase (chitinase) and protein (protease) [12].

These enzymes degrade complex organic material into simple material to use the product as sources of carbon, energy and nutrient [13].

Enzymes activity (esterase, phosphatase, pectinase and trehalase) were used as an index to detect changes in the microbial functioning in the soil by myorrrhizal and other inoculation treatment [14].

Rizospheric bacteria community belong to genus *proteobacteria*, *Bacillus* and *pseudomonas* have plant growth promoting activity and were demonstrated other ecologically important activities like production of hydrolytic enzymes including cellulase pectinase, protease, chitinase and lipase indicated beneficial relationship between R*hizobacteria* and *Zeamays* [15].

Figure 2 (A)-*Pseudomonas putida* produce pectinase enzyme pbvjjjpjjjjjproducehh hgg pectinase pictensae enzyme

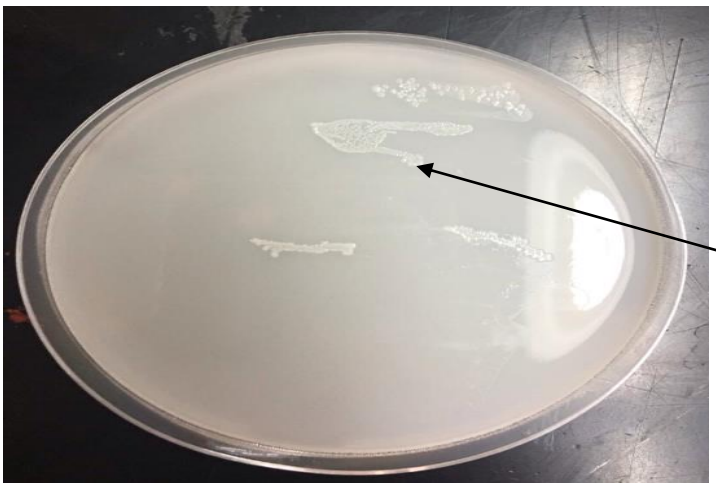

Figure 2(B)-*P. putida* produced phosphatse enzyme(growth appearance)

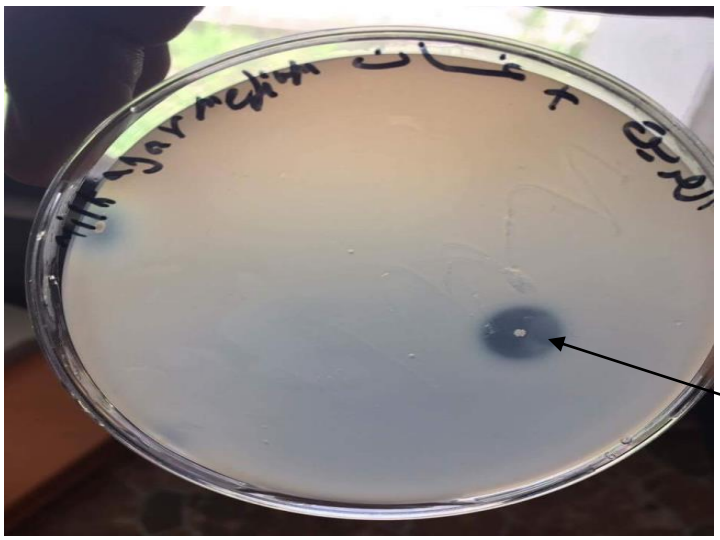

Figure 2 (C)- P. *putida* produced protease enzyme (clear zone around colony)

Phychrotrophic *Pseudomonas* produced proteolytic and lipolytic enzymes in all media investigated (skim milk, cheese, whey casein broth and tryptone,soy broth)[16].

Soil microorganisms play a key role in soil dynamic and availability of phosphate to plant [17].

Bacteria are more impact in phosphorus solubilization than fungi phosphate solubilization bacteria (PSB) constitute 1 to 50% while phosphorus solublization fungi (PSF) are only 0.1 to 0.05% in p soubilization potential [18].

Bacillus, *Enterobacter*, *Penicillium*, *Aspergillus*, and *Azospirillum* are the most powerful p soubilization (white low, 2000).

Plant insufficient in phosphorus are powerless (weakly) in growth and frequently have an violent dark – green color [19]. Phosphorus is frequently recommended as a row - applied onset fertilizer for stimulating premature growth. [20]

Phosphatase enzyme helps plants to obtain inorganic phosphate they needs, soil phosphatase play a major in the mineralize process of organic phosphorus substrates, [21].

The activity of soil phosphatase can be influenced by numerous factors and soil properties and forming system play a key role among them.

Soil acid phosphatase plays a vital role in controlling phosphate mineralization, and its activity reflects the capacity of organic phosphate mineralization Potential in soils.

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