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Production of the phytohormone Indole Acetic acid by some rhizospheric bacteria associated with the Egyptian flora

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Abstract: Indole acetic acid (IAA) is a phytohormone that regulates plant growth and development via cell elongation, cell enlargement, and cell division. IAA is synthesized both in plants and microorganisms. For microorganisms, the production of phytohormone IAA is one of the essential criteria for plant growth promotion. The present work involves the isolation of bacteria from the rhizosphere of different localities of the Egyptian flora and screening these isolates for the production of Indole acetic acid. Thirty-three bacterial isolates were obtained from clay and sandy soils. 67% of bacterial isolates were obtained from clay soil while 33% were obtained from sandy soil. The isolated bacteria produced a high amount of IAA in the range of 6.36 and 62.59 µg /ml. Thus, these bacteria are recommended as sustainable biofertilizers for their high production of IAA.

Key words: Rhizobacteria- Indole-3-acetic acid- Salkowski assay-clay soil-sandy soil

1. Introduction

Plant growth in soil depends upon a number of biotic and abiotic factors. The thin layer of soil immediately surrounding the root of a plant is an extremely important area for root activity and metabolism and is known as the rhizosphere. Plants select bacteria that are beneficial for their growth by the release of particular organic compounds through root exudates. Thus, creates a very selective environment where only a limited species of bacteria can survive, and hence diversity is low [1]. This happens due to the corresponding ability of bacteria to utilize these compounds.

Rhizosphere acts as a unique ecological niche for each plant and those beneficial bacteria associated with plants are referred to as plant growth-promoting bacteria. A number of bacterial species belonging to *Azospirillum, Alcaligenes, Arthrobacter, Acinetobacter, Bacillus, Burkholderia, Bradyrhizobium, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium,* and *Serratia* have been found to be associated with rhizosphere and are able to exert beneficial effects on plant growth [2].

Indole-3-Acetic Acid (IAA) is one of the most important and physiologically active phytohormones [3]. It is a secondary metabolite of L-tryptophan that acts as a regulator of many biological processes for plant development while acting on organogenesis, trophic responses, and cellular responses such as cell expansion, division, differentiation, and regulation of genes [4]. The majority of rhizobacteria can produce IAA which is the most abundant type of auxins [5]. Under natural conditions, plant roots excrete organic compounds, including L-Trp that can be used by rhizobacteria for IAA biosynthesis which can help non-native plant species to resist biotic and abiotic stress conditions [6].

IAA has been well-documented as an essential phytohormone known primarily for its ability to stimulate plant growth and development [7]. Indeed, IAA synthesized by rhizobacteria affects mostly the root system by increasing its size, weight, lateral root number, and area of contact with the soil. This mechanism contributes to increasing nutrient research and acquisition in soil, which in turn improves plant development and yield [8]. IAA can act

as a reciprocal signaling molecule by affecting gene expression in many bacteria and also plays a critical role in the plant-bacteria interaction [9]. Moreover, it has been shown that nodulated roots contain more IAA than non-nodulated roots [10], and auxins could be essential for maintaining a root nodule function [11].

The current study, aimed to isolation some of the plant growth-promoting bacteria from the rhizosphere of different plants located in different cities in Egypt and screening these isolates for the production of Indole acetic acid.

2. Materials and methods

Collection of soil samples

Clay and sand soil samples were collected from the rhizosphere of different plants located in different cities in Egypt (30° 1' 59.9988" N, 31° 14' 0.0024" E). Clay soils were collected from Zagazig, Shibin Elkom and Benha cities while sandy soils were obtained from Alwadi Aljadid, El-Tur and Raas Sedr cities. The intact plant with root was dug out carefully with 15 cm soil slab. The clumps of soil tightly bound to the roots were carefully stored in sterile bags and used for the isolation of bacteria.

Isolation of bacteria from rhizospheric soil

A standard tenfold serial dilution method was used for the bacterial isolation from the soil. Soil was air-dried to remove the excess moisture. 1 gm of soil was suspended in 10 ml autoclaved distilled water and 1 ml of soil solution from each tube was passed on to a next tube and subsequently, a dilution range of 10^{-1} to 10^{-10} was prepared. One ml of soil solution was spread on sterile Luria broth (LB) agar plates and incubated at 37˚C for 24 h. Several bacterial colonies appeared whereby the morphologically distinguishable colonies were picked and streaked on nutrient agar plates. Re-streaking was carried out until pure cultures were obtained. Pure cultures were maintained in nutrient agar slants at 4˚C in sterile conditions for further use.

Screening for Indole-3-acetic acid production

For the determination and quantification of IAA production by rhizospheric bacteria, the bacterial isolates were inoculated into Luria broth

(LB) media. Media was supplemented with 1mg/mL L-tryptophan. Then, cultures were incubated at 28 ± 2°C, with continuous shaking at 125rpm for 5d. Nearly 2mL of culture filtrate was centrifuged at 15000rpm for 1min, and IAA production was detected in the filtrate using Salkowski's reagent (7.5mL 0.5M $FeCl_3.6H_2O$, 150mL concentrated H_2SO_4, and 250mL distilled water). A 1mL aliquot of the supernatant was added to 2mL of Salkowski's reagent. The mixture was incubated in the dark for 20 min at room temperature [12]. The appearance of a pinkish-red color confirmed the presence of IAA. Absorbance was measured using a spectrophotometer at 530nm. The concentration of the IAA produced by rhizosphere bacteria was determined from the standard curve of a pure solution of IAA [13].

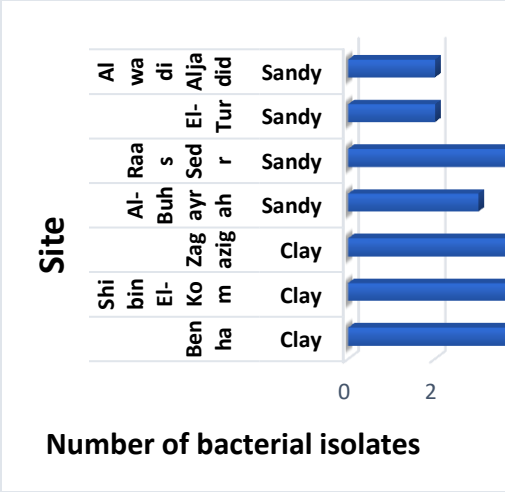
3. Results and Discussion

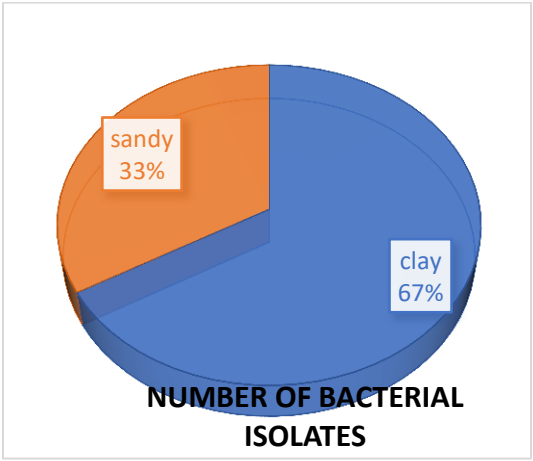
Data in Figs. 1&2 shows that thirty-three bacterial isolates were isolated from clay and sandy soils. Pie chart demonstrates that 67% and 33% of bacterial isolates were obtained from clay and sandy soils respectively. Our results assure the predominance of bacterial isolates in clay than saline soil.

In this regard, Sessitsch et al. [14] revealed that the clay fraction has a more diverse bacterial community than silt or sand fractions.

Bacterial isolates were tested for their ability to produce IAA on LB media. The 33 bacterial isolates exhibit their positive reaction by developing pink color when reacted with Salkowski's reagent which indicates a positive result for IAA production (Fig. 3). Data in Table 1. Demonstrated that bacteria produced IAA with varying amounts between 6.36 - 62.59 µg /ml. Isolate code 23N produced the highest of IAA (62.59 µg /ml followed by 29N which produced (49.54 µg /ml). While the least amount (6.36 µg /ml) was produced by isolate code 12N.

In this regard, Sehim and Dawwam [15] isolated 90 endophytic bacterial isolates from different genotypes of *Populus tomentosa*. Among various bacterial isolates, IAA production ranged from 0.42±0.06 to 150.84±1.15µg/ml. Also, **Widawati** et al [16] isolated 19 bacterial isolates from the soil on the Peatlands area. The bacteria produced IAA with varying amounts between 2.88 - 5.14 µg /ml.


Site
Al wadi Aljadid
El-Tur
Raas Sedr
Al-Buhayrah
Zag azig
Shibin El-Kom
Benha
Sandy
Sandy
Sandy
Sandy
Clay
Clay
Clay
0
2
Number of bacterial isolates


sandy 33%
clay 67%
NUMBER OF BACTERIAL ISOLATES


23 N
29 N
31 N
25 N
28 N
22 N
10 N
19N
Control

Sample code	IAA production (µg/ml)	Sample code	IAA production (µg/ml)
1 N	10.22±0.25	17 N	27.72±0.39
2 N	33.27±0.17	18 N	9.72±0.25
3 N	30.59±0.31	19 N	35.63±0.17
4 N	10.50±0.29	20 N	7.22±0.28
5 N	8.54±0.28	21 N	32.54±0.36
6 N	16.63±0.17	22 N	37.04±0.27
7 N	7.18±0.24	23 N	62.59±0.25
8 N	22.09±0.18	24 N	8.18±0.27
9 N	13.77±0.19	25 N	45.90±0.22
10 N	37.27±0.24	26 N	35.27±0.27
11 N	24.18±0.39	27 N	37.18±0.14
12 N	6.36±0.28	28 N	40.31±0.19
13 N	17.77±0.25	29 N	49.54±0.27
14 N	31.40±0.24	30 N	38.40±0.24
15 N	19.27±0.17	31 N	46.18±0.18
16 N	16.51±0.25	32 N	26.13±0.36
		33 N	31.40±0.33

In addition, Dawwam et al [17] isolated endophytic bacteria from the roots of potato plants and found that all the bacterial isolates produce IAA. The produced mounts of IAA ranged from 10.73 to 0.6 µg/ml.

Conclusion

In conclusion, Thirty-three bacterial isolates were obtained from the rhizosphere of clay and saline soils. These bacteria produced IAA with different proportions. Further studies are required to explore more production of IAA by the most potent isolates and study their effect on various crop plants in the field where plants are exposed to various abiotic and biotic factors.

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