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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: The phytohormone indole acetic acid (IAA) is synthesized both by plants and microorganisms. This phytohormone performs its activity on plant growth and development by regulating cell elongation, enlargement, and division. The production of IAA is one of the essential criteria that discriminate the plant growth-promoting microorganisms. 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.

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

1. Introduction

Several biotic and abiotic factors control plant growth in soil. The rhizosphere, or the thin layer of soil closely to a plant root, is a crucial location for root activity and metabolism. In this location, plants secrete certain organic compounds through the root exudates to designate the bacterial strains that will promote their growth. This plant strategy produces an extremely selective environment where only a few numbers of bacterial species can survive, leading to minimal diversity in the soil microbial population [1]. This preferable selection is owing to the varied ability of bacterial species towards the secreted exudates.

The rhizosphere is an exceptional ecological niche for each plant and those useful bacteria which coexist with plants are known as plant growth-promoting bacteria (PGPB). Some bacterial species belonging to *Azospirillum, Alcaligenes, Arthrobacter, Acinetobacter, Bacillus, Burkholderia, Bradyrhizobium, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium,* and *Serratia* have been recorded to promote plant growth [2].

Indole-3-acetic acid (IAA) is one of the most vital and physiologically dynamic phytohormones [3]. It is a secondary metabolite synthesized from the precursor molecule, L-tryptophan. IAA regulates various biological activities for plant development such as organogenesis, tropic responses, and cellular responses including cell expansion, division, differentiation, and gene regulation [4]. Most of the rhizobacteria can produce IAA, especially those of the auxins category [5]. Rhizobacteria can use L-Trp, which is normally secreted by plant roots to synthesize IAA. IAA secretion enables the non-native plant species to elevate the deleterious effects of biotic and abiotic stresses [6].

Despite, IAA has been well-known basically for its activity to stimulate plant growth and development [7], it is synthesized by rhizobacteria and affects typically the root system by increasing root size, masses, lateral root number, and area in contact with the soil particles. This strategy enhances nutrient search and acquisition from soil, which consequently reinforces plant growth, development, and yield [8]. Because of its operational role in gene expression in

several bacterial species which strongly affects the plant-bacteria interaction, IAA is considered a mutual signaling molecule [9]. Besides, it has been found that nodulated roots contain higher IAA content than non-nodulated ones [10], and auxins could be crucial for preserving root nodulation [11].

The current study, aimed to isolate 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 **a** 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 to isolate the examined bacteria from the soil. Firstly, and to remove the excessive moisture, the soil was air-dried. Then, 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 the 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 distinctive colonies were chosen 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. 1mg/mL L-tryptophan was added to the media. Following that, cultures were incubated for five days at 28 ± 2°C while being shaken constantly at 125 rpm. Using Salkowski's reagent (7.5mL 0.5M $FeCl_3.6H_2O$, 150mL concentrated H_2SO_4, and 250mL distilled water), about 2 mL of the culture filtrate was centrifuged for 1 minute at 15000 rpm to detect the formation of IAA in the filtrate. 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 formation of a pinkish-red color indicated that IAA was present. At 530 nm, absorbance was measured. The IAA concentration generated by rhizosphere bacteria was calculated by utilizing the standard curve of an IAA-pure solution. [13].

3. Results and Discussion

Data in Figs. 1&2 shows that thirty-three bacterial isolates were isolated from clay and sandy soils. A 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 bacterial community in the clay fraction is more diversified than that in the silt or sand ones.

The ability of bacterial isolates to form IAA on LB media was examined. The 33 bacterial isolates positively reacted with Salkowski's reagent by producing a pink color, this denotes the synthesis of IAA (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*. The authors recorded varied production of IAA across the different bacterial isolates, ranging 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. In their study, the quantities of IAA generated by the bacteria ranged from 2.88 to 5.14 µg/ml.

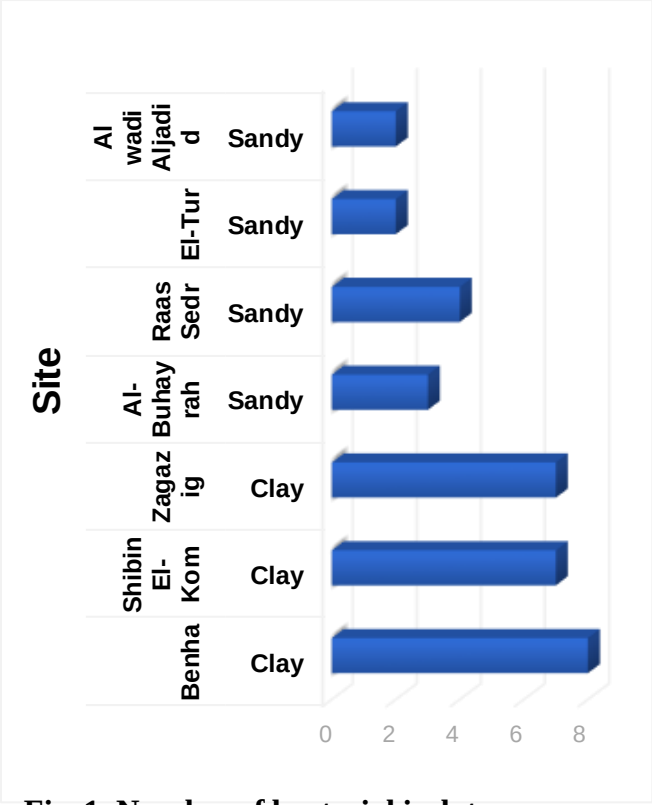

Fig. 1. Number of bacterial isolates obtained from different sites.

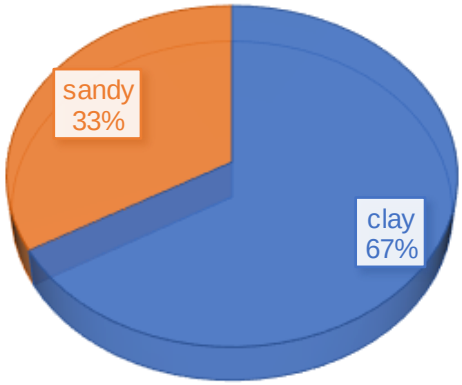

Fig. 2. Pie chart demonstrates the abundance of bacterial isolates in sandy and clay soil.

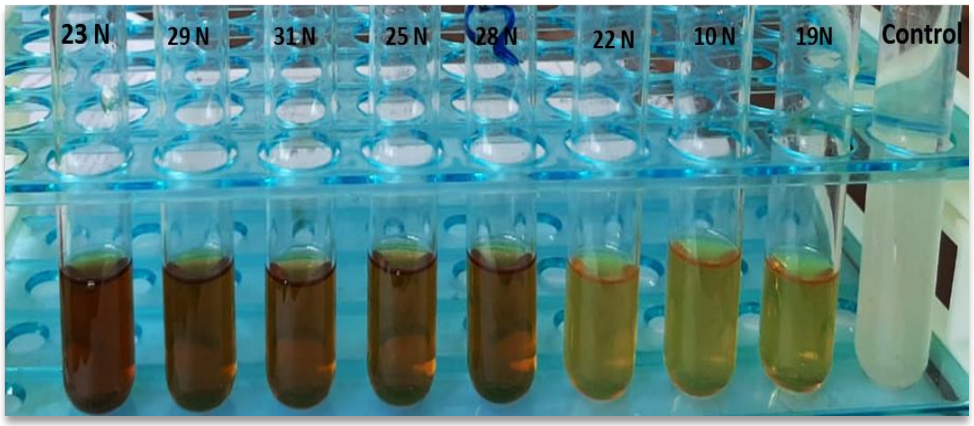

Fig. 3. IAA production by bacterial isolates.

Table 1. IAA production by different bacterial isolates.

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

*Results were expressed as mean ± standard deviation.

In addition, Dawwam et al [17]isolated endophytic bacteria from the roots of potato plants and found that IAA is produced by all examined bacterial isolate and The IAA amounts that varied 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. More research is needed to investigate the most potent isolates' ability to produce IAA and to examine their impact on different crop plants under varied abiotic and biotic stimuli in the field.

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