Screening of rhizospheric actinobacteria isolated from different cultivated plants in Egypt

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ABSTRACT

In present study, four hundred and seventy-three actinobacterial isolates were isolated from five different rhizospheric soil samples including clover, potato, wheat, mint, and nalata jute plants. Culture characterization of all actinobacterial isolates was performed using starch casein agar medium. Microscopic examination of all isolates was carried out using slide culture technique. All isolates were identified morphologically. The most dominant actinobacteria isolated from rhizospheric soil was genus Streptomyces. The key factor of shaping microbial population in rhizosphere is plant species. Fifteen (3.1%) isolates only from all isolated actinobacteria were able to produce indole acetic acid. Out of fifteen actinobacteria, fourteen isolates belonged to genus Streptomyces, while the last one belonged to genus Saccharopolyspora. The fifteen actinobacteria could be used as plant growth-promoting rhizospheric bacteria instead of chemical fertilizers, since it enhances growth and development of plants through developing specific communication pathways with the plant and may influence plant physiology.

Key words: Actinobacteria, Rhizospheric soils, PGPB, IAA.

INTRODUCTION

Actinobacteria are one of the largest bacterial phyla. They are gram positive bacteria having high DNA G+C content ranged from under 50% to over 70%. They may be aerobic, facultative anaerobic, or anaerobes. Phylum actinobacteria have morphological diversity from cocci shape to well-developed aerial and substrate mycelia. This phylum of bacteria is classified into 6 classes, 6 orders, 14 suborders, and 56 family (Parte et al., 2012). Actinobacteria are widely distributed in numerous habitats in ecosystems all over the world such as soil habitat, aquatic ecosystems including sediment, marine, and fresh water, and in extreme environments such as desert ecosystems, and antarctica region (Alam et al., 2021; Lipko & Belykh, 2021; Solans et al., 2022; Tistechok et al., 2021).

In 1904, Hiltner described that soil portion around root system where is affected by microorganism’s metabolism is called rhizosphere that play vital role in plant nutrition, health, and quality. Plants excrete from 10% up to 44% of carbon compounds synthesized from photosynthesis such as organic acids, amino acids, lipids, proteins, enzymes, flavonoids, aliphatics, and aromatics by root (Bais et al., 2006). These compounds act as substrate for nutrition of rhizospheric microbial community. As root exudates composition and amount vary according to plant family or species, microbial community in rhizosphere is shaped according to type of plant. Therefore, key factors for microbial population enrichment in rhizosphere are plant species and root exudates (Dastogeer et al., 2020).
Actinobacteria are important group of bacteria that is well known due to their unique characteristics and biotechnological applications, especially their ability to produce a wide range of natural products called secondary metabolites. Secondary metabolites are bioactive compounds produced by living organisms that is not essential for their own metabolic process (Bérdy, 2012). *Streptomyces* produce more than 50% of all known bioactive secondary metabolites such as antibiotics, antitumor agents, anti-helminthics, herbicides, insecticides, pigments, enzymes, plant protectants, growth promoters of animals and plants, and other several vital secondary metabolites (Hwang et al., 2014). Approximately 75% of all antibiotic products on the earth produced by this powerful bacterial group such as streptomycin, anthracyclines, macrolides, and beta-lactams (Procópio et al., 2012). Novel secondary metabolites that have never been observed before could be discovered by mining of several *streptomyces* genome (Ikeda et al., 2003). Streptomycetes are considered massive reservoirs of several secondary products made them the most important microbial genus used in industrial fields (Harir et al., 2018). Secondary metabolites as antibiotics and other bioactive metabolites that have diversity in chemical structures reflecting on their biological activities that used in human, pharmaceutical, industrial, medical, agricultural sectors (Harir et al., 2018).

Auxin production ability is the most powerful mechanism explaining effect of plant growth promoting rhizospheric bacteria on plant growth. 80% of rhizospheric microbes can produce auxin as secondary metabolites according to Patten & Glick, (1996). Indole-3-acetic acid (IAA) is the most important active member of auxin group that regulate many basic cellular processes (Spaepen et al., 2007). Auxins have vital role in geotropism and phototropism, cell division, epical dormancy, stem and root elongation, vascular differentiation, and lateral and adventitious root initiation (Grobelak et al., 2015). IAA increase number of nodulation and stablish symbiosis of rhizobia with legumes (Remans et al., 2008). Auxin concentration and type of microorganism are vital factors for plant seedling stimulation (Ahmad et al., 2005). The microorganism that produce the highest amount of auxin concentrations in non-sterilized soil result in maximum increase in growth and yield of wheat plant (Khalid et al., 2004) even the microorganism that produce low amount of auxin concentration and produce it continuously can enhance plant growth (Elena A Tsavkelova et al., 2007).

**MATERIALS AND METHODS**

**Collection of samples**

Soil samples were collected from different rhizospheric plants (clover, potato, wheat, mint, and nalta jute plants). Rhizospheric soil samples were collected in sterile polyethylene bags. Soil samples were dried in room temperature before isolation for 5-7 days.

**Isolation and purification of Actinobacteria**

Each rhizospheric soil sample was suspended into flask containing 100 ml sterile water. Serial dilution method was used to dilute microbial concentration in each sample (Hayakawa & Nonomura, 1987). Inoculation into agar medium from each dilution was done by surface and pour plate methods. Starch casein agar medium (SCA) and Yeast extract – malt extract agar medium (YMEA) were used for isolation. All petri dishes were incubated at 30°C for 2 weeks to allow growth of slow growing actinobacteria.

The actinobacteria from isolated plate were purified into SCA and YMEA then incubated at 30°C for 7 days. Purified actinobacteria was transferred into slants of SCA and YMEA.
Morphological characterization

Morphological and cultural characterization of all isolated actinobacteria was detected by using starch casein agar. Presence of aerial mycelium, color of aerial mycelium, color of substrate mycelium, texture of culture, and soluble pigment production were recorded.

Morphology of aerial mycelium, substrate mycelium, and spore were recorded through slide culture technique by inserting sterile glass slide in starch casein agar at 45° angle and actinobacteria were streaked at the line between slide and media then incubated at 30°C for 7 days (Williams, 1989). Slides were examined under light microscope.

Indole acetic acid (IAA) production

All actinobacterial isolates were tested for IAA production by growing them in Starch casein broth amended with 500 µg/ml tryptophan for 7 days at 30°C. The pink color observed after adding Salkowski reagent (1ml of 0.5M FeCl3 in 50ml of 35% HClO4) in broth culture indicated the ability of actinobacterial isolates to produce IAA (Gordon and Weber, 1951).

RESULTS

Collection and isolation of actinobacteria

Four hundred and seventy-three isolates were isolated from different plant rhizospheric soil including 121 isolates from clover plant (C), 149 isolates from potato plant (P), 108 isolates from wheat plant (W), 50 isolates from mint plant (M), and 45 isolates from nalta jute plant (O).

Fig. (1): Number of isolated actinobacteria from different plants rhizospheric soil.

Culture characterization

Presence of aerial mycelium, color of aerial mycelium, soluble pigment production, and texture of culture were recorded for all isolated actinobacteria as shown in Table (1). In case of actinobacteria isolated from clover plant, all isolates had ability to produce aerial mycelium. The rhizospheric isolates were grouped into 7 groups. Group (1) comprises 35 isolates that form powdery and gray aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced. Group (2) comprises 40 isolates that form powdery and gray aerial mycelium, brown substrate mycelium, and red pigment was produced. Group (3) comprises 18 isolates that form powdery and green aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced. Group (4) comprises 25 isolates that form powdery and pink aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced. Group (5) comprises 7 isolates that form powdery and white aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced.
pigment was produced. Group (6) comprises 10 isolates that form cottony and white aerial mycelium, creamy substrate mycelium, and yellow pigment was produced. Group (7) comprises 3 isolates that form cottony and white aerial mycelium, creamy substrate mycelium, and brown pigment was produced.

While in case of actinobacteria isolated from potato plant, all isolates had ability to produce aerial mycelium. The rhizospheric isolates were grouped into 5 groups. Group (1) comprises 70 isolates that form powdery and gray aerial mycelium, brown substrate mycelium, and no soluble pigment was produced. Group (2) comprises 36 isolates that form powdery and gray aerial mycelium, creamy substrate mycelium, and red pigment was produced. Group (3) comprises 15 isolates that form powdery and green aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced. Group (4) comprises 9 isolates that form powdery and pink aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced. Group (5) comprises 20 isolates that form powdery and white aerial mycelium, creamy substrate mycelium, and yellow pigment was produced.

In case of actinobacteria isolated from Mint plant, all isolates didn’t have ability to produce soluble pigment. The rhizospheric isolates were grouped into 4 groups. Group (1) comprises 3 isolates that didn’t form aerial mycelium, had leathery texture and orange substrate mycelium. Group (2) comprises 31 isolates that form powdery and gray aerial mycelium, creamy substrate mycelium. Group (3) comprises 10 isolates that form powdery and yellowish white aerial mycelium, creamy substrate mycelium. Group (4) comprises 7 isolates that form powdery and white aerial mycelium, creamy substrate mycelium.

In case of actinobacteria isolated from Nalta jute plant, all isolates had ability to produce aerial mycelium. The rhizospheric isolates were grouped into 5 groups. Group (1) comprises 28 isolates that form powdery and gray aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced. Group (2) comprises 3 isolates that form powdery and gray aerial mycelium, creamy substrate mycelium, and red pigment was produced. Group (3) comprises 7 isolates that form powdery and pink aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced. Group (4) comprises 2 isolates that form powdery and yellowish white aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced. Group (5) comprises 7 isolates that form powdery and white aerial mycelium, creamy substrate mycelium, and yellow pigment was produced.
Table (1): Culture characterization of rhizospheric actinobacteria isolated from different cultivated plants.

| Cultivated Plant | Groups       | Texture | Aerial mycelium | Substrate mycelium | Pigment production |
|------------------|--------------|---------|-----------------|--------------------|--------------------|
| Clover plant     | Group (1)    | Powdery | Gray            | Creamy             | Non                |
|                  | Group (2)    | Powdery | Gray            | Creamy             | Red                |
|                  | Group (3)    | Powdery | Green           | Creamy             | Non                |
|                  | Group (4)    | Powdery | Pink            | Creamy             | Non                |
|                  | Group (5)    | Powdery | White           | Creamy             | Non                |
|                  | Group (6)    | Cottony | White           | Creamy             | Yellow             |
|                  | Group (7)    | Cottony | White           | Brown              | Brown              |
|                  | Group (1)    | Powdery | Gray            | Creamy             | Non                |
|                  | Group (2)    | Powdery | Gray            | Creamy             | Red                |
|                  | Group (3)    | Powdery | Green           | Creamy             | Non                |
|                  | Group (4)    | Powdery | Pink            | Creamy             | Non                |
| Potato plant     | Group (5)    | Powdery | White           | Creamy             | Yellow             |
| Wheat plant      | Group (1)    | Powdery | Gray            | Creamy             | Non                |
|                  | Group (2)    | Powdery | Green           | Creamy             | Non                |
|                  | Group (3)    | Powdery | Green           | Creamy             | Yellow             |
|                  | Group (4)    | Powdery | White           | Creamy             | Non                |
|                  | Group (5)    | Powdery | White           | Creamy             | Pink               |
| Mint plant       | Group (1)    | Leathery | Non           | Orange             | Non                |
|                  | Group (2)    | Powdery | Gray            | Creamy             | Non                |
|                  | Group (3)    | Powdery | Yellowish white | Creamy             | Non                |
|                  | Group (4)    | Powdery | White           | Creamy             | Non                |
| Nalta jute plant | Group (1)    | Powdery | Gray            | Creamy             | Non                |
|                  | Group (2)    | Powdery | Gray            | Creamy             | Pink               |
|                  | Group (3)    | Powdery | Pink            | Creamy             | Non                |
|                  | Group (4)    | Powdery | Yellowish white | Creamy             | Non                |
|                  | Group (5)    | Powdery | White           | Creamy             | Yellow             |
Microscopic characterizations

Slide culture technique was carried out using starch casein agar. The microscopic examination of actinobacteria produced only substrate mycelium that isolated from Mint plant showed short chains of spores on substrate mycelium only indicating that isolate mainly may belong to genus *Saccharopolyspora*. The left isolates showed long chains of spores varied between hooks, Spiral, and loops on aerial mycelium so largely can belong to genus *Streptomyces*.

**Fig. (3): Some cultures of actinobacteria isolated from rhizospheric soils**

**Fig. (4): Microscopic examination of actinobacteria.**

a- showed short chains of spores on substrate mycelium of *Saccharopolyspora*.

b- c and d showed different shapes spore chains of *Streptomyces* isalates.
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Qualitative and quantitative IAA production
All isolates were screened for qualitative determination of IAA production. Fifteen (3.1%) isolates only were able to produce IAA. The IAA producing isolates were 2 (1.65%) isolates from clover plant, 8 (5.36%) isolates from potato plant, 4 (3.7%) isolates from wheat plant, 1 (2%) isolate from mint plant, and no isolates from nalta jute plant. Out of fifteen actinobacteria, fourteen isolates belonged to genus Streptomyces while the last one belonged to genus Saccharopolyspora.

Fig. (5): Percentage of actinobacteria that had ability to produce IAA isolated from different rhizospheric soil samples (Clover, Potato, Wheat, Mint, and Nalta jute plants).

DISCUSSION
The most dominant phylum in rhizosphere that have important economic impact due to its secondary metabolites production is actinobacteria (Yadav et al., 2018). More than 30% of total rhizospheric soil microbiota belong to actinobacteria. Among actinobacterial genera, Streptomyces have been represented in rhizosphere. Genus Streptomyces represent more than 95% of all rhizospheric actinobacteria (Ventura et al., 2007).

From 5 plants rhizosphere soils, 473 actinobacterial isolates were obtained. Number of actinobacteria isolated from potato plant were more than other rhizosphere soils. It is possible that wheat plant might synthesizes and secrete compounds in its root exudates that activate actinobacterial spore, promote actinobacterial growth and inhibit microbial soil bacteria and fungi that is in line with results reported in (Khamna et al., 2009). Number and diversity of actinobacteria isolated from wheat plant were more than those in nalta jute plant rhizosphere although the two soil samples were collected from the same field because plant root exudates are vital key for microbial population enrichment in rhizosphere (Uren, 2000). Streptomycetes group was the dominant actinobacteria isolated from all rhizospheric plant soil that is in line with (Pandey and Palni, 2007). Moreover, Saccharopolyspora was isolated from rhizosphere of mint plant that in line with (El-Tarabily and Sivasithamparam, 2006; Tsavkelova et al., 2006) who reported that several actinobacteria isolated from different rhizospheres including streptomycetes group and rare-actinomycetes group.

Out of 473 actinobacterial isolates, 15 (3.1%) actinobacteria isolated from different rhizospheric soils have ability to
produce IAA which agree with El-Tarabily and Sivasithamparam, 2006; Tsavelkova et al., 2006, who reported that several actinobacteria including Streptomyces group and rare-actinomycetes group isolated from different rhizospheres have ability to produce IAA. Potato rhizospheric soil was the most soil containing actinobacteria that had IAA production ability, since the activity of microorganism for production of IAA is supported by root exudate of plants which vary according to plant species (Frankenberger and Arshad, 2020). It is possible that potato plant secretes root exudate containing high level of tryptophan as IAA precursor supporting and enhancing IAA biosynthesis in rhizosphere agreeing with (Khamna et al., 2010).

Conclusion

The dominant actinobacteria in soil is streptomyces. Abundance and diversity of actinobacteria in rhizosphere is changed according to plant type. Plant species is the key factor for microbial population enrichment in rhizosphere. Actinobacteria that produce IAA can used as plant growth promoting rhizospheric actinobacteria for enhancing soil fertility and increasing crop productivity.

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Research conducted at the University of Sheria, Faculty of Microbiology, Department of Microbiology, Ayeen University Science Foundation.

The aim of the study was to isolate and characterize rhizospheric actinobacteria from different cultivated plants in Egypt. 173 actinobacteria strains were isolated and characterized using starch-casein agar culture media and slide culture techniques. The actinobacteria were identified as Streptomyces spp. with varying activities, including indole acetic acid production and growth promotion.}

The results showed that these actinobacteria had positive effects on plant growth and yield, indicating their potential for use in biological control of plant diseases.

This research provides valuable information for the development of new biocontrol agents and for the optimization of agricultural practices in Egypt.