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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 nalta 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 *etal*., 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 promotors 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-3acetic 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^{o}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^{o}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^{o} angle and actinobacteria were streaked at the line between slide and media then incubated at 30^{o}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℃. 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).

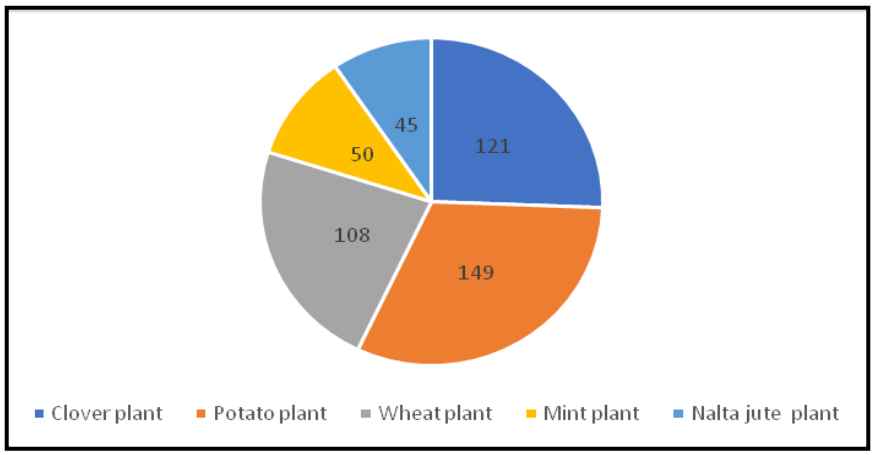

Fig. (1): Number of isolated actinobacteria from different plants rhizospheric soil.

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. 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 wheat plant, all isolates had ability to produce aerial mycelium. The rhizospheric isolates were grouped into 5 groups. Group (1) comprises 81 isolates that form powdery and gray aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced. Group (2) comprises 12 isolates that form powdery and green aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced. Group (3) comprises 4 isolates that form powdery and green aerial mycelium, creamy substrate mycelium, and yellow pigment was produced. Group (4) comprises 8 isolates that form powdery and white aerial mycelium, creamy substrate mycelium, and no soluble pigment was produced. Group (5) comprises 3 isolates that form powdery and white aerial mycelium, creamy substrate mycelium, and pink 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.

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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
Potato plant	Group (1)	Powdery	Gray	Brown	Non
	Group (2)	Powdery	Gray	Creamy	Red
	Group (3)	Powdery	Green	Creamy	Non
	Group (4)	Powdery	Pink	Creamy	Non
	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

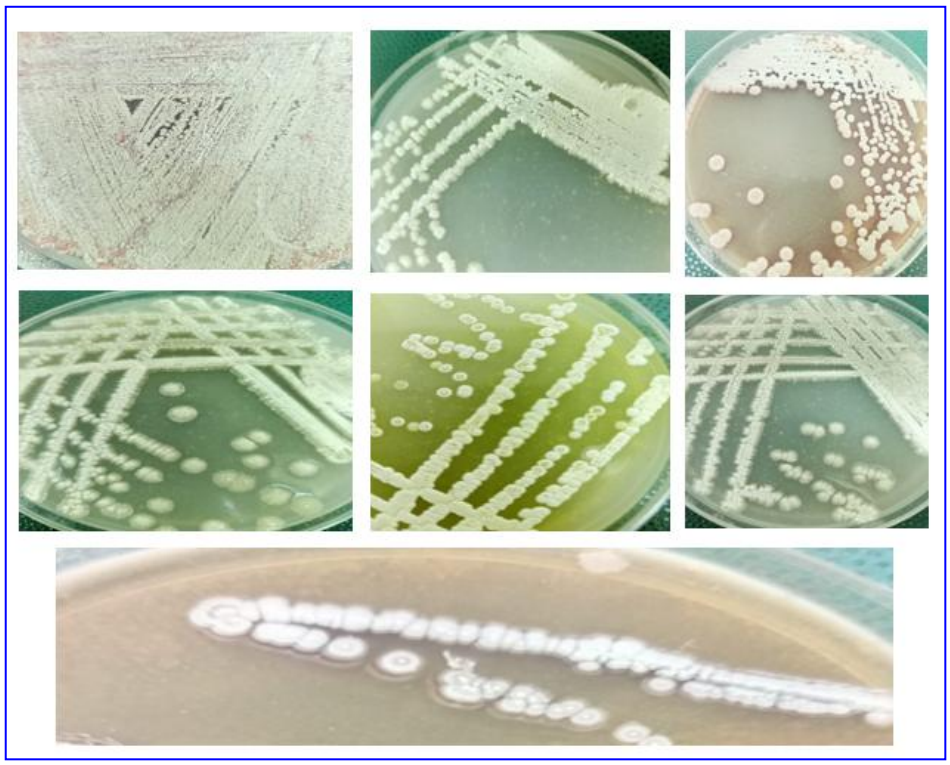

Fig. (3): Some cultures of actinobacteria isolated from rhizospheric soils

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. (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* isoalates.

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.*

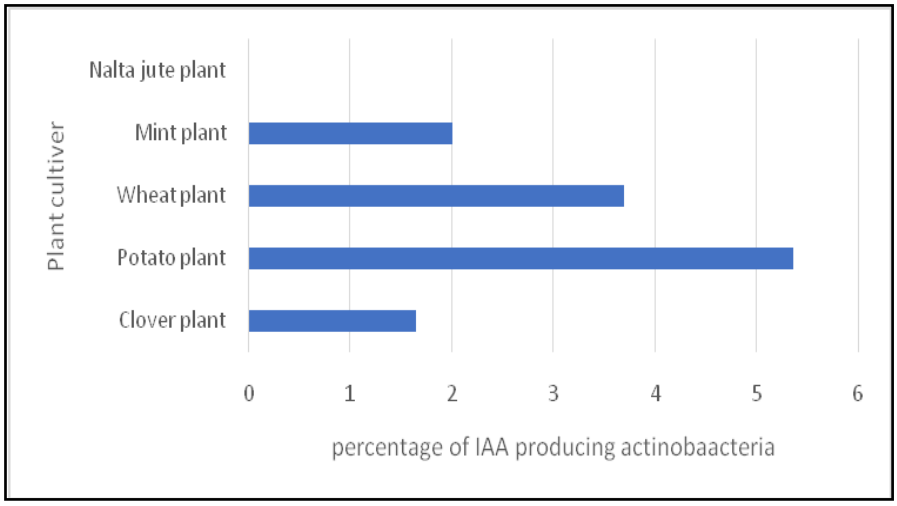

Fig. (5): Percentage of actinobacteria that had ability to produce IAA isolated from different rhizospheric soil samples (Clover, Potato, Wheat, Mint, and Nalta jute plants).

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 juta 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; Tsavkelova *et al*., 2006, who reported that several actinobacteria including Streptomycetes 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 streptomycetes. 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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مسح على الاكتينوبكتريا المحيطة بالجذر المعزولة من نباتات مختلفة مزروعة في مصر

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قسم الميكروبيولوجي، كلية العلوم، جامعة عين شمس

المستخلص

في الدراسة الحالية تم عزل 173 كائن من الاكتينوبكتريا وذلك من خمس عينات تربة مختلفة محيطة بالجذر لنباتات البرسيم والبطاطس والقمح والنعناع والملوخية . وتم عمل Culture characterization لجميع الاكتينوبكتريا المعزولة باستخدام وسط starch casein agar. كما تم عمل الفحص الميكروسكوبي لجميع الكائنات باستخدام تقنية slide culture. جميع العينات تم التعرف عليها ظاهريا. الاكتينوبكتريا السائدة التي تم عزلها من التربة المحيطية بالجذر كانت تنتمي الى جنس *Streptomyces*. يعتبر العامل الأساسي لتغيير البيئة الميكروبية في المنطقة المحيطة بالجذر هو نوع النبات. وقد وجد ان خمسة عشر (3.1%) كائن فقط من جميع الاكتينوبكتريا التي تم عزلها لديها القدرة على انتاج indole acetic acid. ومن الخمسة عشر كائنوجد ان أربعة عشر كائن ينتمون الى جنس *Streptomyces* في حين ان واحد فقط كان ينتمي الى جنس *Saccharopolyspora*. من الممكن استخدام الخمسة عشر اكتينوبكتريا كبكتريا جذر محيطية محفزة لنمو النبات بدلا من الأسمدة الكيمائية، وذلك لتحفيز النمو وتطور النباتات من خلال تحفيز مسارات تواصل محددة مع النبات ومن الممكن ان يؤثر هذا على تحسين فسيولوجيا النبات.