Overproduction of Some Plant Growth Promoters by Rhizospheric Microorganisms on Natural Medium

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GJSFR-G Classification: FOR Code: 060599
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Abstract - Considering the nutritional values of Mentha viridis L. and Aloe vera plants, these plants can be utilized for the production of alternative cultivation media. The cost of artificial culture media is very high, and some components may be unavailable. Use of the plant-based culture media would drastically reduce the expense of the synthetic media. Fifteen bacterial isolates were isolated from Aloe vera rhizosphere, nine bacterial and nine actinomycetes isolates were isolated from Mentha viridis rhizosphere both cultivated in Sirs El Layan, El-Menoufia governorate, Egypt. In-vitro screening was done for the production of indole acetic acid (IAA) and phosphorus solubilization. Results revealed that bacterial isolate No. MB4 produced a high amount of IAA(36.51 µg/ml) on the Aloe vera-based culture medium, No. A6 showed maximum IAA production (16.25µg/ml) on the Mentha-based culture medium, No. A6 showed maximum IAA production (16.25µg/ml) on the Aloe vera-based culture medium and isolate No. MA6 was efficient in phosphorus solubilization (867.85µg/ml) that was isolated from the Mentha viridis L rhizosphere.16s rRNA analysis of these isolates revealed they are (Pseudomonas monteilii strain CIP 104883, Streptomyces rochei strain DW3 and Kosakonia radicincitans strain DSM 16656) respectively. Bio-control ability of selected strains screened by antagonistic activity against pathogenic fungi revealed that Pseudomonas monteilii strain CIP 104883 had shown maximum zone of inhibition against Rhizoctonia solani (19 mm) while Streptomyces rochei and Kosakonia radicincitans showed highly antifungal activity against Fusarium solani (18 mm and 11 mm zone of inhibition, respectively).

Keywords: plant-based culture media, menthe viridis, l, aloe vera, rhizospheric microorganisms, plant growth-promoting activities, biocontrol.

1. INTRODUCTION

Microbial culture media can be of different types, depending on the nutritional growth requirements of the microorganisms. Microorganisms require about ten macroelements, namely (C, O, H, N, S, P, K, Ca, Mg and Fe). The first six components are needed for the synthesis of Carbohydrates, Lipids, Proteins, and Nucleic acids, and the remaining four exist in the cell as cations and play a variety of roles. Additionally, all microorganisms require several microelements like (Mn, Zn, Co, Mo, Ni, and Cu) that are generally part of enzymes and cofactors. They also require growth factors, which are organic compounds such as vitamins Basu et al. (2015). Uthayasooriyan et al. (2016) stated that the feasibility of evolving alternate culture media to artificial media, namely Potato Dextrose Agar and Nutrient Agar, were assessed by using locally obtainable materials due to the use of synthetic culture media in colleges, research centers and laboratories have limitations in finance. In general, the inexpensive locally obtainable materials such as rice, chickpea, corn, and natural soy flour may serve as replacing rich nutrient media to the growth of bacteria and fungi, and reduce the cost of microbial media. Commercially available media such as Nutrient Agar and MacConkey Agar, which used for the growth of microorganisms but these, are very expensive. Plant-based culture medium is a natural environment that has been introduced for the culturing of rhizobacteria as a sole growth milieu Nour et al. (2012).

In this study, Mentha and Aloe vera extracts were used as a natural medium for isolation and growth of plant growth-promoting rhizobacteria (PGPR). Mentha and Aloe vera are perineal plants that are locally available, cheap plant material, and nutritionally rich. (Mainasara et al., 2018) revealed that the fresh peppermint leaves contained 92.31% carbohydrates, 2.19% protein, 0.50% lipid, 1.5% fiber, 3.57% ash, 89.5% moisture, and the most predominant mineral found was potassium and sodium while the other minerals also found in low values such as calcium, magnesium, and phosphorus. Aloe vera, a succulent plant that grows in arid and subtropical climates, the leaves of this nutraceutical medicinal plant contains numerous vitamins, natural sugars, enzymes, and amino acids Lanka (2018).

The use of chemical fertilizers, especially nitrogenous and phosphorous, led to water, soil, and airpollution. The excessive use of these fertilizers cause harmful effects on rhizospheric microorganisms, effects on the fertility of agriculture lands, pollutes the environment, and also declines the productivity of the crops and yield Kumar et al. (2015) and Ahmad et al. (2016). In addition to its cost, the production of these
fertilizers causes shortage in nonrenewable resources like natural gas and oil that used to produce these chemical fertilizers, and poses a lot of environmental and human dangers Prakash and Verma (2016). To achieve a clean agriculture with low cost and produce crops with desired properties, one possibility is to use soil microorganisms (bacteria, fungi, actinomycetes, algae, etc.) that increases the nutrient uptake capacity and water use efficiency. Among these potential soil microorganisms, bacteria known as plant growth-promoting rhizobacteria (PGPR) are the most promising. In this sense, PGPR may be used to enhance plant health and regulate plant growth rates without environmental contaminations (Saharan and Nehra, 2011). Plant Growth-Promoting Rhizobacteria (PGPR) are the rhizospheric bacteria that can positively affect plant growth by several mechanisms like phosphate solubilization, siderophore production, biological nitrogen fixation, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), and inhibition of biofilm formation, phytohormone production, exhibiting antimicrobial activity, induction of systemic resistance (ISR), promoting beneficial plant-microbe symbioses, and many others mechanisms Benaissa (2019).

Current studies aimed to develop and evaluate culture media from plants because the synthetic culture media have become very expensive in the local market and, most instances, are not available and also the production of plant growth promoters on this natural medium to use in clean agriculture instead of chemical fertilizers.

II. Materials and Methods

a) Sampling sites

Samples from the rhizosphere of Mentha vividis, L and Aloe vera plants were collected from Sirs EL-Layan, El-Menoufia governorate, Egypt, from the upper surface layer of the soil (5 cm), where maximum populations of microorganisms concentrated.

b) Tested plants

The tested plants, Mentha vividis and the succulent plants Aloe vera, are cultivated in Sirs El-Layan, El-Menoufia governorate, Egypt. Both plants were perineal and rich in nutrient content, Aloe vera was chosen for its availability in arid and semi-arid environments as well as their copious juicy nature, while Mint is one of the most common herbs traditionally produced in Egypt for hundreds of years and widely distributed and cultivated in the temperate and subtropical regions of the world.

The chemical compositions and nutritional contents of the tested succulent plant (A. vera) were available by Tiwari and Upadhayay (2018). Therefore, special attention was given to the analysis of the juice of Spearmint (Mentha viridis L) for the application in isolation of rhizobacteria. Macro- and micro-nutrients were detected by atomic absorption analysis, total protein by Tru Spec N instrument AOAC International and Latimer (2012), carbohydrate Dubois et al. (1965), mineral Horwitz (2012) amino acids by performic oxidation method and vitamins by GC/MS/MS analyses Lehotay and Hajslova’s, (2002). Total crude fiber and ash were also determined AOAC International (1998).

c) Preparation of plant-based culture media

Wash the succulent leaves of A. vera and the vegetative parts (leaves and stems) of M. vividis, slice, and then blend with equal aliquots of distilled water (w/v) for 5 min in the blender. The resulting slurry homogenate was coarse-filtered through cheesecloth to obtain plant juice; almost 73–82% of the plant fresh weight was recovered as juice. The plant juices from the tested plants further diluted with distilled water (v/v); 1:10, 1:20, 1:40, 1:80, and 1:100. The pH for M. vividis diluted juices were in the range of 5.8-6.5 and A. vera diluted juices 4-6.2. Exclusively, use these diluted juices to prepare the plant-based agar culture media (2% agar, w/v). Adjust the pH of all media to 7.0, then autoclave at 1.5 atm., 121°C for 20 min.

Use Serial dilution techniques to isolate rhizospheric microorganisms, prepare the suspensions of samples by adding 1g of the rhizospheric soil that obtained from Sirs El-Lian, El-Menoufia governorate, Egypt to 10 ml of sterile distilled water under aseptic conditions and shake vigorously for 10 minutes by using a vortex at 150rpm, then let to settle for a short period of time. Serial dilution sequentially made, started from stock and 10 till 10⁶. From each diluted tube, 0.1 ml was transferred into the surface of the plant-based agar media at different diluted juice of both Mentha and A. vera (in triplicate) and spread with an alcohol sterilized L shape glass rod then incubated at 30°C for 24 - 48 hours. After the successful growth of microorganisms, pick up the individual colonies and purify them.

d) Comparison between the growth of microbial isolates on plant-based culture medium and synthetic media

Bacterial isolates that developed on plant-based culture were tested to grow in nutrient agar medium at 30°C for 24 – 48hr; on the other hand, actinomycetes isolates also developed on starch nitrate medium at 30°C for 5-7days. After the incubation period, the microbial growth and pigmentation were observed in all plates, and then compared to the plant-based culture medium.

e) Indole acetic acid production by microbial isolates on both plant-based culture and synthetic medium

Estimate the Indole acetic acid (IAA) production quantitatively according to Salkowski method Brick et al. (1991). For bacterial isolates, inoculate the conical flasks (250 ml capacity), that contain 100ml of both plant-based and nutrient broth medium supplemented with
(500µg/ml) tryptophan (sterilized by the bacterial filter) with 1 ml of 24 h old rhizobacterial cultures, incubate the flasks on a rotary shaker (150 rpm) 36±2°C for 72 h. Centrifuge the flasks at 4000 rpm for 15 min to separate supernatant. On the other hand, IAA from actinomycetes was quantified using the method of (Rafik et al., 2014). Streak the actinomycetes isolates on plant-based and starch nitrate agar medium and incubate at 28°C. After five days. Transfer the agar discs of actinomycetes mycelia to conical flasks (250 ml capacity) containing 100ml of both plant-based and starch nitrate liquid medium containing 500µg/ml tryptophan. After seven days of culture at 30°C and stirring at 150 rpm, centrifuge at 3000 rpm for 15min. Estimate the production of IAA in the supernatants by using a colorimetric assay. Mix one milliliter of supernatant with 2 ml of the Salkowski reagent, incubate at room temperature for 30min in the dark. The appearance of pink or red color indicates the production of IAA. Measure the absorption a spectrophotometrically at 530 nm against control of 1 ml culture medium and 2 ml of Salkowski reagent (Glickmann and Dessaux, 1995). The amount of IAA produced per milliliter culture was estimated using a standard curve.

f) Quantitative assay of phosphate solubilizing activity

Quantitative estimation of P-solubilization was carried out as per standard methodology Mehata and Nautiyal (2001), by inoculating 1 ml of bacterial suspension and discs of actinomycetes in 50 ml of National Botanical Research Institute Phosphate broth (NBRIP) and incubating the flasks for three days at 36±2°C and seven days at 30°C respectively. At the end of the incubation period, the cell suspension was centrifuged at 10,000 rpm for 10 min in aliquot 2-5 ml of Barton's reagent was added, and after 10 minutes the intensity of color was measured by a spectrophotometer at 420 nm with standard KH₂PO₄. The amount of IAA produced per milliliter culture was estimated using a standard curve.

g) Identification of selected bacterial and actinomycetes isolates

The efficient bacterial isolates (MB4), (A6), and actinomycetes isolate (MA6) in IAA production and phosphorus solubilization, respectively, were further identified by 16S rRNA at Sigma Scientific Services Company, 6 of October, El Giza, Egypt.

h) Molecular characterization of bacteria and actinomycetes

DNA extraction: Add 200 µl of the sample (liquid media that contain bacteria or actinomycetes) in microcentrifuge tube and add 95 µl water, 95 µl solid tissue buffer (blue) and 10 µl proteinase K. Mix thoroughly and then incubate the tube at 55°C for 2 hours. Mix thoroughly and centrifugation at 15,000 rpm for 1 minute. Transfer supernatant to a 300 µl tube. Add 600 µl DNA Binding Buffer, and mix thoroughly. Move the mixture to a Zymo-Spin™ IIC-XL Column in a Collection Tube. Centrifuge at 15,000 rpm for 60 sec. Discard the collection tube with the flow through. Add 400 µl of DNA Pre-Wash Buffer to the column in a new Collection Tube then centrifuge at (15,000 rpm) for 60 sec. Add 700 µl gDNA Wash Buffer and centrifuge at (12,000 xg) for 60 sec. Discard the Collection Tube. Add 200 µl of DNA Washing Buffer to the column and centrifuge at (15,000 rpm) for 60 sec., get rid of the flow through. Add 30 µl of elution buffer then incubate for 5 minutes, and centrifuge at (15,000 rpm) for 60 sec.

i) PCR amplification and phylogenetic analysis

Dissolve the extracted DNA in 20 µl TE buffer to be used as a template in the PCR reactions. PCR amplifications is to be performed in a total volume of 50 µl by mixing 20 ng of the genomic DNA with 2.5 mM concentrations of each dNTPs (deoxynucleotide triphosphate), 1 mM concentrations of each primer of pA (50 -AGAGTTTGATCTGCTGCA-30) and pH (50 - AAGGAGGTATCCAGCGCA-30) as described by Edwards et al. (1989). Also, and 3 U of Taq DNA polymerase in 10X Taq buffer (A (GeNetI). The PCR products separated using 0.8% agarose gel. 16S rRNA gene sequence of the isolate compared with 16S rRNA gene sequences available online by using BLASTN search program in the NCBI website, (http://www.ncbi.nlm.nih.gov). Clustal X used for multiple sequence alignment (Thompson et al., 1997). The method of Jukes and Cantor (1969) used to calculate evolutionary distances. Phylogenetic analysis was constructed by the neighbor-joining manner and tree topologies were evaluated by performing bootstrap analysis of 1,000 data sets using MEGA 3.1.

j) Effect of different incubations periods on the growth of the most efficient isolates on both natural and synthetic medium

This study was carried out to determine the optimum incubation period at which the selected isolates showed maximum growth on both natural and synthetic medium. Inoculate Pseudomonas monteilii and Kosakonia radicincitans on the natural medium and artificial (nutrient broth) medium with pH 7.2 ± 0.2. Incubate the flasks at 30°C for different incubation periods (1, 2, 3, 4, 5, and 6 days). Determine the bacterial growth at the end of each incubation period spectrophotometrically by measuring the optical density at 600nm (OD₆₀₀). Koch (1970). Inoculate the conical flasks, that contain 20ml of both Mentha-based broth medium and starch nitrate broth medium with Streptomyces rochei, then incubate it at 30°C for 1-10 days, take the culture daily, filitate, then dry it in oven at 70°C for three days to determine the dry weight. The
growth, as indicated by dried biomasses plotted against
time Sejiny (1991).

k) In-vitro screening of efficient microbial isolates for
antagonistic activities against root rot fungi

The possible interaction between the selected
bacterial strains and pathogenic fungi (Fusarium sp. and
Rhizoctonia sp.), which are responsible for root rot in a
variety of legume and non-legume crops was monitored
on potato dextrose agar (PDA) medium using a
modified agar plate inhibition zone technique (Silosuch
et al., 1994). Initially, grow the pathogenic fungi
(Fusarium solani, Fusarium oxysporum, and Rhizoc-
toniasolani) in 15 cm Petri dishes contains PDA medium
and incubate at 28°C for 48 hr. Then, cut 0.5 cm disks
from the edge of the actively growing colonies, and
transfer one of them on the center of a Petri dish
from the edge of the actively growing colonies, and
incubate at 28°C for (4-7 days). Zones of inhibition of
fungal growth were observed. On the other hand, Potato
dextrose agar plates were prepared and inoculated with
Streptomycyes rochei by streaking in a single line at the
center of the petri dish containing PDA medium. After
seven days of incubation at 28°C, seed the plates with a
disk of test fungi at an angle of 90° to the actinomycetes
isolate and incubate at 28°C for five days, microbial
interactions were recognized by the determination of the
diameter of inhibition zone (Madigan et al., 1997).

III. Results and Discussion

A total of fifteen bacterial isolates were isolated
from Aloe vera rhizosphere on Aloe vera-based culture
medium with different dilutions 1:10, 1:20, 1:40, 1:80
and 1:100 v/v (juice or sap: distilled water, v/v) (Plate
1a-d and Plate 2a), these results are harmony with
Youssef et al. (2015) who isolated distinctive colonies of
rhizobacteria associated with the roots of Aloe vera
developed on agar plates of plant-based culture media,
that prepared from diluted juices (1:20, v/v) of A. vera.
And nine bacterial and nine actinomycetes isolates were
rhizosphere of M. viridis were developed on agar plates of
Mentha-based culture media prepared from diluted
juices (1:40, 1:80 and 1:100 v/v) of M. viridis
(Plate 2b). The results indicated that both Aloe vera and
Mentha juices were nutritionally rich enough in Table
(1and 2, respectively) to support the better growth of
rhizobacterial isolates associated with the rhizosphere of
both tested plants. Good bacterial growth was obtained
with further dilutions up to 1:100 (juice: distilled water,
v/v) (Plate 1a-d and Plate 2a and b). Such a positive
dilution effect attributes to decreasing the osmotic effect
of concentrated nutrients as well as minimizing the
inhibitory effect of antimicrobial compounds present in
the juices of tested plants Pellizzoni et al. (2012). The tested Mentha (Table 2) contains abundant nutritional
content, and Aloe vera was reported to contain amino
acids, anthraquinones, enzymes, steroids, hormones,
salicylic acid, minerals, sugars, saponins, and vitamins
as main ingredients Upadhyay (2018) that essential for
the microbial growth. The results also showed that
actinomycetes isolate (Gram-positive) developed on
Mentha-based medium rather than Aloe vera-based
medium. This may occur due to the presence of steroid,
triterpenoid, flavonoid, phenol, tannin, alkaloid, saponin
and acid in the Aloe vera juice responsible for its
antibacterial activity. An earlier report suggested that a
large number of Aloe vera extracts to be active against
Gram-positive bacteria McCutcheon et al. (1992),
probably due to the absence of the outer of the bacterial
cell wall.

Table 1: The chemical compositions and nutrient contents of Aloe vera Tiwari and Upadhayay (2018)

| Chemical Group | Constituents |
|----------------|-------------|
| Amino Acids    | Provides 20 of the 22 required amino acids and 7 of the 8 essential ones. |
| Enzymes        | Anthranol, barbaloin, chrysophanic acid, ethereal oil, ester of cinnamic acid, isobarbaloin, resistannol |
| Anthraquinones | Provides aloe emodin, aloe tic acid, alovin, Anthracine. |
| Steroids       | Cholesterol, lupeol, camp sterol, sistosterol |
| Hormones       | Auxins and gibberellins |
| Salicylic Acid | Aspirin like compounds |
| Saponins       | Glycosides |
| Minerals       | Calcium, chromium, copper, iron, manganese, potassium, sodium and zinc |
| Sugars         | Monosaccharides: Glucose and Fructose Polysaccharides: Glucomannans/polymannose |
| Vitamins       | A, B, C, E, choline, B12, folic acid |
Table 2: The chemical compositions and nutritional contents of *Mentha*

| parameters                     | mg/ 11g fresh weight of mint | parameters                     | mg/ 11g fresh weight of mint |
|--------------------------------|------------------------------|--------------------------------|------------------------------|
| Protein and Amino Acids        | Carbohydrate                 |                                |                              |
| Protein                        | 350                          | Fiber                          | 790                          |
| Tryptophan                     | 5.3                          | Ash                            | 175                          |
| Threonine                      | 15.1                         | Fats & Fatty Acids             |                              |
| Isoleucine                     | 15.1                         | Fat                            | 190                          |
| Leucine                        | 26.8                         | Omega-3 fatty acids            | 38.2                         |
| Lysine                         | 15.4                         | Omega-6 fatty acids            | 5.8                          |
| Methionine                     | 5.1                          |                                |                              |
| Cysteine                       | 4.2                          | Vitamin C                      | 1.5                          |
| Phenylalanine                  | 18.6                         | Niacin                         | 0.1                          |
| Tyrosine                       | 11.0                         | Folate                         | 0.012                        |
| Valine                         | 17.5                         | Retinol                        | 0.022                        |
| Arginine                       | 17.2                         |                                |                              |
| Histidine                      | 7.1                          | Calcium                        | 21.4                         |
| Alanine                        | 18.7                         | Iron                           | 1.3                          |
| Aspartic acid                  | 43.4                         | Magnesium                      | 7.0                          |
| Glutamic acid                  | 40.0                         | Phosphorus                     | 6.4                          |
| Proline                        | 15.2                         | Potassium                      | 50.5                         |
| Serine                         | 14.4                         | Sodium                         | 3.2                          |
| Glycine                        | 17.4                         | Zinc                           | 0.1                          |
| Phytosterols                   | 1.1                          | Manganese                      | 0.1                          |

Plate 1: a-d) Colonies of rhizobacteria associated with the roots of *Aloe vera* developed on agar plates of plant-based culture media prepared from diluted juice (1:10, 1:20, 1:40, 1:80 v/v) of *A. vera*
Plate 2: a) Colonies of rhizobacteria associated with the roots of Aloe vera developed on agar plates of plant-based culture media prepared from diluted juice (1:100 v/v) of A. vera. b) Colonies of microorganisms associated with the roots of Mentha viridis developed on agar plates of plant-based culture media prepared from diluted juice (1:20, v/v) of M. viridis.

a) Comparison between the growth of microbial isolates on plant-based culture medium and synthetic media

The actinomycetes isolates showed higher rate of growth on Mentha-based agar plates than starch nitrate agar plates (Plate 3). This may be due to Mentha-based medium possesses a considerable amount of nutritional contents like proteins, carbohydrate and fates and also growth factors such as vitamins and amino acids (Table 2) that essential for their growth while synthetic media provides only limited growth factors. In general, the growth of actinomycetes isolates on the plant-based culture media was good enough and very much comparable to the standard culture medium (starch nitrate medium). It is clear from the results that the growth is greatly influenced by the nature and type of the nitrogen source supplied in the culture medium. In comparison with inorganic nitrogen sources, organic nitrogen sources (protein and amino acids) induced relatively higher biomass yield. This is in accordance by the findings of Yu et al. (2008) who found that the Streptomyces spp. records maximum growth rates when peptone use as nitrogen source while ammonium chloride showed moderate growth when it supplied as inorganic nitrogen source in medium, Amendment of amino acids, in combination with carbon source, promoted the growth of actinomycetes, which correlated with Kumar and Kannabiran (2010). Although Mentha contains antibacterial and phenolic compounds, the growth of actinomycetes was not affected by them; this may be because actinomycetes are resistant to these compounds or their concentrations are not enough to inhibit their growth. On the other hand, some bacterial isolates exhibited maximum growth and pigmentation on nutrient agar plates like A13 (Plate 4) than Mentha and Aloe vera-based culture medium because nutrient agar medium provides balanced salt solution with specific pH and osmotic pressure. While other bacterial isolates showed maximum growth on Aloe vera-based culture medium and high pigmentation on nutrient agar like (A6 isolate) (Plate 4). In addition, MB4 (Plate 5) isolate also yielded significant growth on Mentha-based culture medium but less biomass yield when compared with nutrient agar medium. The phenolic compounds in Mentha and Aloe vera juice may limit the growth of bacterial isolates. These results are in line with similar findings of previous reports of (Jadhav et al., 2018).
Plate 3: Comparison between the growth of actinomycetes isolates on Mentha viridis-based medium and starch nitrate medium

Plate 4: Comparison between the growth of rhizobacterial on Aloe vera-based medium and nutrient agar medium

Plate 5: Comparison between the growth of rhizobacterial isolates on Mentha viridis-based medium and nutrient agar medium
Not only the growth of actinomycetes affected by the type of medium but also by the concentration of the nutritional content. The growth and pigmentation of actinomycetes isolate (MA6) and (MA11) were high at dilution (1:20 v/v) and (1:10 v/v) respectively while both growth and pigmentation of the two isolates were decreased at dilution (1:80 v/v) (Plate 6). This due to the nutritional content was high at lowest dilutions and low with highest dilutions of plant extract. These results are in agreement with Saleh et al. (2017), who found that the diluted plant juice (1:10, v/v) supported better growth compared to further diluted plant juices.

Plate 6: The growth of actinomycetes isolates (MA6 & MA11) at different dilutions of Mentha extract

In-vitro screening of isolates for production of some plant growth-promoting (PGP) activities

b) Indole acetic acid (IAA) production by microbial isolates on both plant-based culture and synthetic medium

Auxin is the most investigated hormone among plant growth regulators. The most common, best characterized, and physiologically most active auxin in the plant is indole-3-acetic acid (IAA). IAA is known to stimulate both a rapid response (e.g., increased cell elongation) and a long term response (e.g., cell division and differentiation) in plants. The production of IAA was examined with the use of Salkowski reagent. The development of pink color was first visible within minutes and continued to increase in its intensity for 30 min in the dark, so the optical density was then measured. All bacterial and actinomycetes isolates produce IAA on both plant-based medium and synthetic medium. MB4 and A6 were efficient producers of IAA (36.51 and 16.25 ppm, respectively) on Mentha and Aloe vera-based culture medium respectively Table (3), while the quantity of IAA decreases (10.53 and 8.69 ppm) on nutrient broth medium, this due to the presence of tryptophan, vitamins, salt, carbon source and nitrogen in a plant-based culture medium that were contributing factors in the IAA biosynthesis which correlated with (Apine and Jadhav, 2011) and also the tryptophan supplemented in the medium. It was observed that the ideal concentration of tryptophan needed for maximum IAA production differed between the isolates (Serjeeva et al., 2007). (Narayana et al., 2009; Malhotra and Srivastava, 2009) mentioned that plant extracts influence bacterial IAA biosynthesis, and organic nitrogen sources observed to promote IAA production better than inorganic nitrogen sources. Furthermore, isolate no. MA13 produce the smallest amount of IAA (2.64ppm) on Mentha-based culture and the highest amount on nutrient broth medium (25.05ppm) Table (3), this due to the high concentration of tryptophan in Mentha-based culture (Mentha content Table (2) and 0.5 mg/ml supplemented to medium) reduce the production of IAA, while nutrient broth medium contains the optimum amount of tryptophan. This result is in consistence with (Shokri and Emiazi, 2010) where they revealed that some strains grown on high concentrations of tryptophan actually reduced IAA production.
Table 3: Production of IAA on Mentha and Aloe vera -based medium and synthetic medium by bacterial and actinomycetes isolates isolated on Mentha and Aloe vera extract medium

| Mentha extract medium | Synthetic medium | Aloe vera extract medium | Synthetic medium |
|-----------------------|------------------|--------------------------|------------------|
|                       | No of isolates   | IAA (µg/ml)              | No of isolates   | IAA (µg/ml) | No of isolates   | IAA (µg/ml) | No of isolates   | IAA (µg/ml) |
| MB1                   | 16.77            | MB1                       | 12.35            | A1          | 6.02            | A1          | 11.47            |             |
| MB2                   | 6.17             | MB2                       | 8.74             | A2          | 4.84            | A2          | 18.32            |             |
| MB3                   | 9.75             | MB3                       | 10.64            | A3          | 3.55            | A3          | 8.66             |             |
| MB4                   | 36.51            | MB4                       | 10.53            | A4          | 0.79            | A4          | 0.82             |             |
| MB5                   | 8.2              | MB5                       | 7.88             | A5          | 14.27           | A5          | 4.19             |             |
| MA6                   | 10.06            | MA6                       | 7.47             | A6          | 16.25           | A6          | 8.69             |             |
| MA7                   | 4.35             | MA7                       | 2.82             | A7          | 0.69            | A7          | 5.86             |             |
| MA8                   | 10.06            | MA8                       | 10.77            | A8          | 0.74            | A8          | 0.9              |             |
| MA9                   | 31.31            | MA9                       | 15.26            | A9          | 3.34            | A9          | 8.43             |             |
| MA10                  | 6.48             | MA10                      | 9.75             | A10         | 0.27            | A10         | 6.51             |             |
| MA11                  | 3.15             | MA11                      | 1.52             | A11         | 1.18            | A11         | 7.68             |             |
| MA12                  | 31.83            | MA12                      | 16.32            | A12         | 0.53            | A12         | 0.58             |             |
| MA13                  | 2.64             | MA13                      | 25.05            | A13         | 1.08            | A13         | 4.19             |             |
| MB14                  | 15.42            | MB14                      | 12.71            | A14         | 0.97            | A14         | 5.39             |             |
| MB15                  | 5.02             | MB15                      | 5.13             | A15         | 5.94            | A15         | 4.82             |             |
| MB16                  | 6.92             | MB16                      | 14.17            |             |                 |             |                  |             |
| MB17                  | 23.52            | MB17                      | 7.02             |             |                 |             |                  |             |
| MA18                  | 12.56            | MA18                      | 10.79            |             |                 |             |                  |             |

c) Quantitative assay of phosphate solubilizing activity

In NBRIP, broth phosphate solubilization activity of microbial isolates was varied from 44.69 to 867.85µg/ml using tricalcium phosphate as a source of insoluble P. Soil microorganisms can solubilize insoluble mineral phosphate by producing various organic acids (Illmer et al., 1995; Jones, 1998). In our study all bacterial and actinomycetes isolates were able to solubilizing phosphorus, which shows an agreement with the estimation of (Kucey, 1983) and (Chabot et al., 1993) and according to them 20 to 40% of the cultivable bacterial population of soil solubilize P. Our results showed that bacterial isolate No A6 was shown maximum phosphorus solubilization on NBRIP broth medium after grown on Aloe vera-based culture as compared to after grown on nutrient broth medium (Figure 1), furthermore actinomycetes No MA6 and bacterial No MB5 isolates were efficient in phosphorus solubilization on NBRIP broth medium after grown on Mentha-based culture 867.85 and 767.2 µg/ml respectively than after grown on starch nitrate and nutrient broth medium 648 and 613.46µg/ml respectively (Figure 1). The high phosphorus solubilization on Mentha-based culture and Aloe vera-based due to the lower pH of M. viridis juices was in the range of 5.8-6.5 and A. vera juices 4-6.2 that lowers the pH of the medium and increases phosphorus solubilization by microorganisms that isolated on Mentha and Aloe vera-based media, indicating the organic acid secretion that similar with the estimation of Humaira and Asghari (2011). The inverse relationship between pH and soluble phosphate was reported earlier by (Rashid et al., 2004).
d) Identification of Selected plant growth-promoting rhizobacteria isolates (PGPR) by 16s rRNA Gene Sequencing

The sequences of 16S rRNA gene of our isolates compared with the sequences in GenBank through BLAST search (http://www.ncbi.nlm.nih.gov/BLAST). The results of the BLAST were, MA6 showed 98.9% similarity with Streptomyces rochei strain DW3 while the MB4 showed 91.4% similarity with Pseudomonas monteilii strain CIP 104883 and A6 showed 99.36% similarity with Kosakonia radicincitans DSM 16656. These sequences from strains MA6, MB4, and A6 have been deposited in the Gen Bank and accession numbers were obtained (Table 4).

Table 4: Genetic similarity of the selected strains with different species of the bacteria and actinomycetes determined by 16S rRNA gene sequencing

| Isolate code | Source of isolation, Plant | Identified as | Similarity (%) | ACC number |
|--------------|---------------------------|--------------|----------------|------------|
| MA6          | Rhizosphere soil, Mentha viridis | Streptomyces rochei strain DW3 | 98.9 | MN135856.1 |
| MB4          | Rhizosphere soil, Mentha viridis | Pseudomonas monteilii strain CIP 104883 | 91.4 | NR114223.1 |
| A6           | Rhizosphere soil, Aloe vera | Kosakonia radicincitans DSM 16656 | 99.36 | NR117704.1 |

e) A phylogenetic relationship based on 16S rRNA nuclear gene

The construction of the phylogenetic tree (Figure 2, 3&4) described the phylogenetic relationship of the three species, namely Streptomyces rochei strain DW13, Pseudomonas monteilii strain CIP 104883 and Kosakonia radicincitans DSM 16656.

The above results are agree with those of Meliani et al. (2017) who investigate that the plant growth-promoting traits of a PGPR P. fluorescens and P. putida like production of IAA, siderophore and phosphate solubilization and found that P. fluorescens and P. putida can produce 3-indole-acetic acid (IAA) in-vitro, at concentrations of 89 µg.ml⁻¹ and 116 µg.ml⁻¹, respectively. The above results were harmony with Jog et al. (2012), who found that Streptomyces rochei, from the wheat rhizosphere, can produce IAA and phosphate solubilization and improve plant growth by increases seed germination, root elongation, and root dry weight and PO₄. The results of Zamoum et al. (2017) showed
that *Streptomyces rochei* was positive for IAA (100.3 µg ml⁻¹). This not harmony with our results. Furthermore, the *in-vitro* analyses, which demonstrated by Schilling et al. (1998) showed that *Kosakonia radicincitans* can solubilize rock phosphates. Moreover, this strain produces phytohormones as auxin and cytokine-like compounds Scholz and Ruppel (1992).

Figure 2: The phylogenetic tree based on the sequencing of the 16S rRNA gene using MEGA4 software (Tamura et al., 2007) illustrating the genetic relationship of *Streptomyces rochei* and closely related *Streptomyces* species.

Figure 3: The phylogenetic tree based on the sequencing of the 16S rRNA gene using MEGA4 software (Tamura et al., 2007) illustrating the genetic relationship of *Pseudomonas monteilii* strain CIP 104883 and closely related *Pseudomonas* species.
When the growth of *Pseudomonas monteilii*, *Kosakonia radicincitans*, and *Streptomyces rochei* on plant extract-based medium was compared with nutrient and starch nitrate respectively, it was found that maximum growth was in plant-based medium and less growth recorded on synthetic medium. It is clear from (Figure 5) that the *Pseudomonas monteilii*, and *Kosakonia radicincitans* grow exponentially during two and three days of incubation on both natural and synthetic media. The highest growth was observed on the third day of incubation. After the exponential phase at the fourth and fifth day, then slightly decreases (stationary phase). On the other hand, *Streptomyces rochei*, and grow exponentially during the first 4 to 7 days of incubation and at the eighth day, the growth slightly decreased.

The three efficient isolates tested for their ability to produce inhibitory activity against pathogenic fungi (*Fusarium solani*, *Fusarium oxysporum*, and *Rhizoctonia solani*). Rhizobacteria can produce plant growth-promoting substances that enhance the growth of plants, and also have an antagonistic activity against

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**Figure 4:** The phylogenetic tree based on the sequencing of the 16S rRNA gene using MEGA4 software (Tamura et al., 2007) illustrating the genetic relationship of *Kosakonia radicincitans* and closely related *Kosakonia* species
pathogenic fungi. The results showed that *Pseudomonas monteilii* and *Streptomyces rochei* (Plate 7) were more active against *Rhizoctonia solani* and *Fusarium solani* respectively, that shown inhibition zone 19 and 18mm respectively Table (5), which in line with similar findings of previous reports (Asgharet *al.*, 2019). Additionally, *Kosakonia radicincitans* was suppressing the growth of *Fusarium solani* due to *K. radicincitans* have genes that responsible for salicylic acid or the jasmonate/ethylene signaling pathways to protect plants against potential pathogen attack *Brock et al.* (2013).

Table 5: Antimicrobial activity of *Pseudomonas monteilii*, *Kosakonia radicincitans* and *Streptomyces rochei* isolates

| Pathogenic fungi Isolates | Diameter of zone of Inhibition (mm) |
|---------------------------|-------------------------------------|
|                          | *Fusarium solani* | *Fusarium oxysporum* | *Rhizoctonia solani* |
| *Pseudomonas monteilii*   | 17                   | 15                   | 19                   |
| *Kosakonia radicincitans* | 11                   | 3                    | 5                    |
| *Streptomyces rochei*     | 18                   | 13                   | 12                   |

Plate 7: Streptomyces isolates showed antimicrobial activity against the test organisms a) control plate with (*Fusarium* sp. and *Rhizoctonia solani*), b) assay plate with (*Fusarium* sp. and *Rhizoctonia solani*) and potential *Streptomyces rochei*

As shown in (Table 5), the cost of natural liquid media is drastically less than the synthetic liquid media. The natural media containing agar for solidification is also very cost-effective as compared to synthetic solid media. The rise in the price of natural agar media is due to the addition of agar, which raises the cost. The finding cheap source instead of agar as solidifying agents needs to found to low this cost.

Table 6: Economic comparison of the media

| Media type       | Cost in E£/100L |
|------------------|-----------------|
| Synthetic medium | 4600            |
| Natural medium   | 4100            |
| Liquid Media     | 600             |
| Natural medium   | 100             |

IV. Conclusions

The natural media of *Mentha* and *Aloe vera* extracts supported the growth of bacteria and actinomycetes such as *Pseudomonas monteilii*, *Kosakonia radicincitans*, and *Streptomyces rochei*, respectively. The plant-based culture media could use as inexpensive alternate medium for the routine practical experiments. The plant-based culture increases the cultivability of rhizobacteria than synthetic medium, and was highly cost-effective. Results suggest that PGP isolates developed on plant-based culture medium, which can produce multiple PGP activities like IAA production, solubilize the phosphate, and existed antagonistic activity against pathogenic fungi may improve the growth of plants. Moreover, in the present study, PGPRs were used with a lower dose of fertilizer; thus, it is an environmentally-friendly technology that can minimize soil pollution and maximize crop returns.

Acknowledgments

This work was sponsored by Al-Azhar University, Faculty of science, Botany and Microbiology Department, Cairo, Egypt.
References Références Referencias

1. Ahmad M, Nadeem SM, Naveed M, Zahir ZA (2016): Potassium-solubilizing bacteria and their application in agriculture. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, New Delhi, pp 293–313.

2. Apine OA, Jadhav JP (2011): Optimization of medium for indole-3-acetic acid production using Pantoea agglomerans strain PVM. Journal of Applied Microbiology, 110:1235–1244.

3. Basu S, Bose C, Ojha N, Das N, Das J, Pal M, Khurana S (2015): Evolution of bacterial and fungal growth media. Bioinformation, 11(4): 182-184.

4. Benaisa A (2019): Plant Growth Promoting Rhizobacteria A review. Algerian Journal of Environmental Science and Technology, 5(1): 872-880.

5. Bric JM, Bostock RM, Silverstone SE. (1991): Rapid in situ assay for in dole acetic acid production by bacteria immobilized on a nitrocellulose membrane. Applied and Environmental Microbiology, 57(2): 535-8.

6. Brock AK, Berger B, Mewis I, Ruppel S (2013): Impact of the PGPB Enterobacter radicictans DSM 16656 on growth, glucosinolate profile, and immune responses of Arabidopsis thaliana. Microbial Ecology, 65:661–670.

7. Chabot R, Antoun H, Cescas MP (1993): Stimulation de la croissance du maïs et de la laitue romaine par des microorganismes dissolvant le phosphate inorganique. Canadian Journal of Microbiology, 39: 941–947.

8. Edwards U, Rogall T, Bloëcker H, Emde M, Bottrger EC (1989): Isolation and direct complete nucleotide determination of enterogenes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Research, 17: 7843–7853.

9. Glickmann E, Dessaux Y (1995): A critical reagent for indolic compounds produced by phytopathogenic bacteria. Applied and Environmental Microbiology, 61: 793-796.

10. Humaira Y, Asghari B (2011): Isolation AND Characterization of Phosphate Solubilizing Bacteria From Rhizosphere Soil Of Weeds Of Khewra Salt Range And Attock. Pakistan Journal of Botany, 43(3): 1663-1668.

11. Illmer P, Barbato A, Schninner F (1995): Solubilization of hardly soluble APO4 with P-solubilizing microorganisms. Soil Biology & Biochemistry,27: 265–270.

12. Jadhav P, Sonne M, Kadam A, Patil S, Dahigaonkar K, Oberoi JK (2018): Formulation of cost effective alternative bacterial culture media using fruit and vegetables waste. International Journal of Current Research and Review, 10 (2): 6-15.

13. Asghar JT, Ahmed B, Ali MA, Ismail M, Usman MA and Muhammad R (2019): Characterization and Bio-Antagonistic Activity of Rhizobacteria against Fusarium Oxysporum F. Sp. Cepae. Pakistan Journal of Agricultural Research, 32 (2): 353-358.

14. Jog R, Nareshkumar G, Rajkumar S (2012): Plant growth promoting potential and soil enzyme production of the most abundant Streptomyces spp. from wheat rhizosphere. Journal of Applied Microbiology, 113: 1154-1164.

15. Jones DL (1998): Organic acids in the rhizosphere — a critical review. Plant Soil, 205: 25–44.

16. Jukes TH, Cantor CR (1969): Evolution of protein molecules. In Mammalian protein metabolism (ed. H.N. Munro). Academic Press, New York. pp 21-123.

17. Koch AL (1970): Turbiditiy measurements of bacterial cultures in some available commercial instruments. Analytical Biochemistry, 38: 252–259.

18. Kucey RMN (1983): Phosphate-solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. Canadian Journal of Soil Science,63: 671–678.

19. Kumar SAURAV, Kannabirann K(2010): Diversity and Optimization of process parameters for the growth of Streptomyces VITSVK9 spp. isolated from Bay of Bengal, India. Journal of Natural & Environmental Sciences, 1(2):56-65

20. Kumar A, Bahadur I, Maurya BR, Raghuwanshi R, Meena VS, Singh DK, Dixit J (2015): Does a plant growth-promoting rhizobacteria enhance agricultural sustainability. Journal of Pure and Applied Microbiology, 9:715–724.

21. Lanka, S (2018): A review on Aloe vera-the wonder medicinal plant. Journal of Drug Delivery & Therapeutics, 8(5-s):94-99

22. Madigan MT, Martinko JM, Parker J (1997): Antibiotics: isolation and characterization. In: Brock Biology of Microorganisms, 8th (ed), Prentice-Hall International Inc. New Jersey, 440-442.

23. Mainasara MM, Abu Bakar MF, Waziri AH, Musa AR (2018):Comparison of Phytochemical, Proximate and Mineral Composition of Fresh and Dried Peppermint (Mentha piperita) Leaves. Journal of Science and Technology, 10 (2): 85-91.

24. Malhotra M, Srivastava S (2009): Stress-responsive indole-3-acetic acid biosynthesis by Azospirillum brasilense SM and its ability to modulate plant growth. European Journal of Soil Biology, 45:73–80

25. McCutcheon AR, Ellis SM, Hancock REW, Towers GHN (1992): Antibiotic screening of medicinal plants of the British Columbian native people. Journal of Ethnopharmacology, 37: 213-223.

26. Mehta S and Nautiyal CS (2001): An efficient method for qualitative screening of phosphate...
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28. Meliani A, Bensoltane A, Benidire L, Oufdou K (2017): Plant Growth-Promotion and IAA Secretion with *Pseudomonas fluorescens* and *Pseudomonas putida*. *Journal of Botanical Sciences*, 6(2): 16-24.

29. Narayana KJ, Peddikotla P, Krishna PSJ, Yenamandra V, Muova V (2009): Indole-3-acetic acid production by *Streptomyces albidoflavus*. *Journal of Biological Research*, 3(1): 35-43.

30. Prakash S, Verma JP (2016): Global perspective of potash for fertilizer production. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, New Delhi, pp 327–331.

31. Pellizzoni M, Ruzickova G, Kalhotka L, Lucini L (2012): Antimicrobial activity of different *Aloe barbadensis* Mill. And *Aloe arborescens* Mill. leaf fractions. *Journal of Applied medicinal plant research*, 6: 1975–1981.

32. Rafik E, Rahal E, Ahmed L (2014): Isolation and screening of actinomycetes strains producing substances plant growth promoting. *Indo-American Journal of Agricultural and Veterinary Sciences*, 2(4): 1-10.

33. Rashid M, Khalili S, Ayub N, Alam S, Latif F (2004): Organic acid production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. *Pakistan Journal of Biological Sciences*, 7: 187-196.

34. Saharan BS, Nehra V (2011): Plant growth promoting rhizobacteria: a critical review. *Life Sciences and Medicine Research*, 21:1–30.

35. Saleh MY, Sarhan MS, Mourad EF, Hamza MA, Abbas MT, Othman AA, Youssef HH, Morsi AT, Youssef GH, El-Tahan M, Amer WA, Fayed M, Ruppel S, Hegazi NA (2017): A novel plant-based-sea water culture media for in vitro cultivation and in situ recovery of the halophyte microbiome. *Journal of Advanced Research*, 8: 577–590.

36. Schilling G, Gransee A, Deubel A, Lőzović G, Ruppel S (1998): Phosphor availability, root exudates, and microbial activity in the rhizosphere. *Journal of Plant Nutrition and Soil Science*, 161 (4): 465–478.

37. Scholz SC, Ruppel S (1992): Nitrogenase activities and phytohormone activities of *Pantoea agglomerans*in culture and their reflection in combination with wheat plants. *Zentralblatt fuer Mikrobiologie*, 147(5):319–28.

38. Sejiny MJ (1991): Growth phases of some antibiotics producing *Streptomyces* and their identification. *Journal of King Abdulaziz University - Science*, 3:21-29.

39. Sergeeva E, Hirkala DLM, Nelson LM (2007): Production of indole-3-acetic acid, aromatic amino acid aminotransferase activities and plant growth promotion by *Pantoea agglomerans* rhizosphere isolates. *Plant Soil*, 297:1–13.

40. Shokri D, Emtiaz G (2010): Indole-3-acetic acid (IAA) production in symbiotic and non-symbiotic nitrogen-fixing bacteria and its optimization by taguchi design. *Current Microbiology*, 61:217–225

41. Silosuch LA, Lethbridge BJ, Raffet SJ, He H, Clardy J, Handelsman J (1994): Biological activities of two fungi static antibiotic produced by *Bacillus cereus* UW85. *Applied and Environmental Microbiology*, 60: 2023-2030.

42. Tamura K, Dudley J, Nei M, Kumar S (2007): MEGA4, Molecular Evolutionary Genetics Analysis (MEGA) Software version 4.0. *Molecular Biology and Evolution*, 24 (8): 1596-1599.

43. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997): The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25:4876–4888.

44. Tiwari M, Upadhayay M (2018): The medicinal plant components and applications (*Aloe vera*). *Journal of Medicinal Plants Studies*, 6(3): 89-95.

45. Upadhyay RK (2018): Nutraceutical, therapeutic, and pharmaceutical potential of *Aloe vera*: A review. *International Journal of Green Pharmacy*, 12 (1): 1-20.

46. Uthayasooriyam M, Pathmanathan S, Ravimannan N, Sathyaruban S (2016): Formulation of alternative culture media for bacterial and fungal growth. *Der Pharmacia Lettre*, 8 (1):431-436.

47. Youssef HH, Hamza MA, Fayed M, Mourad EF, Saleh MY, Sarhan MS, Siker RM, Eltahlawy AA, Nmrm RA, El-Tahan M, Ruppel S, Hegazi NA (2015): Plant-based culture media: Efficiently support culturing rhizobacteria and correctly mirror their in-situ diversity. *Journal of Advanced Research*, http://dx.doi.org/10.1016/j.jare.2015.07.005.

48. Yu J, Liu Q, Liu Q, Liu X, Sun Q, Yan J (2008): Effect of liquid culture requirements on antifungal antibiotic production by *Streptomyces rimosus* MY02. *Bioreource Technology*99:2087-2091.

49. Zamouma MB, Goudjala YB, Sabaoua N, Mathieuc F, Zitouni A (2017): Development of formulations based on *Streptomyces rochei* strain PTL2 spores for biocontrol of *Rhzoctonia solani* damping-off of tomato seedlings. *Biocontrol Science and Technology*, 27(6):723-738.