Egypt. J. Chem. **Vol. 67**, No. 2. pp. 79 - 89 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Biotechnological Studies on Some Bacteria from Avocado Fruits

Nada Mansour*, Amany Kzamel, Ashraf Elsayed

Botany Department, Faculty of Science, Mansoura University

Abstract

Limited agricultural lands and water resources scarcity in Egypt lead to that the food supply would be insufficient to meet over-population needs so that we targeted to take part in solving the food gap problem through increasing wheat crop productivity by applying endophytic bacteria as biofertilizers. This study mainly aims to study the potentiality of endophytic bacteria isolated from avocado fruits (*Persea americana*) as biofertilizer in promoting of wheat plant (*Triticum aestivum*) growth via estimation of plant growth promoting criteria. From wheat seed experiment, out of 13 bacterial isolates from avocado fruits, five isolates increased germination percent as well as number and length of adventurous roots and shoot length. It was concluded that endophytic bacterial isolates from avocado fruits promote wheat seedling growth.

Keywords: Endophytic bacteria; Biofertilizer; Avocado fruit; Wheat plant.

1. Introduction

Due to the growing number of people on the planet, there is an increase in the need for agricultural goods [1]. Various agricultural alternatives, including the use of chemical or synthetic fertilisers, pesticides, and insecticides, have been used to produce crops with a high yield in the shortest amount of time possible and to protect them from insects and pest attack during and after harvest in order to address the challenges of food scarcity brought on by the increase in population [2]**.** However, the public's worry about the sustainability, security, and safety of the food supply has increased as a result of the usage of these fertilizers and pesticides [3, 4]. Studies have revealed that even when crops are removed from farms for human consumption, there is still a large quantity of pesticide residue in them [5].

Hence, the necessity for alternatives like biofertilizer to ensure the security and safety of food was increased [2]. Additionally, beyond the required amount, synthetic fertilisers that include different nutrients including nitrogen (N2), phosphorous (P), potassium (K), and sulphur may become toxic [6]. These fertilisers have negative effects, such as weakening plant roots, a high rate of disease incidence, and soil acidity [7]. Future generations will also be impacted by the effects of these substances, in addition to the present. Therefore, it is necessary to look for eco-friendly methods, such as biofertilizers, which are crucial to sustainable agriculture [6]. When applied to seeds, plants, or soil, biofertilizers are microscopic organisms that promote the host plant's nutrition supply, hence promoting plant development [6, 8, 9].

Various microbes are typically linked to plants. The organisms that infiltrate a plant's internal tissues are called endophytic organisms exhibiting no visible symptoms of infection or harm to their host [10]. Endophytic organisms find a large array of niches in plants. Every one of the over 300,000 plant species that exist on the planet hosts one or more endophytes [11].

De Bary was the first to use the term endophyte (Endon, within; phyton, plant), According to De Bary (1866), an endophyte is a bacterial or fungal microbe that spends all or part of its life cycle colonizing within the healthy tissues of the host plant, usually without showing any outward signs of illness [12].

*Corresponding author e-mail: nadaalaaali1@gmail.com, (Nada Mansour)
Received date 27 May 2023; revised date 09 July 2023; accepted date 31 July 2023
DOI: 10.21608/ejchem.2023.213603.8031

Based on their sorts of activity, endophytic bacteria in plants can be categorised into two groups: those that promote growth and those that manage disease. One of two mechanisms is thought to be involved in endophytic bacteria's encouragement of plant growth: Directly by creating phytohormones like auxin or cytokinin, or indirectly by creating the enzyme 1- aminocyclopropane- 1-carboxylate (ACC) deaminase, which lowers plant ethylene levels, by directly producing phytohormones like auxin or cytokinin, by competing with pathogens for nutrients by producing siderophores, or by developing the plant's systemic resistance. In addition to helping plants acquire nutrients by nitrogen fixation, phosphate solubilization, or iron chelation, endophytes also boost drought tolerance, provide heat protection, and help plants survive osmotic stress [12].

One or more of these mechanisms may be used by a specific bacterium to influence plant growth and development, and it may employ different ones at various points throughout the plant's life cycle. Endophytic bacteria must not only be compatible with host plants and capable of colonising their tissues without being identified as pathogens in order to promote plant development [13]. Endophytes are likely present in every plant species. As a result, there is a great chance that researchers may discover novel and helpful endophytic microorganisms among the variety of plants in various habitats.

Avocado (*Persea americana*) is a tree or shrub belongs to family Lauraceae [14], native to Mexico and Central America [15], has a green peel, and fleshy body that may be pear-shaped or spherical with a central seed [16]. Avocado fruit is a source of nutrients containing protein (1.9%), water (72.3%), fibres (6.8%), fat (15.4%) and carbohydrate (8.6%) and high energy value of 167 kcal [17]. Microorganisms were found to colonize Avocado fruits due to their high nutritional content that can support their growth [18]. In Domlur Layout, India Avocado fruit samples were collected from the regional fruit outlet and transported in sterile bags to Research Labs for further processing. Three endophytic bacteria were isolated from avocado fruit samples and tested for plant growth-promoting traits which observed that three isolates were positive for ammonia, Indole-3-acetic acid (IAA), and siderophore production [19].

Common wheat (*Triticum aestivum*), also known as bread wheat, is a plant belonging to the family of grasses (Poaceae), which is an important and cheap source of carbohydrates. Additionally, some wheat is used by industry to produce starch, paste, malt, dextrose, gluten, alcohol, and other products [20]. The main goal of this study is biopriming of common wheat seeds with the endophytic bacteria that will be isolated from avocado fruits to promote wheat productivity.

2. Experimental

2.1. Plant sampling:

Avocado fruit samples from local fruit stores would be collected and transported in bags to Research Labs of the Botany Department in the Faculty of Science, Mansoura University for further processing.

Wheat seeds, (Sakha 8) obtained from agricultural research center in Giza, Egypt.

2.2. Isolation and purification of endophytic bacteria:

The surface-sterilized Avocado fruit samples would be rinsed with autoclaved distilled water and disinfected with 70% Ethanol, peeled by using a sterile forceps and 1 g of the macerated samples will be immersed in 1 ml of Luria Bertania (LB) liquid media (Peptone 10 g, Yeast extract 5.0 g, NaCl 10 g, dissolved in one litre of distilled water, and autoclaved at 121°C for 20 m) under aseptic conditions and incubated at 37˚± 2 for 24hr. 100 mL of broth media plated on (LB) agar media solidified by adding 20 g of agar-agar [21] under aseptic conditions and incubated at 37˚± 2 for 24 h. Sub-culturing of endophytic bacterial growth several times on new LB plates until to obtain pure single colonies, then maintained in glycerol stocks in -20°C freezer.

2.3. Cultural, microscopic, and biochemical characterization of bacterial isolates:

The selected isolates were examined morphologically and by Gram staining [22]. The enzymatic production of the endophytic bacterial isolates was studied in relation to lipase, protease, amylase, and cellulase.

2.3.1. Lipase Activity:

The idea behind this technique is the precipitation of calcium salts. Fatty acids are released during tween hydrolysis and combine with the calcium in the medium to create insoluble crystals near the site of inoculation. To verify lipolytic activity, a precipitation test using Tween20 agar plates was performed (Peptone 10 g, NaCl 5 g, $CaCl_2$ 1 g, Tween20 10 g, solidified by adding 20 g of agar-agar and dissolved in one liter of distilled water, and autoclaved at 121°C for 20 m). The organisms were inoculated on Tween20 agar plates and incubated for 2-4 days at 28 °C. White

precipitation surrounding the colony's perimeter indicated the presence of lipase [23].

2.3.2. Protease Activity:

On skimmed milk agar plates (Beef 3 g, Peptone 10 g, NaCl 10 g, Skimmed milk 10 g, solidified by adding 20 g of agar-agar and dissolved in one liter of distilled water, and autoclaved at 121°C for 20 m), one purified colony was inoculated. At 28 °C, plates were incubated for 24 hours. The presence of a definite clear zone around the colonies provided evidence that the tested bacteria could make protease [24].

2.3.3. Amylase activity (Starch hydrolysis):

To investigate the amylase's capacity for production, a starch hydrolysis test was performed. The endophytic isolates were streaked on starch agar medium (Peptone 5 g, Beef extract 3 g, Soluble starch 10 g, solidified by adding 20 g of agar-agar and dissolved in one liter of distilled water, and autoclaved at 121°C for 20 m) and kept at 28 °C for two to three days of incubation. Iodine solution flooding was used to test for starch hydrolysis, and the existence of clear zones surrounding the colonies on the plates was taken as a sign of success [25].

2.3.4. Cellulolase activity (Cellulose degradation):

Endophytic bacterial isolates were streaked over Carboxy methyl cellulose (CMC) agar medium (CMC 2 g, $NaNO_3$ 1 g, K_2HPO_4 1 g, KCl 1 g, $MgSO_4.7H_2O$ 0.5 g, $FeSO_4$ 0.01 g, Yeast extract 5 g, solidified by adding 20 g of agar-agar and dissolved in one litre of distilled water, and autoclaved at 121°C for 20 m) [26] and incubated at 28 °C for 2–5 days in order to determine the potential of the cellulase enzyme for the hydrolysis of cellulose. Plates were flooded with 0.2% aqueous Congo red and destained with 1M NaCl for 15 minutes. Then, the plates were examined to see clean zones surrounding the colonies. A distinct zone encircling the colony showed that there was active cellulase [27].

2.4. Wheat seeds biopriming and germination tests:

Seeds were treated separately with each isolate, 30 wheat seeds were surface-sterilized in 97% ethyl alcohol for 60s, washed 5 times with dist. water, and then immersed into 24 h bacterial isolates broth with the same adjusted inoculum at OD_{600} as well as in dist. water and free LB medium as two controls for a day long, then the treated seeds were put on clean cotton for a week and watered continuously with dist. Water under a long-day photoperiod (16 /8 h light to dark), at room temperature [28] . After biopriming experiment, six endophytic bacteria were selected for further studies.

2.5. Salinity tolerance test of six endophytic bacteria isolates:

In salinity tolerance test the endophytic bacterial isolates (Avo-A3, Avo-A4, Avo-E1, Avo-L1, Avo-L2, and Avo-K4) were cultured on LB agar medium with different NaCl concentrations (1, 5, 10, 15, and 20 %), and incubated at 37˚± 2 for 24hr to detect sublethal doses for each organism.

3. Results

3.1. Isolation and purification of endophytic bacteria from Persea americana fruits:

Thirteen bacterial isolates were obtained from avocado fruit samples were collected from different local fruit stores. The endophytic bacterial isolates grown on LB agar medium under aseptic conditions were selected, purified according to differences in their morphology and have been coded as Avo-A1, Avo-A2, Avo-A3, Avo-A4, Avo-E1, Avo-L1, Avo-L2, Avo-L3, Avo-K1, Avo-K2, Avo-K3, Avo-K4, and Avo-K5 (Figure 1).

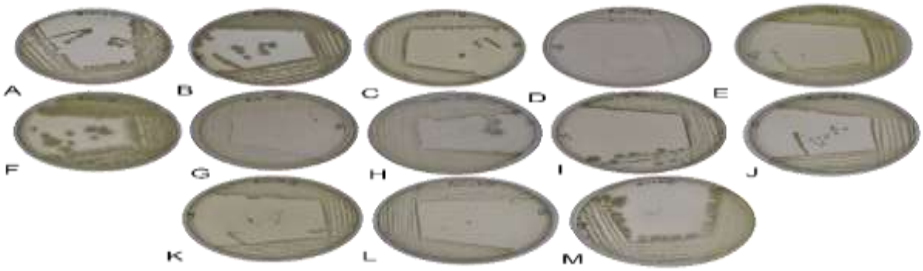

Figure 1: Purified culture of endophytic bacteria isolated from *Persea americana* fruits on LB media coded as follow: (A) Avo-A1, (B) Avo-A2, (C) Avo-A3, (D) Avo-A4, (E) Avo-E1, (F) Avo-L1, (G) Avo-L2, (H) Avo-L3, (I) Avo-K1, (J) Avo-K2, (K) Avo-K3, (L) Avo-

3.2. *Cultural, morphological, and microscopic characterization of the endophytic bacterial isolates from Persea americana fruits*

The colonies morphology of thirteen bacterial isolates on LB agar medium varied in colure from creamy-white and white, to yellow and orange, different in form and size from circular or irregular large colonies to small punciform colonies, with mixed entire, lobate, filamentous, and rhizoid margins and flat and convex elevation (Figure 2). All cultures morphology details are recorded in Table 1. The microscopic examination showed that four isolates were rods shaped, and stained Gram- negative bacteria, four isolates were rods shaped and stained Gram- positive bacteria, six isolates were rounds shaped, and stained Gram- positive bacteria, and only one isolate was round shaped, and stained Gram-negative bacteria (Figure 2 & Table 1).

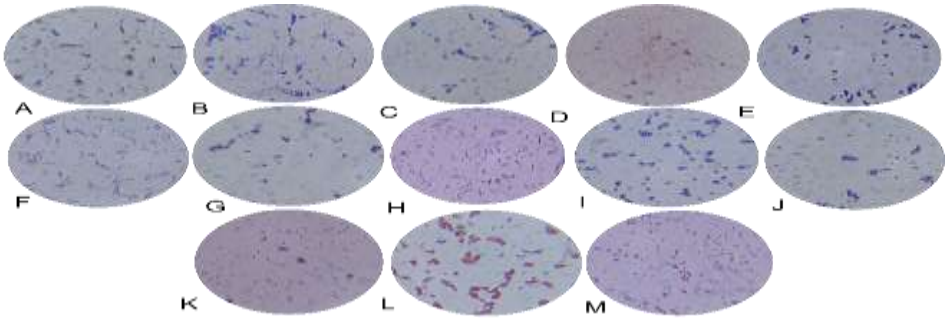

Figure 2: Microscopic view of endophytic bacterial isolates after Gram staining coded as follow: (A) Avo-A1, (B) Avo-A2, (C) Avo-A3, (D) Avo-A4, (E) Avo-E1, (F) Avo-L1, (G) Avo-L2, (H) Avo-L3, (I) Avo-K1, (J) Avo-K2, (K) Avo-K3, (L) Avo-K4, and (M) Avo-K5 (1000X).

3.3. Screening for hydrolytic enzymes:

The enzymatic productivities of the endophytic bacterial isolates were studied in relation to lipase, amylase, cellulase and protease. The results are presented in Table 2.

3.3.1. Screening for lipase production by endophytic bacterial isolates:

In lipase test, the positive result represents a white precipitate formed around the boundary of the colony indicating lipase activity as shown in Figure (3). In this study, the endophytic bacterial isolates Avo-A2, Avo-A3, Avo-K4, Avo-K5, Avo-L1, Avo-L3 were able to produce lipase.

3.3.2. Screening for amylase production by endophytic bacterial isolates production (Starch hydrolysis):

The amylase enzyme is indicated by a clear zone around colonies on starch-agar medium, all bacterial isolates were able to produce amylase enzyme except Avo-A3, Avo-L2, and Avo-K2 as shown in Figure (4).

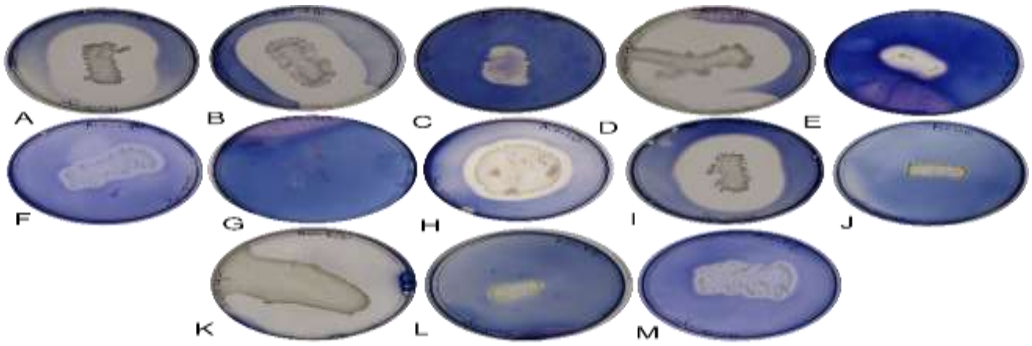

Figure 4: In starch hydrolysis test, the positive result is indicated by a clear zone around colonies on starch-agar medium, all bacterial isolates were able to produce amylase enzyme except (C) Avo-A3, (G) Avo-L2, and (J) Avo-K2.

3.3.3. Screening for cellulases production by endophytic bacterial isolates (cellulose degradation):

All the endophytic bacterial isolates were not able to produce cellulases except Avo-A2 and Avo-K2. Positive results are determined by the formation of clear zone around colonies (Figure 5). The results clearly showed that Avo-A2 was the most productive for cellulases followed by Avo-K2.

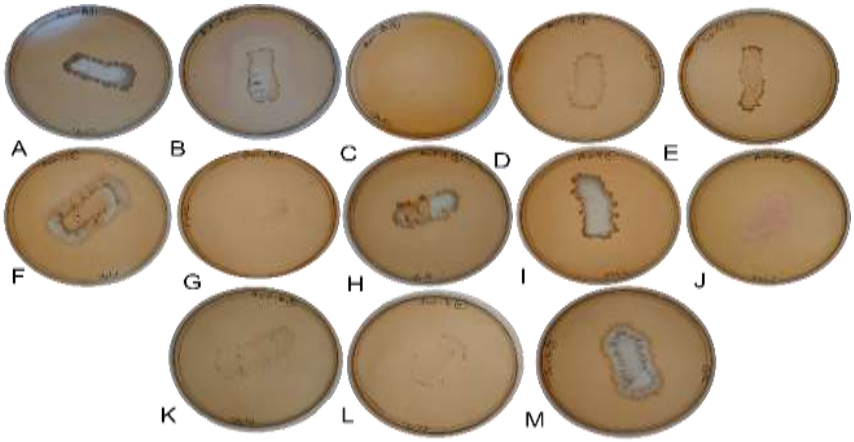

Figure 5: All the endophytic bacterial isolates were not able to produce cellulases except (B) Avo-A2 and (J) Avo-K2. Positive results are determined by the formation of clear zone around colonies.

3.3.4. Screening for protease production by endophytic bacterial isolates:

In this test clear zones were observed around colonies of six isolates (Avo-A1, Avo-L1, Avo-K1, Avo-K2, Avo-K3, and Avo-K5) showing to positive result, while no clear zone on the rest isolate plates as shown in Figure (6). These results proved that Avo-K3 was the most productive one.

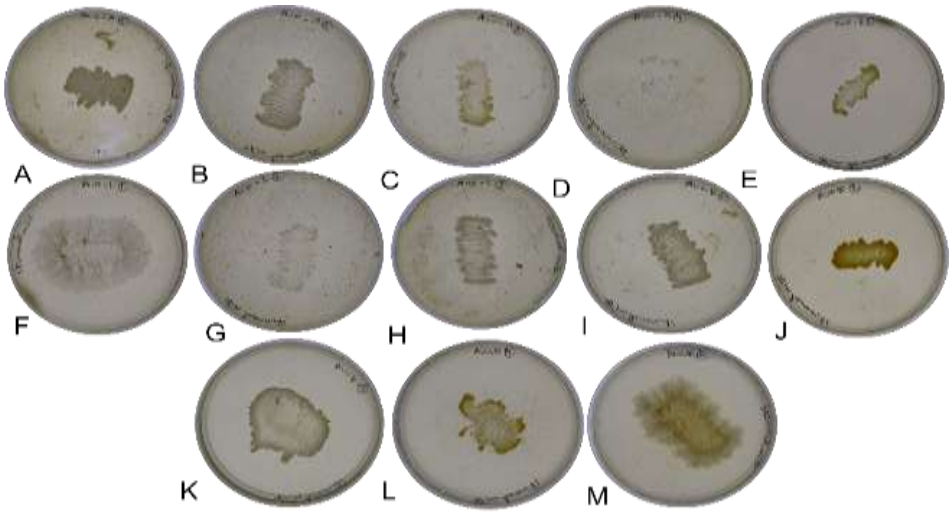

Figure 6: In protease test, the positive result is indicated by a clear zone around colonies on medium observed in (A) Avo-A1, (F) Avo-L1, (I) Avo-K1, (J) Avo-K2, (K) Avo-K3, and (M) Avo-K5.

3.4. Wheat seeds biopriming and germination:

The effect of endophytic bacterial isolates was tested for enhancing the germination of wheat seeds, and the results as shown in Figure (7) and Table 3.

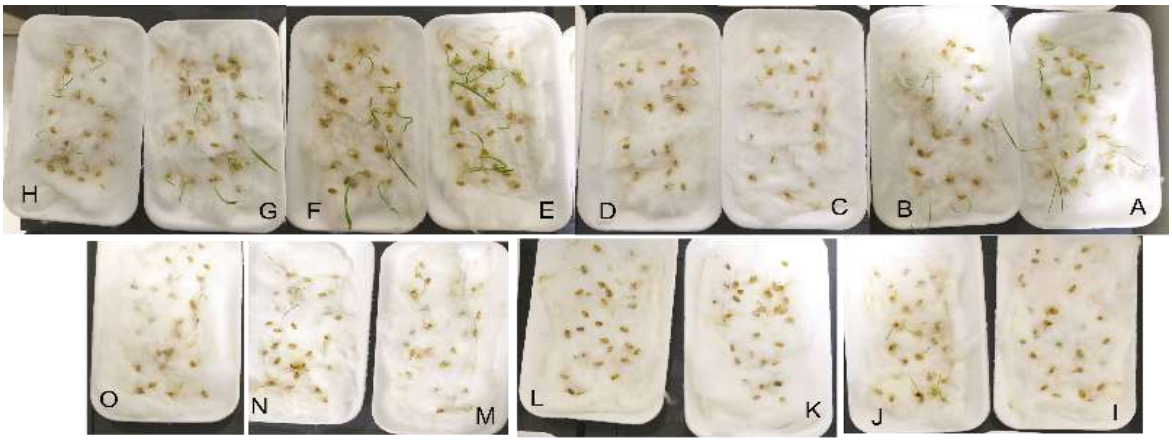

Figure 7: Primary wheat seeds biopriming by endophytic bacteria isolated from *Persea americana* fruits and germinated on cotton after a week coded as follow: (A) dist. Water control, (B) LB medium control, (C) Avo-A1, (D) Avo-A2, (E) Avo-A3, (F) Avo-A4, (G) Avo-L, (H) Avo-L2, (I) Avo-L3, (J) Avo-E1, (K) Avo-K1, (L) Avo-K2, (M) Avo-K3, (N) Avo-K4, and (O) Avo-K5.

3.5. Salinity tolerance of six endophytic bacteria isolates:

In salinity tolerance test according to a control plate (LB agar media with 1% NaCl concentration), all endophytic bacterial isolates managed to grow in 5% NaCl concentration, in 10% NaCl concentration only Avo-A3, Avo-K4, Avo-L1 were able to grow, in 15% and 20% NaCl concentration no bacterial isolates managed to grow as shown in Figure (8).

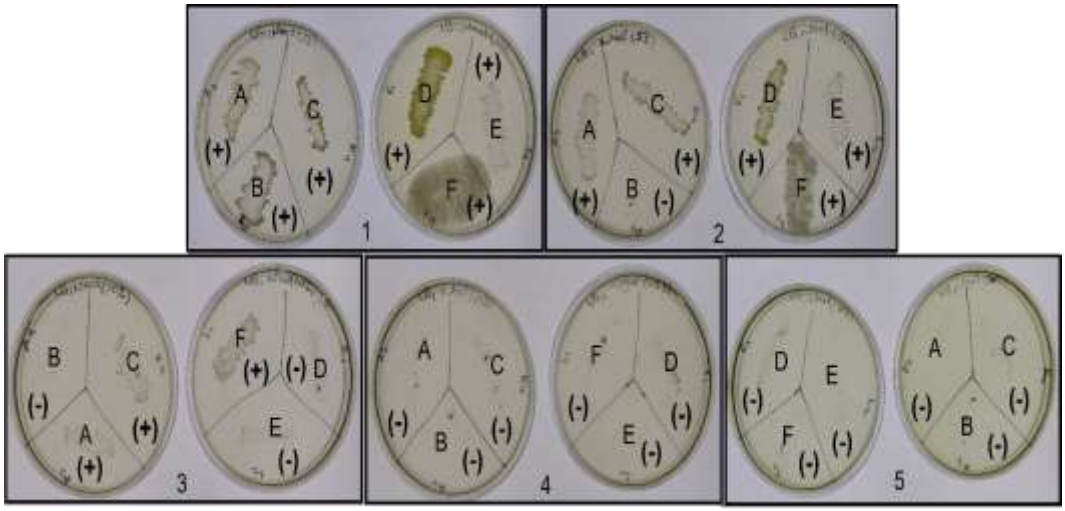

Figure 8: (1) plates represented 1% NaCl concentration (control), (2) plates represented 5% NaCl concentration, (3) plates represented 10% NaCl concentration, (4) plates represented 15% NaCl concentration, (5) plates represented 20% NaCl concentration, and six bacterial isolates were coded as follow: (A) Avo-A3, (B) Avo-A4, (C) Avo-K4, (D) Acvo-E1, (E) Acvo-L2, and (F) Avo-L1, The results were reported as plus (+) for growth or minus (-) no bacterial ability to grow.

Table1.Whole characteristics of the endophytic bacterial isolates from *Persea americana* fruits:

Isolates	Colour	Form	Margin	Elevation	Size	Opacity	Gram stain	Cell shape
Avo-A1	Creamy- White	Irregular	Lobate	Convex	Large	Opaque	+VE	Bacillus
Avo-A2	Creamy- White	Irregular	Filamentous	Flat	Large	Opaque	+VE	Coccus
Avo-A3	White	Punciform	Entire	Flat	Small	Translucent	+VE	Coccus
Avo-A4	Creamy- White	Punciform	Entire	Flat	Moderate	Opaque	+VE	Bacillus
Avo-E1	Yellow	Punciform	Lobate	Convex	Moderate	Opaque	+VE	Coccus
Avo-L1	Creamy- White	Irregular	Rhizoid	Flat	Large	Opaque	+VE	Bacillus
Avo-L2	White	Punciform	Entire	Flat	Small	Opaque	+VE	Coccus
Avo-L3	White	Irregular	Rhizoid	Flat	Large	Opaque	-VE	Bacillus
Avo-K1	White	Circular	Entire	Flat	Large	Opaque	+VE	Coccus
Avo-K2	Creamy- White	Punciform	Lobate	Convex	Moderate	Opaque	+VE	Coccus
Avo-K3	Creamy- White	Punciform	Entire	Convex	Small	Opaque	-VE	Bacillus
Avo-K4	Orange	Punciform	Entire	Convex	Moderate	Opaque	-VE	Coccus
Avo-K5	Creamy- White	Irregular	Rhizoid	Flat	Large	Opaque	+VE	Bacillus

Table 2. Summary of hydrolytic enzyme production of the endophytic bacterial isolates from *Persea americana* fruits.

Isolates	Lipase	Amylase	Cellulase	Protease
Avo-A1	-Ve	++Ve	-Ve	+Ve
Avo-A2	++Ve	++Ve	++Ve	-Ve
Avo-A3	-Ve	-Ve	-Ve	-Ve
Avo-A4	-Ve	+++Ve	-Ve	-Ve
Avo-E1	-Ve	-Ve	-Ve	-Ve
Avo-K1	-Ve	++Ve	-Ve	+Ve
Avo-K2	-Ve	-Ve	+Ve	+Ve
Avo-K3	-Ve	+++Ve	-Ve	++Ve
Avo-K4	+Ve	+Ve	-Ve	-Ve
Avo-K5	++Ve	+Ve	-Ve	+Ve
Avo-L1	++Ve	+Ve	-Ve	+Ve
Avo-L2	-Ve	-Ve	-Ve	-Ve
Avo-L3	++Ve	+Ve	-Ve	-Ve

-Ve indicated negative results, +Ve indicated weak positive results, ++Ve indicated moderate positive results, +++Ve indicated high positive results.

Table3. The result of different treatments with endophytic bacterial isolates after a week on number of germinated seeds, germination ratio (%), average shoot length (cm), and average fibrous root number and length(cm), Avo-A3 and Avo-A4 samples were marked with (**) give the best result after seeds treatment following by Avo-L1, Avo-L2 and Avo-K4 which were marked with (*).

Sample	Germination (%)	Shoot length (cm)	Roots number	Root length (cm)
dist. Water	70	3.5	4	4
LB medium	60	3	3	4
A1	40	2	3	2
A2	50	2	3	2
A3 **	70	5	5	5
A4 **	60	5	5	5
L1 *	70	3	4	4
L2*	60	2.5	4	3
L3	50	2	3	1.5
E1	60	3	3	2
K1	50	1.5	3	2
K2	60	1.5	3	2
K3	40	1	3	2
K4 *	60	2.5	4	3
K5	60	2	3	1.5

Avo-A3, and Avo-A4 samples were marked with (**) gave the best result after seeds treatment following by Avo-L1, Avo-L2 and Avo-K4 which were marked with (*).

4. Discussion

Due to simpler methods for isolation and identification as well as already-existing molecular biology tools, studies on endophytic microorganisms have drawn increasing interest from researchers. A variety of bioactive substances that have advantageous to pharmaceuticals, the environment, agriculture, and industry can be found in endophytes. As a result of their enormous significance to plants, people, and the environment, scientists have started using them for newer compounds and functions in the environment and human beings. In order to help the scientific community, it is critical to concentrate on achievements in the field of endophytic science [29].

Recently, endophytes have been viewed as a great source of secondary metabolites that are bioactive and important enzymes. Enzymes from microbial origin have great interest in the biotechnology industry, such as in the textile and food industries as they can create more stable enzymes than enzymes derived from plants or animals [30, 31].

The use of bacterial endophytes in agriculture has a significant potential to lessen the negative environmental effects of chemical fertilizers and to promote plant growth. Microorganisms, one of the most significant organisms that can create advantageous plant interactions to survive in their natural environments can develop relationships with plants. By triggering plant defense systems, producing chemicals that are hostile to diseases, or by competing for colonization sites and nutrients, endophytes may defend plants from infections and by producing plant growth regulators such auxins, cytokinins, and gibberellins, bacteria can promote plant development. [32].

Thirteen bacterial isolates found in avocado fruits in this study varied in shape between rod and round shaped and Gram staining between Gram-negative and Gram-positive bacteria. Most of the isolates (9 from 13) were Gram-positive bacteria, but previous literature noted that Gram-negative bacteria predominate in the tissues of different plants [33]. The isolates included in this study were chosen for their dominance as well as for any distinctive characteristics or distinctions in colony shape from other isolates.
Around half of the bacterial isolates were bacillus shaped after microscopic examination of Gram staining. These results matched to another study in which *Bacillus spp*. were the most prevalent bacterial

species isolated from healthy avocado roots, which were collected from diverse sites in South Africa. The least common bacteria found in avocado roots include those from the species *Lysinibacillus sp.*, *Paenibacillus polymyxa*, and *Enterobacter sp*. The primary biological constraint on avocado output globally is the oomycete *Phytophthora cinnamomi*, which causes the plant disease known as Phytophthora root rot [34].

The hydrolytic enzyme activity of the four enzymes Lipase, Protease, Amylase, and Cellulase was investigated in the endophytic bacterial isolates, five isolates (Avo-A2, Avo-K4, Avo-K5, Avo-L1, Avo-L3) give positive results for lipase production, only Avo-K3 showed good results for the protease assay, nine isolates (Avo-A1, Avo-A2, Avo-A4, Avo-K1, Avo-K3, Avo-K4, Avo-K5, Avo-L1, Avo-L3) give positive results for starch hydrolysis, and six isolates (Avo-A1, Avo-K1, Avo-K2, Avo-K3, Avo-K5, Avo-L1) were able to degrade cellulose.

McDonald (1999) defined seed priming as the beginning of the germination process, after soaking the seeds in any solution containing our needed priming ingredients [35]. Biopriming is a concept used to describe hydration utilizing any biological ingredient among other priming strategies [36]. Seed biopriming provides the perfect environment for bacterial colonization and inoculation in the seed [37].

In wheat seeds biopriming experiment by 13 bacterial isolates in this study, comparing with dist. water and free LB media controls, germinated wheat seeds which were treated by Avo-A3, and Avo-A4 isolates gave longer average shoot length seedlings (5 cm), more average roots number (5), and longer average root length (5 cm), following by Avo-L1, Avo-L2 and Avo-K4 treatments which shown results like or less than controls but more than other treatments.

In another study three endophytic bacterial isolates, Enterobacter E1S2, Klebsiella MK2R2, and Bacillus B2L2, were used either singly or in consortia for grain bio-priming. The results revealed that the best growth vigour and a substantial increase above the control were seen in the maize grains treated with Bacillus B2L2, Klebsiella MK2R2 + Bacillus B2L2 + Enterobacter E1S2, Bacillus B2L2 + Enterobacter E1S2, and Bacillus B2L2 + Enterobacter E1S2 [38].

A different study tested the ability of isolates from various plant tissues to promote plant development revealed that rhizobia-coinhabiting root nodule-dwelling strains were more effective than their endophytic counterparts. This was demonstrated not just by in vitro tests but also by seed biopriming tests [39].

Bacterial endophytes have been effectively employed to counteract salt's harmful effects and improve plant development in challenging environments. Osmotic adjustment, detoxification, phytohormone modulation, and nutrient acquisition in plants are all positive effects triggered by endophytes [40].

We would be better able to comprehend the plant-microbe interaction in detail with more molecular characterization, plant growth promoting traits estimation, and their impacts on plant development in the field.

5. Conclusions:

Endophytic organisms are found in a large array of niches in plants. As a result, there is a great chance that researchers may discover novel and helpful endophytic microorganisms among the variety of Plants in various habitats. This study mainly aims to study the potentiality of endophytic bacteria isolated from avocado fruits (*Persea americana*) as a biofertilizer in promoting wheat plant (*Triticum aestivum*) growth. From wheat seed experiment, out of 13 bacterial isolates from avocado fruits, five isolates increased germination percent as well as number and length of adventurous roots and shoot length. It was concluded that endophytic bacterial isolates from avocado fruits promote wheat seedling growth. We would be better able to comprehend the plant-microbe interaction in detail with more molecular characterization, and plant growth promoting traits estimation.

6. Conflicts of interest

The authors declare no conflict of interest.

7. Formatting of funding sources

This research received no external funding.

8. Acknowledgments

My completion of this project could not have been accomplished without the support of my lab mates and botany department members, many thanks for them.

9. References:

1. Batista, B.D., et al., *Screening of tropically derived, multi-trait plant growth-promoting rhizobacteria and evaluation of corn and soybean colonization ability.* 2018. **206**: p. 33-42.
2. Egamberdieva, D., S. Shrivastava, and A. Varma, *Plant-growth-promoting rhizobacteria (PGPR) and medicinal plants*. 2015: Springer.
3. Bruinsma, J., *By how much do land, water and crop yields need to increase by 2050? FAO Expert meeting on how to feed the world in 2050, 24–26 June 2009. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy*. 2009.
4. Liu, Y., et al., *Current agricultural practices threaten future global food production.* 2015. **28**(2): p. 203-216.

5. Lyu, X., et al., *Sustainable intensification to coordinate agricultural efficiency and environmental protection: a systematic review based on metrological visualization.* 2021. **16**(3): p. 313-338.
6. Kumar, M.S., et al., *Role of bio-fertilizers towards sustainable agricultural development: A review.* 2018. **7**(6): p. 1915-1921.
7. Wang, C., et al., *Present situation and future perspectives of biofertilizer for environmentally-friendly agriculture.* 2010(634).
8. Bardi, L. and E.J.A.s.N.r. Malusà, *Drought and nutritional stresses in plant: alleviating role of rhizospheric microorganisms.* 2012: p. 1-57.
9. Malusa, E., N.J.A.m. Vassilev, and biotechnology, *A contribution to set a legal framework for biofertilisers.* 2014. **98**(15): p. 6599-6607.
10. Schulz, B. and C. Boyle, *What are endophytes?*, in *Microbial root endophytes*. 2006, Springer. p. 1-13.
11. Strobel, G., et al., *Taxomyces andreanae, a proposed new taxon for a bulbilliferous hyphomycete associated with Pacific yew (Taxus brevifolia).* 1993. **47**: p. 71-80.
12. Wilson, D.J.O., *Endophyte: the evolution of a term, and clarification of its use and definition.* 1995: p. 274-276.
13. Rosenblueth, M. and E.J.M.p.-m.i. Martínez-Romero, *Bacterial endophytes and their interactions with hosts.* 2006. **19**(8): p. 827-837.
14. Morton, J.F., *Fruits of warm climates*. 1987: JF Morton.
15. Chen, H., et al., *Tracing the geographic origins of major avocado cultivars.* 2009. **100**(1): p. 56-65.
16. Storey, W.J.C.A.S.Y., *What kind of fruit is the avocado*. 1974. **7071**.
17. Dreher, M.L., A.J.J.C.r.i.f.s. Davenport, and nutrition, *Hass avocado composition and potential health effects.* 2013. **53**(7): p. 738-750.
18. Wogu, M.J.A.S.A.I.J.o.S. and Technology, *Microorganisms associated with the spoilage of avocado pear, Persea americana fruits.* 2014. **3**(2): p. 244-258.
19. Prasad, M. and S.J.I.J.C.M.A.S. Dagar, *Identification and characterization of Endophytic bacteria from fruits like Avacado and Black grapes.* 2014. **3**(8): p. 937-947.
20. Brenchley, R., et al., *Analysis of the bread wheat genome using whole-genome shotgun sequencing.* 2012. **491**(7426): p. 705-710.
21. Bertani, G.J.J.o.b., *Studies on lysogenesis I: the mode of phage liberation by lysogenic Escherichia coli.* 1951. **62**(3): p. 293-300.
22. Arthi, K., B. Appalaraju, and S.J.I.J.o.M.M. Parvathi, *Vancomycin sensitivity and KOH string test as an alternative to gram staining of bacteria.* 2003. **21**(2): p. 121-123.
23. Rai, B., et al., *Screening, optimization and process scale up for pilot scale production of lipase by Aspergillus niger.* 2014. **2**: p. 54-59.
24. Chang, W.-T., et al., *Conversion of crude chitosan to an anti-fungal protease by Bacillus cereus.* 2009. **25**(3): p. 375-382.
25. Bhagya, M., et al., *Isolation and Characterization of Endophytic bacteria from nodule, root and seeds of Greengram (Vigna radiata L.).* 2019. **7**(4): p. 319-328.
26. Samanta, R., D. Pal, and S.J.B.u.P.d.P. Sen, *Production of hydrolases by N2-fixing microorganisms.* 1989. **185**(1-2): p. 75-81.
27. Khianngam, S., et al., *Screening and identification of cellulase producing bacteria isolated from oil palm meal.* 2014. **4**(4): p. 090-096.
28. Lastochkina, O., et al., *Effects of Bacillus subtilis on some physiological and biochemical parameters of Triticum aestivum L.(wheat) under salinity.* 2017. **121**: p. 80-88.
29. Nair, D.N. and S.J.T.S.W.J. Padmavathy, *Impact of endophytic microorganisms on plants, environment and humans.* 2014. **2014**.
30. Falch, E.A.J.B.a., *Industrial enzymes—developments in production and application.* 1991. **9**(4): p. 643-658.
31. Jalgaonwala, R. and R.J.J.o.A.T. Mahajan, *Evaluation of hydrolytic enzyme activities of endophytes from some indigenous medicinal plants.* 2011. **7**(6): p. 1733-1741.
32. Santoyo, G., et al., *Plant growth-promoting bacterial endophytes.* 2016. **183**: p. 92-99.
33. Sudhir, A., et al., *Isolation, biochemical and PGP characterization of endophytic Pseudomonas aeruginosa isolated from chilli red fruit antagonistic against chilli anthracnose disease.* 2014. **3**(2): p. 318-329.
34. Pérez, C., G. Hernández, and C.J.R.C.d.C.A. Fuentes, *Use of endophytic bacteria as biological control on Phytophthora cinnamomi Rands causing root rot in avocado (Persea americana Mill.).* 2014. **6**(1): p. 213-222.
35. McDonald, M.J.S.S. and technology, *Seed deterioration: physiology, repair and assessment.* 1999. **27**(1): p. 177-237.
36. Ashraf, M. and M.J.A.i.a. Foolad, *Pre-sowing seed treatment—A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions.* 2005. **88**: p. 223-271.
37. Burges, H.D., *Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatments*. 2012: Springer Science & Business Media.
38. Mowafy, A.M., et al., *Endophytic Bacillus, Enterobacter, and Klebsiella enhance the growth and yield of maize.* Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, 2021. **71**(4): p. 237-246.
39. Mowafy, A.M., S. Khalifa, and A. Elsayed, *Brevibacillus DesertYSK and Rhizobium MAP7 stimulate the growth and pigmentation of Lactuca sativa L.* Journal of Genetic Engineering and Biotechnology, 2023. **21**(1): p. 17.

40. Vaishnav, A., et al., *Endophytic bacteria in plant salt stress tolerance: current and future prospects.* 2019. **38**(2): p. 650-668.