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Isolation of Potent Endophytic Bacteria Able to Boost Plant Growth and Control Pathogens

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ABSTRACT

Endophytic bacteria are promising source of plant probiotics due to their ability to promote plant growth and control pathogens. This study aims to isolate diverse endophytic bacteria capable of boosting plant growth and controlling pathogens. A total of 62 endophytic bacteria were isolated from the stems, leaves and roots of the flowering plant (*Matthiola incana*), the potato plant (*Solanum tuberosum*), the Prickly pear plant (*Opuntia- ficus indica*), and seedlings of *Acacia* sp. trees. Endophytic bacterial isolates were screened for their production of indole acetic acid (IAA) and cellulase enzyme, as well as their antagonistic activities against potato pathogens such as *Ralstonia solanacearum* and *Fusarium oxysporum*. The most promising endophytic isolates ML7 and PL10 showed the highest productivity of IAA, 69.1 and 64.8 µg/ml respectively. They also exhibited high cellulase activities on Congo red plates showing clear zone/colony diameters ratios of 3.36 and 2.8 respectively. The antagonistic activities of ML7 and PL10 against *R. solanacearum* were represented as 6 and 16*mm* inhibition zones diameters, while the inhibition zones diameters representing the antagonistic activities of ML7 and PL10 against *F. oxysporum* were 58 and 7*mm*, respectively. Isolates ML7 and PL10 exhibited a good ability to survive in a broad range of temperatures from 15 to 45 °C and upon exposure to direct UV radiation for 3 hours. Finally, isolates ML7 and PL10 were identified as *Achromobacter marplatensis* and *Bacillus velezensis*, respectively. Therefore, it is highly recommended that they can be used as plant probiotics in future field studies.

INTRODUCTION

The endosymbiotic group of microorganisms living in internal plant tissues which inhabit host plant tissues without causing harmful effects to the plant is called Endophytes **[1].** They have been recently used in agriculture as biofertilizers, biocontrols, and inducers of abiotic stress. The effective colonization of the host plant by the endophyte is a necessary step in order to start these advantageous plant–microbe interactions that are engaged in boosting plant growth **[2]**. In general, all plants and their parts are inhabited by endophytic microbes which play a crucial role in plant growth and stress resilience. Harnessing endophytic services can provide effective solutions for a sustainable increase in agriculture productivity and can be used as a complement/alternative to agrochemicals. Several species of cultivable endophytic bacteria have been isolated, identified, and reported from different environments. They varied according to the plant genotype, tissue type, plant age, microclimate conditions, and anthropogenic factors **[3].**

Plant probiotics are defined as plant-associated microorganisms which cause beneficial effects to the health of plants. They enhance crop production safely due to the avoidance of applying chemical fertilizers and pesticides. **[4].** Plant hormones can be released into the rhizosphere where they stimulate plant-interacting microorganisms and the root microbiome for metabolite signaling. The synthesis of plant hormones occurs in a wide range of tissues or cells within tissues. Auxin, the first identified plant hormone, is able to induce a growth response at a distance from its site of synthesis and thus fits the definition of a transported chemical messenger **[5].**

Cellulases have been widely used for a variety of bio-processing and bio-transformation purposes and are

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acknowledged as essential enzymes for industrial applications. The enzyme system of cellulose manages cellulolysis, which is a naturally occurring biological process. Bacteria are widely used for the production of cellulases due to their rapid growth, expression of multi-enzyme complexes, , capacity to colonize a wide variety of environmental niches, and ability to withstand varieties of environmental stresses as well as their stability at extremes of temperature and pH, lesser feedback inhibition **[6]**.

Ralstonia solanacearum is the causal agent of bacterial wilt disease. Biocontrol agents have been recently used as safe and environmentally friendly alternatives to the traditional means to control this disease. Traditional means include applying bactericides, using resistant varieties and implementing cultural practices to control this disease **[7]**. Endophytes play important roles in supporting plant growth and health. Healthy plants can be enriched with beneficial endophytic bacteria for their antagonistic activity against *R. solanacearum* **[8]**.

Dry rot diseases are caused by different strains of *Fusarium oxysporum* which are found in many soils where plants are cultivated and can survive in the soil for very long periods. Starting symptoms of *Fusarium* dry rot look like shallow dark depressions on the outside of the tuber of a potato, which can develop and become wrinkled in concentric rings as the underlying dead tissue desiccates **[9]**. Endophytes help plants to tolerate under stress conditions by regulating plant hormones and inducing systemic resistance. A wide variety of synthetic agents produced by endophytic bacteria are widely used to offset the economic losses caused by pathogens in agriculture **[10].** Tolerance to stress and potential application of endophytes against potato late blight were tested by many researchers **[11]** who conducted that most of them are highly tolerant to stresses such as temperature and UV.

This study aims to isolate and characterize some endophytic bacteria as potent plant probiotics able to produce IAA, cellulase enzymes and control pathogens.

MATERIAL AND METHODS

Preparation of Plant Samples for Isolation of Endophytic Bacteria

Plant parts of *Matthiola incana*, *Solanum tuberosum*, *Opuntia ficus –indica*, seedlings of *Acacia sp*. trees, and *Calotropis procera* were obtained from different localities in Egypt, then transferred to the laboratory in sterile sampling bags and treated immediately after collection. The collected plant parts were washed several times under running tap water in order to remove soil particles, then separated into fragments such as (leaves, stems, and roots). The fragments were washed with 70% ethanol for 1 min, washed with sterile distilled water several times for 5 min, then the fragments were immersed in sodium hypochlorite (0.9% chlorine) for 10 min, and finally washed with sterile distilled water again several times according to **[12].**

A surface sterility test was performed on nutrient agar (NA) media to ensure the removal of epiphytic bacteria. One ml of the last wash water runoff was inoculated into sterile NA plates to evaluate the effectiveness of the disinfection process then incubated at 30 °C for 7 days. Samples were discarded if any growth was detected in the sterility check **[13].**

Isolation of endophytic bacteria from the plant samples

Under asceptic conditions, the plant parts including root, stem, and leaf parts were cut into small pieces and macerated separately in one milliliter of sterile distilled water using a sterile pestle and mortar, then serially diluted to 10^{-6}. Finally, 100 µl of each dilution of the different samples was separately inoculated into Petri dishes containing NA media. Plating was performed in triplicate for each dilution. The plates were incubated at 30 °C for 7 days. The bacterial isolates were purified by the streaking plate method **[14].** The purified bacterial isolates were examined using the light microscope under an oil immersion lens.

Screening of endophytic bacterial isolates as potent plant probiotics:

Indole Acetic Acid (IAA) Production

Endophytic bacterial isolates were grown in 20 ml aliquots of sterilized nutrient broth medium (NB) and incubated at 28°C for 48 h with vigorous shaking at 100 rpm. The non-inoculated broth culture was used as control. Using the Salkowski method, IAA production was determined **[15].** Salkowski reagent was prepared by mixing 2 ml 0.5M $FeCl_3$, 49 ml water, and 49 ml 70% perchloric acid. The cultures were centrifuged at 3300 rpm for 12 min, then 1 ml of the supernatant was

transferred to a tube containing 2 ml of Salkowski reagent. The mixture was finally incubated in the dark at 25°C for 30 min. The development of a red color was considered an indication of IAA production. Absorbance of the mixture was measured at 530 nm using a spectrophotometer. The concentrations of IAA were calculated using the standard curve which was constructed by plotting known concentrations of standard pure tryptophan against the corresponding values of absorbance.

Cellulase enzymatic activity of the endophytic bacterial isolates

The cellulolytic activity of all isolates was carried out using Cellulose Congo Red agar medium (CCA) (g/l): CMC 1.88, gelatin 2.0, K_2HPO_4 0.5, $MgSO_4$ 0.25, Congo red 0.2, trace salt solution 1ml ($MnCl_2.4H_2O$ 0.1 ml – $FeSO_4.7H_2O$ 0.1 ml – $ZnSO_4.7H_2O$ 0.1ml – distilled water 100 ml) agar 20 gm, the isolates were inoculates on Congo red agar plates and incubated at 37˚C for 4 days, flooded with 1M NaCl (58.5 g/l) after incubation and a clear zone was visually observed. The isolates were inoculated on plates using a sterile cork borer with a zone scale of 5 mm in diameter. Clear hydrolysis zones indicate carboxymethyl cellulose degradation. Cellulase activity was determined via measuring the ratio of the clear zone diameter to colony diameter **[16, 17]**.

In *vitro* evaluation of antagonistic activities of endophytic isolates

The antagonistic activities of 24 h endophytic bacteria grown on NA at 30°C were separately tested against *F. oxysporum* and *R. solanacearum.* Fungal strain *F. oxysporum* was provided by the Radiation Microbiology Department, NCRRT, Cairo, Egypt. *Fusarium oxysporum* was routinely grown at 28℃ for 7 days on Saboraud dextrose agar medium SDA. Bacterial strain *R. solanacearum* was provided by Potato Brown Rot Project (PBRP), Dokki, Egypt and routinely grown on NA medium at 28℃ for 2-3 days. In order to examine antagonistic properties of endophytic isolates, two groups of plates were prepared. One ml aliquots of *F.oxysporum* were added to the plates of the first group, while 1ml aliquots of *R. Solanacearum* were added to the second group, and then NA medium was poured into all plates. After drying of NA in all plates a rounded disk of 5 mm in diameter was removed from the center of each plate using a sterile cork borer. Each well was then filled with 50 ul of endophytic bacterial suspension of absorbance of 0.6 at OD_{600}. For the detection of antifungal activity, the plates were incubated at 28°C for 4 days, whereas for the detection of antibacterial activity, the plates were incubated at 30°C for 2 days. Control plates were prepared by adding 50 ul of 0.85% sterile saline solution to the wells instead of the endophyte suspensions. After incubation, clear zones around inoculated wells were detected and measured **[18]**.

Growth of endophytic bacterial isolates at different temperatures

The growth of the endophytic bacterial isolates was tested at different temperatures (15, 20, 40, and 45 °C) on NA media. The growth abundance was detected and recorded after incubation for 24 h.

Molecular identification of bacterial endophytes

The most potent isolates for IAA production, cellulase and antagonistic activity were genetically identified using 16S rRNA gene sequence to provide genus and species for isolates by Sigma Scientific Services Company, Giza, Egypt, according to **[19].** DNA extraction was carried out by using protocol of Gene Jet genomic DNA purification Kit. PCR purified products were obtained using Gene JET™ PCR Purification Kit according to **[20]**. Sequences of 16S rRNA were matched with sequences of reference strains in a public repository NCBI GenBank database (http://www.ncbi.nlm.nih.gov) and given accession numbers OQ457665 and OQ457694.

Effect of UV-radiation on selected isolates

Endophytic bacterial suspensions in saline solution were UV-irradiated separately in open Petri dishes in the dark under sterile conditions. The Petri plates were placed under a 365 W.L. ultraviolet radiation lamp at a distance of 30 cm, (emitting an energy of 1.6 x 10^2 $J/m^2/s$) for 1, 2, and 3 h. The dose-response was determined directly by plating 1 ml of the bacterial suspension exposed to UV radiation for 1, 2, and 3 h separately into NA plates to count the number of cfu that could grow after radiation exposure **[21].** Unirradiated plates served as controls. After 24 h, the plates were examined for bacterial counts.

Statistical analysis

Statistical analysis of the data was performed using Microsoft Excel and has been expressed in mean along with standard deviation.

RESULTS

Isolation of endophytic bacteria

Results in (Table 1) show that a total of 62 endophytic bacterial strains were isolated from different plant parts of the collected plants. Twenty-two endophytic bacterial strains were isolated from *Matthiola incana* (M), 26 strains were isolated from *S. tuberosum* (P) (potatoes), 7 strains were isolated from *Opuntia ficus –indica* (O) species of cactus plants and 7 isolates were isolated from *Acacia sp*. seedlings (A). No endophytic bacterial isolates were obtained from *Calotropis procera* (C) plants.

Screening of the most potent plant probiotics

Indole Acetic Acid (IAA) production

Endophytic bacterial isolates were grown separately on NA media, the pink color developed upon treatment with the Salkowski reagent was photometrically analyzed, and the concentrations were calculated and presented (Table 2). It is shown that all 62 isolates produced IAA. The concentrations of IAA ranged from 69.1 µg/ml produced by isolate ML7 to 20.4 µg/ml produced by MS3. It was found that 58.1% of isolates were able to produce IAA at concentrations above 40 µg/ml.

Cellulose Congo red assay (CCA) for cellulase production

Out of 62 bacterial isolates, only 44 isolates exhibited clear zones around the colonies indicating their abilities to produce cellulase (Table 3). Sixteen isolates showed a clear zone-to-colony diameter ratio (z/c) > 2 mm. The highest cellulolytic activities showed a clear zone-to-colony ratios (z/c) of 4.5, 4.4, and 4.0 mm by the endophytic isolates PR1, ML7, and PL10 respectively.

Antagonistic activity of endophytic bacteria against *F. oxysporum*

It was found that 25 isolates have an antagonistic effect, out of the 62 tested ones. The diameters of the zones of inhibition are listed in (Table 4). Isolate ML7 exhibited a remarkable antagonistic effect against *F. oxysporum* where the recorded clear zone diameter was 58 mm.

Antagonistic activity of endophytic bacteria against *R. solanacearum*

It was found that 18 isolates have an antagonistic effect out of 62 tested isolates. The diameters of inhibition zones are listed in (Table 5). Isolate PL10 exhibited a remarkable antagonistic effect against *R. solanacearum* with a recorded clear zone of 16 mm.

Table (1): A brief survey of the endophytic bacteria isolated from different plants

Plant	No. of Isolates	Isolates
***Matthiola incana* (M)**	22 Isolates	ML1, ML2, ML3, ML4, ML5, ML6, **ML7**, ML8, ML9, ML10, ML11, MS1, MS2, MS3, MR1, MR2, MR3, MR4, MR5, MR6, MR7, MR8
***Solanum tuberosum* (P)**	26 Isolates	PS1, PS2, PS3, PS4, PS5, PS6, PS7, PS8, PR1, PR2, PR3, PR4, PL1, PL2, PL3, PL4, PL5, PL6, PL7, PL8, PL9, **PL10**, PL11, PL12, PP1, PP2
***Opuntia ficus –indica* (O)**	7 Isolates	OS1, OS2, OS3, OS4, OS7, OS6, OS7.
Acacia trees (A)	7 Isolates	AL1, AL2, AL3, AL4, AS1, AS2, AS3

Table (2): Concentrations of (IAA) produced by endophytic bacterial isolates

Isolates	(IAA) production (µg/ml)	Isolates	(IAA) production (µg/ml)	Isolates	(IAA) production (µg/ml)	Isolates	(IAA) production (µg/ml)
ML1	33.9	**MR3**	54.4	**PR3**	25.1	**OS1**	54.7
ML2	60.9	**MR4**	45.1	**PR4**	40.5	**OS2**	25.8
ML3	36.2	**MR5**	47.8	**PL1**	44.7	**OS3**	45.5
ML4	37.4	**MR6**	40.5	**PL2**	43.5	**OS4**	33.5
ML5	49.3	**MR7**	29.3	**PL3**	25.8	**OS5**	45.5
ML6	33.5	**MR8**	39.7	**PL4**	47.1	**OS6**	47.06
ML7	69.1	**PS1**	49.7	**PL5**	35.8	**OS7**	34.3
ML8	45.1	**PS2**	40.8	**PL6**	48.2	**AL1**	49.3
ML9	54.7	**PS3**	59.8	**PL7**	29.3	**AL2**	48.2
ML10	44.3	**PS4**	56.7	**PL8**	40.5	**AL3**	25.1
ML11	30.4	**PS5**	35.8	**PL9**	26.6	**AL4**	28.5
MS1	42.0	**PS6**	26.9	**PL10**	64.8	**AS1**	26.2
MS2	48.2	**PS7**	40.1	**PL11**	39.7	**AS2**	24.6
MS3	20.4	**PS8**	44.7	**PL12**	56.7	**AS3**	26.6
MR1	43.9	**PR1**	60.5	**PP1**	43.9		
MR2	38.1	**PR2**	45.1	**PP2**	38.1		

Table (3): Cellulase enzyme activity of endophytic bacterial isolates

Isolates	Zone/colony ratio	Isolates	Zone/colony ratio	Isolates	Zone/colony ratio	Isolates	Zone/colony ratio
ML1	1.63	**MR3**	0	**PR3**	0	**OS1**	1.68
ML2	3.36	**MR4**	0	**PR4**	2.14	**OS2**	2.30
ML3	2.00	**MR5**	2.6	**PL1**	2.30	**OS3**	1.43
ML4	1.45	**MR6**	2.33	**PL2**	0	**OS4**	1.54
ML5	1.56	**MR7**	0	**PL3**	0	**OS5**	1.65
ML6	0	**MR8**	0	**PL4**	1.11	**OS6**	1.91
ML7	4.44	**PS1**	1.60	**PL5**	0	**OS7**	2.80
ML8	1.90	**PS2**	0	**PL6**	0	**AL1**	1.60
ML9	0	**PS3**	2.40	**PL7**	1.33	**AL2**	0
ML10	2.30	**PS4**	1.50	**PL8**	1.63	**AL3**	1.70
ML11	1.88	**PS5**	1.80	**PL9**	1.54	**AL4**	0
MS1	2.53	**PS6**	1.90	**PL10**	4.00	**AS1**	2.00
MS2	2.60	**PS7**	0	**PL11**	0	**AS2**	1.70
MS3	1.60	**PS8**	2.16	**PL12**	0	**AS3**	1.29
MR1	3.10	**PR1**	4.50	**PP1**	1.92		
MR2	1.11	**PR2**	1.90	**PP2**	0		

Table (4): Antagonistic activity of endophytic bacteria against *F. oxysporum*

Isolates	Inhibition zone diameter (mm)	Isolates	Inhibition zone diameter (mm)	Isolates	Inhibition zone diameter (mm)	Isolates	Inhibition Zone Diameter (mm)
ML1	0	**MR3**	7	**PR3**	16	**OS1**	0
ML2	46	**MR4**	8	**PR4**	13	**OS2**	0
ML3	0	**MR5**	0	**PL1**	0	**OS3**	0
ML4	0	**MR6**	0	**PL2**	0	**OS4**	0
ML5	0	**MR7**	0	**PL3**	0	**OS5**	0
ML6	0	**MR8**	0	**PL4**	0	**OS6**	0
ML7	58	**PS1**	0	**PL5**	0	**OS7**	0
ML8	7	**PS2**	0	**PL6**	0	**AL1**	0
ML9	0	**PS3**	22	**PL7**	0	**AL2**	8
ML10	0	**PS4**	0	**PL8**	18	**AL3**	7
ML11	9	**PS5**	0	**PL9**	6	**AL4**	6
MS1	9	**PS6**	0	**PL10**	7	**AS1**	8
MS2	11	**PS7**	10	**PL11**	0	**AS2**	0
MS3	22	**PS8**	8	**PL12**	6	**AS3**	0
MR1	0	**PR1**	17	**PP1**	0		
MR2	0	**PR2**	6	**PP2**	7		

Note: 0 refers to the absence of a clear zone

Table (5): Antagonistic activity of endophytic bacteria against *R. solanacearum*

Isolates	Inhibition zone diameter (mm)	Isolates	Inhibition zone diameter (mm)	Isolates	Inhibition zone diameter (mm)	Isolates	Inhibition zone diameter (mm)
ML1	0	**MR3**	0	**PR3**	6	**OS1**	0
ML2	15	**MR4**	0	**PR4**	6	**OS2**	0
ML3	0	**MR5**	0	**PL1**	0	**OS3**	6
ML4	0	**MR6**	0	**PL2**	0	**OS4**	6
ML5	0	**MR7**	7	**PL3**	0	**OS5**	0
ML6	0	**MR8**	0	**PL4**	0	**OS6**	0
ML7	6	**PS1**	0	**PL5**	0	**OS7**	0
ML8	0	**PS2**	0	**PL6**	0	**AL1**	0
ML9	0	**PS3**	11	**PL7**	9	**AL2**	0
ML10	0	**PS4**	6	**PL8**	10	**AL3**	0
ML11	0	**PS5**	0	**PL9**	0	**AL4**	0
MS1	0	**PS6**	6	**PL10**	16	**AS1**	7
MS2	0	**PS7**	6	**PL11**	0	**AS2**	0
MS3	0	**PS8**	6	**PL12**	0	**AS3**	0
MR1	0	**PR1**	10	**PP1**	0		
MR2	0	**PR2**	6	**PP2**	0		

Note: 0 refers to the absence of clear zone

Preliminary selection of promising endophytic isolates

According to the IAA and cellulase production potentials, as well as antagonistic properties, 13 bacterial endophytes (ML2, ML7, ML8, ML11, MS3, MR8, PS2, PS3, PS6, PR1, PL10, OS7 and AL4) were selected as promising. The ability of these promising endophytic bacteria to grow at a broad range of temperatures was tested. Replica of each isolate was grown on NA plates and incubated at 15, 20, 40, and 45°C for 24 h. All the tested isolates were able to grow at diverse incubation temperatures except MR8 and PS6, which did not grow at 45°C, and 15°C, respectively. Poor growth was detected in all isolates at 15°C. Isolates ML7, ML2, PS3, and PL10 showed moderate to abundant growth in the temperature range from 20 - 45°C (Data not shown).

Selection of the most promising endophytic isolates

According to the previous results, two promising endophytic bacteria (ML7) isolated from the leaves of *Matthiola incana* plant and (PL10) isolated from the leaves of *Solanum tuberosum* plant were selected according to their high potentials to produce IAA, cellulase, as well as their antagonistic abilities against *F.oxysporum* and *R. solanacearum*, in addition to their abilities to grow at a wide range of temperatures. Based on the previous results, isolates ML7 and PL10 were selected to be used as plant probiotics for potato cultivation. No antagonistic effect was detected between isolates ML7 and PL10 (Data not shown).

Identification of isolates ML7 and PL10 as promising plant probiotics

Amplicon 16S rRNA sequences of isolates ML7 and PL10 on 1% agarose gels were 1500 bp. Amplification of bacterial 16S rRNA sequences can produce 1500 - 1600 bp amplicons. Analysis of DNA sequences showed that, isolate ML7 was identified as *Achromobacter marplatensis* whereas isolate PL10 was identified as *Bacillus velezensis*. Isolate ML7 revealed a close phylogenetic relationship with *A. marplatensis*, whereas isolate PL10 revealed a close phylogenetic relationship with *B. velezensis*. Agarose 2.5% gel electrophoresis of restriction fragments obtained after digestion of 16S rDNA amplicons of the most efficient isolates from endophytic bacteria. (ML7) *Achromobacter marplatensis* with *accession no. OQ457665* and (PL10) *Bacillus velezensis* with *accession no. OQ457694*.

Effect of Ultraviolet radiation on *A. marplatensis* and *B. velezensis*

A. marplatensis and *B. velezensis were directly exposed to UV for different periods of time* and then grown on NA medium at 30℃ for 24 h. The bacterial survivors of both isolates were separately counted after different exposure periods and represented in (Fig. 1 a & b) and their D_{10} was found to be 28 and 16, respectively. Both isolates were able to resist radiation exposure for 1 h, and then a gradual decrease in the count was observed with further exposure for up to 3 h until complete death was reached.

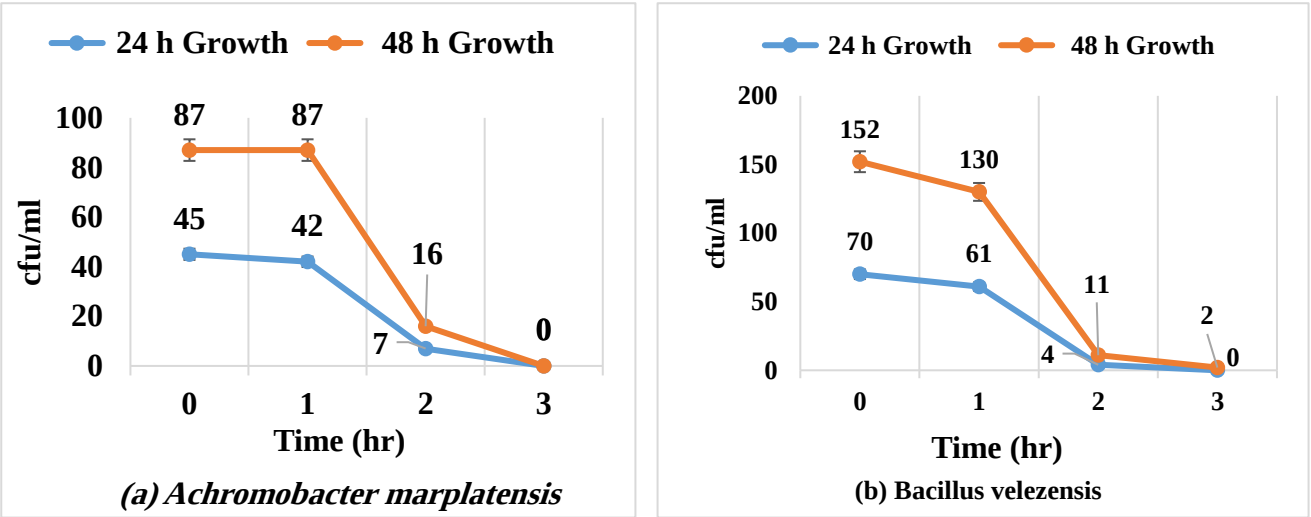

Fig. (1): Effect of UV-irradiation for different periods on the (a) *Achromobacter marplatensis* (b) *Bacillus velezensis*.

DISCUSSION

This study aimed to isolate potent endophytic bacteria for application as plant probiotics that support plant growth and control pathogens. A total of 62 endophytic bacterial strains were isolated on NA medium from diverse plants collected from different areas of Egypt. It was previously reported that endophytic bacteria have been isolated and screened in *vitro* from tubers, stems, roots and leaves of *S. tuberosum*plants **[22],** *Opuntia ficus-Indic* species of cactus plants**[23],** and the roots of different Acacia species **[24]** to produce inhibitory compounds that support plant growth and control pathogens. *Calotropis procera* plants widely used as a possible source of bioactive secondary metabolites**[25].** In contrary, no endophytic bacterial isolates were obtained from *Calotropis procera* plants in the present study.

In this study, all 62 isolates were able to produce IAA. It was found that 58.1% of the isolates were able to produce IAA concentrations > 40 µg/ml. The concentrations of IAA ranged from 69.1 µg/ml produced by isolate ML7 to 20.6 µg/ml produced by MS3. In another study, eleven isolates of endophytic bacteria showed IAA production, and the concentration of IAA ranged between 5 and 39.79 µg/ml **[26].** In a similar study, IAA was produced by *Stenotrophomonas maltophilia* with the highest concentration of 89.73 µg/ml, followed by *Pseudomonas protegens* and *Bacillus firmus* which produced 54.63 and 47.61 µg/ml of IAA, respectively **[27].**

In our study, 62 endophytic bacterial isolates were cultured separately on CCA agar media to test the cellulolytic activities, only 44 isolates showed clear zones around the colonies, indicating cellulolytic activity. Amongst them sixteen bacterial isolates showed a zone-to-colony diameter ratio z/c > 2. The highest cellulolytic activities showed clear zone-to-colony diameter ratios of 4.50, 4.44 and 4.00 by the isolates PR1, ML7, and PL10 respectively. It was previously found that, *Bacillus subtilis* shows a significant clear zone among 40 endophytic bacterial isolates on CCA agar media and exhibited remarkable cellulase activity **[28]**. Similar results were obtained by Siala *et al.* **[29]** who reported that, out of 167 isolates, only 12 isolates (7.2%) that showed cellulase activity with a clear zone over 3.0 mm were selected and named cellulose-degrading bacteria *B. subtilis*.

The results of the current study showed that 25 isolates had antagonistic effects against *F. oxysporum*, representing 42% and 18 isolates had antagonistic effects against *R. solanacearum*, representing 29% of the tested isolates. Mohamad *et al.* **[30]** found that different endophytes varied in their antagonistic action against fungi, with the percentage of inhibition ranging from 12.3 to 75.3%. Of the 44 strains, 38.6% were antagonistic *to F. oxysporum*. In another study, among the 77 isolates screened, 4 isolates showed a large inhibition zone development against *R.solanacearum* strain, namely E7, S25, P7, and E13, with 40, 34, 26, and 23 mm, respectively **[31].**

In this study, the ability of all isolates to grow under the influence of different temperature conditions was tested. All tested isolates could grow at diverse incubation temperatures of 15, 20, 40, and 45˚C except MR8 and PS6 which did not grow at 45˚C and 15˚C, respectively. Poor growth was detected for all isolates at 15˚C. Isolates ML7, ML2, PS3, and PL10 showed moderate to abundant growth in the temperature range from 20˚C to 45˚C. Gulboev *et al* **[32]** mentioned that the temperature effect is important for the growth of endophyte bacteria that can grow at a certain level at all temperature options 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40˚C. The growth traits of endophytic bacteria improve up to 30-32 ˚C, after which the growth rate decreases in inverse proportion to the increase in temperature.

In the present study, two promising endophytic bacteria ML7 and PL10 were selected because of their potential to produce IAA and cellulase, their antagonistic abilities against *F.oxysporum* and *R.solanacearum*,-and-their-abundant-growth-at-different temp-eratures. No antagonistic effect was detected between isolates ML7 and PL10, so they can be applied together as potent plant probiotics to boost growth by increasing the level of the growth hormone IAA and the degrading enzyme cellulase, which degrades cellulose in the soil to serve nutrition of cultivated plants, and to protect the cultivated plants against pathogens as biocontrol agents.

Analysis of 16S rRNA sequences showed that isolate ML7 was identified as *Achromobacter marplatensis*, while isolate PL10 was identified as *Bacillus velezensis*. The bacterium *A. marplatensis* was isolated and screened for its biocontrol efficacy against the selected plant pathogen *F. oxysporum* in *vitro* **[33].** The maximum growth inhibition of *F. oxysporum* was 80%. Siala *et al* **[29]** reported that, in dual culture tests, *B. subtilis* and *Arthrobacter agilis* strains only were shown to present lasting growth inhibition activity against assessed *F. oxysporum* with Percent Growth Inhibition (PGI) reaching 36.48%.

Plant growth promotion was studied using *Bacillus amyloliquefaciens* which showed antifungal activity against *F. oxysporum* **[34].** Another study showed that isolate *B. subtilis* had the highest zone of clearance against *F. oxysporum* **[35].** The isolated strain *Achromobacter sp.*, which produces indole acetic acid, has antifungal compounds and reduces root rot disease, improves yield performance, physiology, and the growth of plants, and effectively suppresses the progress of root rot disease **[36]**.

In the present study, the tolerance of *A. marplatensis* and *B. velezensis* to UV at wave length 365λ radiation was tested. The results showed that both isolates could tolerate up to 2 h of direct UV exposure. There is an evidence that more than half of the bacteria that exist on leaf surfaces are pigmented and able to resist to ultraviolet radiation. If pigmentation is lost, these bacteria are no longer able to survive under high levels of UV radiation **[37].** In another study, selected isolates exhibited high levels of UV radiation resistance among the aerobic subsurface isolates compared to the surface soil bacteria that were tested **[38].** Ultraviolet-A (365 nm, 120 kJ/mz/h) exposure resulted in death of *Pseudomonas aeruginosa* at doses at which *Escherichia coli* cell viability was not affected. Further studies showed that no UVA-induced growth delays or any other sublethal effects were detected **[39].**

CONCLUSION

Based on the results of the present work, the bacterial endophytic isolates *A. marplatensis* and *B. velezensis* can be considered as potent plant probiotics due to their high potential to produce IAA, cellulase enzyme, and control pathogens. They can survive in various environmental conditions. Further field studies are recommended to study their applicability in agriculture

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Not applicable.

DECLARATIONS

Ethics Approval and Consent to Participate

This work does not involve the use of human or animal subjects, as it does not require any international Code of Ethics of the world. Not applicable.

Consent for Publication Not applicable.

Availability of Data and Material

The identified strains in this study have been deposited in NCBI database under the accession numbers OQ457665 and OQ457694.

COMPETING INTERESTS

The authors declare no conflict of interests.

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