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Antagonistic Effect of the Endophytic Bacteria and Against some Phytopathogens

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ENDOPHYTIC bacteria have received a great attention because of their intimate and non-detrimental association with plants. They release an array of bioactive compounds that play important role in the biological control of various phytopathogens. A variety of endophytic bacteria was isolated from a range of plants gathered from Fayoum Government, Egypt. The antagonistic potentiality of the bacterial isolates was evaluated against a number of phytopathogens. A sharp antifungal activity was recorded with isolate H8 against *Rhizoctonia solani* and *Pythium ultimum* while elevated antagonistic potentiality was evidenced with isolate H18 against *Erwinia carotovora* and *Rhizoctonia solani*. Simultaneously, the isolate H40 demonstrated remarkable inhibitory influence against *Erwinia carotovora* and *Fusarium solani*. Using 16S ribosomal DNA technique, the bacterial isolates were identified as *Stenotrophomonas maltophilia*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. In conclusion, the bacterial strains *S. maltophilia* (H8), *B. subtilis* (H18) and *P. aeruginosa* (H40) could be used as biological agents against wide range of phytopathogens.

Keywords: Antagonism , *Stenotrophomonas maltophilia* , *Bacillus subtilis* , *Pseudomonas aeruginosa* , Phytopathogens.

Plant pathogenic microorganisms represent a great danger to crop production and ecosystem (Sabuquillo *et al*., 2006). With respect to phytopathogens, many effective pesticides are available, but they will not be reliable as a long-term solution because of concerns about exposure risks and residue persistence. Moreover, tolerance in the target pathogen may be developed as a result of frequent application of pesticides.

Endophytes are microorganisms that live inside living tissues of plants. In most cases, the microbial relationship with the host plant is symbiotic or mutualistic with no visible damage or morphological changes on their hosts (Schulz and Boyle, 2006). Because endophytes live in a steady environment inside the plant, they have more antagonistic potentiality than microorganisms isolated from rhizosphere, plant surface, or soil (Andrews, 1992).

As an important group of endophytes, endophytic bacteria have received a wide attention on their bioactivities including antibiotics production, biological

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control of plant diseases, plant growth stimulation and nitrogen fixation (Qiao *et al*. 2006 and He *et al*., 2010). Furthermore, endophytic microorganisms have a vast role in the release of insecticidal compounds (Guo *et al*., 2000 and Shi *et al.*, 2013).

The application of microorganisms and their bioactive compounds as biocontrol agents has become a promising approach to manage phytopathogenic microorganisms. Many beneficial microbes, including antagonistic endophytic bacteria, applied as treatments with different formulation provide privileges for crop production and protection against soil-borne pathogens. One of the advantages of using endophytes as biocontrol agents is that they exist in the same place where the plant pathogen survive that provide competition sufficient to inhibit many plant diseases especially vascular diseases. Another advantage is that they do not cause environmental contamination (M Bhattacharjee *et al.*, 2014).

A wide array of endophytic bacteria have been isolated from a variety of plants (Rosenblueth and Martínez-Romero 2006*; Hameed et al., 2015)*. Endophytic bacteria have been recorded to demonstrate an inhibitory effect against many plant pathogenic fungi such as Verticillium longisporum, Rhizoctonia solani, Fusarium oxyporum and Phythium ultimum in balloon flower; *Rhizoctonia solani* and *Fusarium oxyporum* in cotton; *Sclerotium rolfsii* in beans; *Verticillium dahliae*, *Verticillium alboatrum* and *Rhizoctonia solani* in potato and *Rhizoctonia solani* in ginseng (Berg *et al*., 2005 and Cho *et al*., 2007). In addition, Amaresan *et al.* (2015) highlighted the antagonistic influence of some endophytic bacteria isolated from chilli plants (*Capsicum annuum*) against the phytopathogens *Sclerotium rolfsii, Fusarium oxyporum, Phythium sp. and Colletotrichum capsici*

Moreover, endophytic bacteria demonstrated great antibacterial potentiality against many plant pathogens such as *Paenibacillus polymyxa*, *Bacilllus* sp. and *Pseudomonas poae* (*Seo, 2010); Xanthomonas oryzae* and *Burkholderia glumae* (Chung *et al.*, 2015). Endophytic microorganisms offer great advantages to host plant via producing wide variety of bioactive molecules that participate in plant protection (Chakraborty et al., 2010 and Dutta et al., 2014). The objectives of this study were: (1) to examine the population structures of endophytic bacteria of some crop plants; and (2) to investigate the antagonistic activities of the endophytic isolates against some phytopathogenic fungi and bacteria.

Materials and Methods

Isolation of endophytic bacteria

Various crop plants were gathered from diverged sites in Fayoum Governorate, Egypt. Plants were congregated in plastic bags and taken to the laboratory instantly. Healthy plants were selected for the isolation of the endophytic bacteria according to Suryanarayanan *et al*. (2005). Plants were washed with distilled water to get rid of adhered soil particles. Two or three

10 mm ×10 mm segments were cut randomly from stems, leaves, and roots of each plant. Segments were separated, and subjected to sequential immersion of each plant part in 95% (v/v) ethanol for 2 min, sodium hypochlorite for 90 sec and 95 % ethanol for 30 sec followed by three rinses in sterilized distilled water. Plant parts were dried using sterilized paper towels and placed on nutrient agar (NA) medium. Plates were incubated at 25°C for 3-7 days. The emerging bacterial colonies from the plant segments were picked out, streaked on nutrient agar plates and incubated at 28°C for 48 hr to get the pure culture. The purified bacterial isolates were cultivated in 5 mL of nutrient broth with constant shaking (100 rpm) at 28°C for 48 hr. The isolated bacteria, cultures were suspended in 20% glycerol solution and were kept at -80°C.

Phytopathogens

The antimicrobial activity of the isolated endophytic bacteria was carried out against the following plant pathogens *Fusarium solani*, *Fusarium oxysporum*, *Pythium ultimum*, *Sclerotium rolfsii*, *Aspergillus flavus*, *Aspergillus niger*, *Rhizoctonia solani*, *Alternaria solani*, *Erwinia carotovora* I and *Erwinia carotovora* II. These strains were afforded by the City of Science and Technology, Cairo, Egypt. The fungal strains were cultured on potato dextrose agar (PDA) meanwhile bacterial strains were cultured on nutrient agar (NA).

Antimicrobial activity of the endophytic bacterial isolates

At the beginning, the antagonistic activity of the endophytic isolates were evaluated against *F. solani* that cause damping-off and root rot diseases to many vegetable and crop plants in Fayoum, Egypt. The bacterial isolates that demonstrated clear antagonistic activity against *F. solani* were further evaluated against the rest of the phytopathogens. The antimicrobial potentiality was assayed according to Lin *et al*. (2009). For antifungal assay, the endophytic bacteria were grown on nutrient agarplates at 30˚C for 24 hr. 100 µL of spore suspension (200 cell/µL) from each fungus was spread on PDA plates. At equal places of PDA plates, nutrient agar discs of the endophytic bacteria were placed. Triplicate dual-inoculated plates, with the fungus alone as a control were incubated at 28˚C for 7 days. Regarding antibacterial assay, one hundred microliter of the bacterial culture (10^8 CFU/mL) was spread on nutrient agar plates. Then the inoculated plates were kept at 28˚C for 48 hr and diameters of inhibition zones were measured in millimeter.

Morphological characterization of the bacterial isolates

Stock cultures were plated out on nutrient agar plates and single colonies were picked and sub-cultured. Under stereomicroscope, colony morphologies were examined. Gram and endospore staining were carried out according to Prescott *et al*. (1996) while negative stain was used to stain bacterial capsule. At the same time, semisolid medium was used to examine the motility of the bacterial isolates (Soutourina *et al*., 2001).

Biochemical characterization of bacterial isolates

The biochemical characters of the bacterial isolates were investigated using API 20E panel systems according to the manufacturer's instructions (BioMerieux, France). In order to obtain single colonies for each bacterial isolate, stock cultures were streaked onto nutrient agar. 200 µL bacterial suspension of each isolate was transferred into the starting well of the panels. In order to prevent contamination, wells were filled with mineral oil and then were incubated at 30◦ C for 24- 48 hr. The results of the tests were evaluated according to the computer-based program 'IdBact v. 1.1, G. Kronvall, with Matrix for API from BioMerieux, France.

Identification of the bacterial isolates

In order to identify the isolated bacteria, genomic DNA was extracted using standard bacterial procedures (Essa, 2012). Two primers were used to amplify the 16S rDNA gene; (F1) AGAGTTTGATCCTGGCTCAG and (R1) GGTTACCTTGTTAC GACTT. PCR mixture was prepared as the following; 10 µL (10x) PCR buffer, 3 µL (50 mM) $MgCl_2$, 1 µL (20 pmole/µL) of each primer, 1 µL (10 mM) dNTPs mixture, 0.5 µL (2.5U) Taq DNA polymerase, 2 µL total DNA extract and the volume is completed to 100 µL by SD H_2O. Thirty five cycles of PCR were performed under the following conditions: 94°C for 40 sec (denaturation step), 55°C for one min (annealing step), 72°C for 2 min (extension step) and 72°C for 10 min (final extension step). 10 µL aliquots of the PCR products were mixed with 2 µL of DNA loading buffer and analyzed by electrophoresis (15 V/cm, 60 min) on 0.7% horizontal agarose gel in TBE buffer containing 0.5 µg/mL ethidium bromide, then visualized on an UV transilluminator. Sequencing of the amplified fragments were sequenced at GATC Biotech, Constance, Germany and DNA sequences were aligned at NCBI DataBase (www.ncbi.nlm.nlh.gov).

Statistical analysis

The data presented here are the mean value of three replicates. Standard errors were determined using MS Excel 2007.

Results

Isolation of endophytes and their antimicrobial activity

About fifty two bacterial strains were isolated from various crop plants where 23 isolates from roots, 15 isolates from stems and 14 isolates from leaves (Table 1). Antifungal activities of the bacterial isolates were assayed firstly against *F. solani*.

TABLE 1. Isolation sources of the endophytic bacteria.

Isolates	Source	Tissue	Isolates	Source	Tissue
H1	*Capsicum annuum*	Root	H31	*Raphanus sativus*	Leaf
H2	*Raphanus sativus*	Leaf	H32	*Pisum sativum*	Stem
H3	*Cucumis sativus*	Root	H33	*Sesamum indicum*	Stem
H4	*Allium cepa*	Leaf	H34	*Cucumis sativus*	Root
H5	*Sesamum indicum*	Stem	H35	*Cucumis sativus*	Root
H6	*Cucumis sativus*	Root	H36	*Cucumis sativus*	Stem
H7	*Brassica oleracea*	Stem	H37	*Cucumis sativus*	Stem
H8	*Brassica oleracea*	Root	H38	*Cucumis sativus*	Leaf
H9	*Cucumis sativus*	Leaf	H39	*Cucumis sativus*	Root
H10	*Cucumis sativus*	Root	H40	*Pisum sativum*	Root
H11	*Pisum sativum*	Stem	H41	*Cucumis sativus*	Leaf
H12	*Raphanus sativus*	Leaf	H42	*Sesamum indicum*	Stem
H13	*Brassica oleracea*	Leaf	H43	*Pisum sativum*	Root
H14	*Pisum sativum*	Leaf	H44	*Brassica oleracea*	Leaf
H15	*Pisum sativum*	Root	H45	*Cucumis sativus*	Root
H16	*Solanumm elongena*	Root	H46	*Helianthus annuus*	Root
H17	*Pisum sativum*	Root	H47	*Capsicum annuum*	Stem
H18	*Capsicum annuum*	Stem	H48	*Cucumis sativus*	Root
H19	*Cucumis sativus*	Leaf	H49	*Sesamum indicum*	Stem
H20	*Solanumm elongena*	Root	H50	*Cucumis sativus*	Leaf
H21	*Sesamum indicum*	Root	H51	*Cucumis sativus*	Stem
H22	*Brassica oleracea*	Root	H52	*Allium sativum*	Leaf
H23	*Pisum sativum*	Leaf			
H24	*Helianthus annuus*	Root			
H25	*Helianthus annuus*	Stem			
H26	*Raphanus sativus*	Stem			
H27	*Capsicum annuum*	Root			
H28	*Raphanus sativus*	Leaf			
H29	*Sesamum indicum*	Root			
H30	*Brassica oleracea*	Root			

According to the obtained results, the inhibitory effect of the screened isolates was classified into three groups: low, medium or strong. The gained results (Table 2) demonstrated that the maximum antifungal activity against *F. solani* was recorded by strains H8 (29 mm), H18 (37 mm), H40 (41 mm) that were isolated from *Brassica oleraces*, *Capsicum sativum* and *Pisum sativum*, respectively. Moreover, the antimicrobial activity of these isolates was further assayed against some selected phytopathogens. The bacterial isolates demonstrated a wide-spectrum antimicrobial activity against the various phytopathogens as shown in Fig.1.

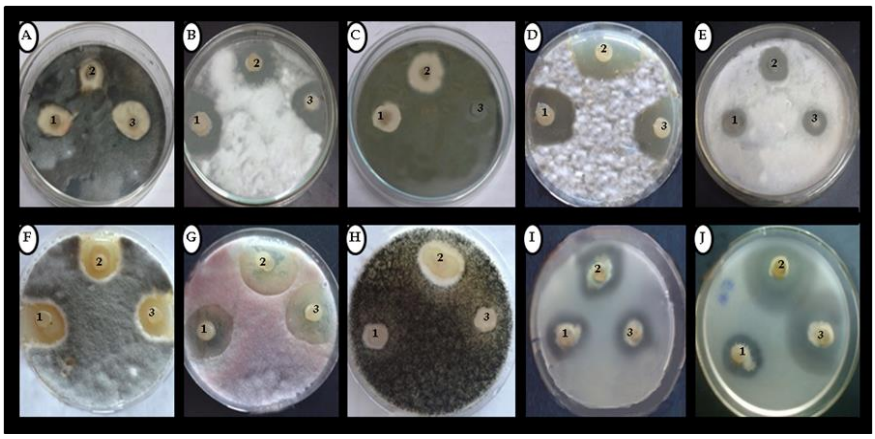

Fig. 1. Antagonistic activity of the endophytic bacterial isolates H8 (1), H40 (2) and H18 (3) against some phytopathogens; *Sclerotium rolfsii* (A), *Pythium ultimum* (B), *Aspergillus flavus* (C), *Rhizoctonia solani* (D), *Fusarium solani* (E), *Fusarium oxysporum* (F), *Aspergillus niger* (G), *Erwinia carotovora* II (I) and *Erwinia carotovora* I (J).

TABLE 2. Antimicrobial activity of selected endophytic isolates against various phytopathogens. Inhibition zones are measured by millimeter and data are the means of three replication ± standard errors.

Phytopathogen	Inhibition Zone diameters (mm)		
	P. aeruginosa **(H40)**	***B. subtilis*** **(H18)**	***S. maltophilia*** **(H8)**
Fusarium solani	41± 6	37 ± 3	29 ± 3
Fusarium oxysporum	23 ± 3	15 ± 1	17± 3
Pythium ultimum	35 ± 4	27 ± 3	39 ± 4
Sclerotium rolfsii	19 ± 3	29 ± 5	25± 1
Aspergillus flavus	15 ± 2	19 ± 2	0.0
Aspergillus niger	19 ± 5	15 ± 4	12 ± 6
Rhizoctonia solani	35 ± 2	39 ± 3	43 ± 3
Alternaria solani	21 ± 4	29 ± 6	25 ± 5
Erwinia carotovora I	49 ± 6	45 ± 3	25 ± 3
Erwinia carotovora II	37 ± 3	25 ± 1	21± 2

Phytopathogens growth was suppressed by the endophytic isolates at different levels. Clear zones of 35, 39 and 43 mm were recorded by bacterial isolates H40, H18, H8 against *R. solani*. Moreover, strain H40 demonstrated a significant antibacterial activity against *Erwinia carotovora* I (49 mm) and

Erwinia carotovora II (37 mm) meanwhile strain H18 recorded 45 mm clear zone against *Erwinia carotovora* I.

Similarly, clear antifungal potentiality was reported by bacterial isolate H40 (35 mm) and isolate H8 (39 mm) against *P. ultimum*. In the main time, a marked inhibition of S. *rolfsii* and *A. solani* was reported with isolate H18. The three endophytic strains (H40, H18 and H8) demonstrated antimicrobial activity with variable extent against the rest of the phytopathogens as shown in Table 2.

Characterization of the bacterial isolates

A variety of morphological and biochemical assays were carried out to have a comprehensive view on the phenotypic characteristics of the bacterial isolates as shown in Table 3. The obtained results showed that bacterial isolate (H40) was Gram-negative motile rods. This isolate demonstrated positive results with arginine dihydrolase, tryptophan deaminase, gelatinase, catalase, oxidase and nitrate reductase tests. Simultaneously, H40 demonstrated an aptitude to utilize arabinose and citrate as carbon source. The bacterial isolate H18 was Gram-positive motile spore producing rods. This strain demonstrated positive results with β-galactosidase, tryptophan deaminase, gelatinase, catalase, oxidase and nitrate reductase and acetoin production tests. In the interim, H18 isolate highlighted the potentiality to exploit various sources of carbon such as sucrose, mannitol, inositol, sorbitol, rhamnose, melibiose and amygdalin. Moreover, isolate H8 was Gram-negative motile non-spore forming rods. This strain demonstrated positive results in β-galactosidase, arginine dihydrolase, orenthine decarbolase, tryptophan deaminase, gelatinase, catalase, oxidase, lipase, nitrate reductase and acetoin production tests. At the same time, the endophytic isolate H8 showed the ability to utilize different carbon sources such as sucrose, manitol, sorbitol, rhamnose, melibiose, amygdalin, arabinose, and citrate. Meanwhile, the three bacterial isolates recorded negative results in urease, amylase, lysine decarboxylase, H_2S production, and indole production tests.

Molecular identification of the bacterial isolates

Beside the phenotypic characteristics of the endophytic isolates, 16S rDNA gene sequencing was used for the molecular identification of the bacterial isolates at higher level. The obtained 16S rDNA sequences were aligned with the corresponding sequences of GenBank using Blast program. The bacterial isolates H40, H18 and H8 were identified as *Pseudomonas aeruginosa* with maximum homology of 99%, *Bacillus subtilis* with maximum homology 99% and *Stenotrophomonas maltophilia* with 99% maximum homology, respectively. The 16S rDNA gene sequences of the bacterial strains were deposited in National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) under accession numbers KF407991 for *Stenotrophomonas maltophilia* H8, KF407989 for *Bacillus subtilis* H18 and KF407990 for *Pseudomonas aeruginosa* H40 (Fig.2).

TABLE 3. Morphological and biochemical characterization of the endophytic bacterial isolates.

Reaction	Bacterial isolate			Reaction	Bacterial isolate		
	H40	H18	H8		H40	H18	H8
Morphological characters				**Fermentation of sugars**			
Gram staining	-ve	+ve	-ve	Glucose	+	+	+
Motility	+	+	+	Sucrose	-	+	+
Cell shape	Rod	Rod	Rod	Mannitol	-	+	+
Endospore formation	-	+	-	Inositole	-	+	-
				Sorbitol	-	+	+
Biochemical characters				Rhamnose	-	+	+
Enzyme profile				Melibiose	-	+	+
β-galactosidase	-	+	+	Amygdalin	-	+	+
Arginine dihydrolase	+	-	+	Arabinose	+	-	+
Lysine decarbolase	-	-	-	Starch	-	-	-
Orenthinedecarbolase	-	-	+	Citrate utilization	+	-	+
Urease	-	-	-				
Tryptophane deaminase	+	+	+	**Other tests**			
Gelatenase	+	+	+	H_2S production	-	-	-
Catalase	+	+	+	Acetoin production	-	+	+
Amylase	-	-	-	Indole production	-	-	-
Lipase	-	-	+				
Oxidase	+	-	+				
Nitrate reduction							
to nitrite-	-	+	-				
to N_2 gas -	+	-	+				

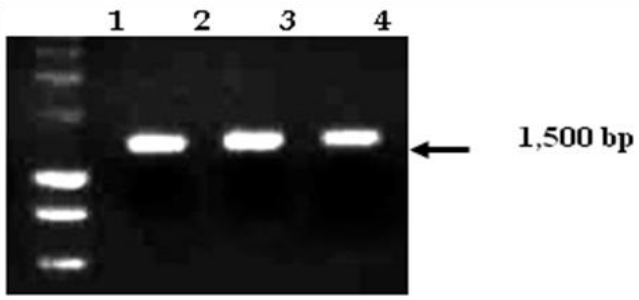

Fig. 2. Gel electrophoresis of PCR products of the 16S rDNA gene (1500 bp) of the endophytic bacterial isolates H40 (lane 2), H18 (lane 3), H8 (lane 4) whereas lane (1) contains Hyperladder I.

Discussion

Production of extremely diverse bioactive compounds by endophytic bacteria and their potential use as biological control agents has been reported to be dependent on many parameters. Among which are taxonomical position, physiological characters, geological conditions (Sharma *et al*., 2009). Endophytic bacteria might either become localized at the entry point or spread throughout the plant tissues (Liu *et al.,* 2015). They can effectively antagonize phytopathogens via releasing various bioactive molecules since both of them reside the same ecological place.

In the present study, different endophytic bacteria were isolated from crop plants in Egypt. These strains were screened in order to find those with strong antagonistic effect against different fungal and bacterial pathogens that cause great losses to crop plants. About half of the bacterial endophytes of this work were isolated from roots of the gathered plants clarifying that most of the endophytic microorganisms exist in the plant roots while their number decreases in stem and leaves as reported by Sharma and Roy (2015). The endophytic bacterial isolates were identified using biochemical characters and the 16S rDNA gene sequence as *Pseudomonas aeruginosa, Bacillus subtilis and Stenotrophomonas maltophilia*. Many studies showed that the genera *Bacillus, Pseudomonas, Agrobacterium* and *Streptomyces* have been considered as main bacteria genera capable of producing antimicrobial active compounds (Raaijmakers *et al*., 2002; Ongena and Jacques, 2008).

The obtained results revealed that *P. aeruginosa* H40 has a noticeable antagonistic action in opposition to the majority of the tested phytopathogens. The suppressive impact of *P. aeruginosa* H40 against the fungal and bacterial phytopathogens was attributed to the capability of this strain to produce bioactive molecules that may act as antimicrobial compounds. In agreement with these findings, Shtark *et al*. (2003) and Reddy *et al*. (2009) proved the high antifungal activity of presence of pyrrolnitrin identified from *P. aeruginosa* against *Rhizoctonia* sp., *Fusarium* sp. In a while, pyrrolnitrin has been used in the development of new phenylpyrrole agricultural fungicides. Besides, additional antibiotics were isolated from *P. aeruginosa* such as pyocyanin Ib, pyocyanin Ic, pyocyanin II, pyocyanin III , phenazines, pyrrolnitrin and pyoluterin (Kumar *et al*., 2005 and El-Fouly *et al., 2015).*

Various members of the genus *Bacillus* are under focus for their broad antagonistic potentiality against wide array of phytopathogenic fungi and bacteria. They release as a minimum 66 diversed antibiotic compounds (Ranjbariyan *et al*., 2011 and Lin *et al*., 2009). This study revealed that the bacterial isolate H18 (*Bacillus subtillus*) that was isolated from pepper stem, verified antimicrobial activity against most of the tested phytopathogens with strong inhibition against *F. solani, S. rolfsii, R. solani, A. solani,* and *Erwina carotovora* . Earlier investigations documented that peptide antibiotics such as

iturins, mycosubtilins and bacillomycins are the principal class of the active compounds with antimicrobial activities produced by *B. subtilis* (Ongena and Jacques, 2008; Ali *et al.,* 2014). At the same time, *Stenotrophomonas maltophilia* that is usually exist in the rhizosphere of cruciferous plants has been found in association with mustard, corn and beet roots (Debette and Blondeau, 1980). This investigation clarified a clear antimicrobial activity of the endophytic bacterial strain *S. maltophilia* H8 that was isolated from cabbage root against the phytopathogens *R. solani* and *P. ultimum*. In agreement with these results, Berg *et al*. (1996) recorded that *S. maltophilia* inhibited the growth of *R. solani*, possibly as a result of antibiosis and production of some lytic enzymes.

At the same time, Kai *et al*. (2007) recorded an apparent reduction of the mycelial growth of *R. solani* exposed to organic volatile compounds of the bacterial culture of *S. maltophilia* R3089. In addition, *Pythium ultimum* damping-off disease of sugar beet seedling was antagonized by *S. maltophilia* isolated from sugar beet (Dunne *et al*., 2000). Jakcobi *et al*. (1996) reported that *S. maltophilia R3089* produces an antibiotic called maltophilin that inhibits the growth of several human pathogens in addition to some phytopathogenic fungi. The gained results showed an obvious antibacterial activity of *S. maltophilia* H8 against the bacterial phytopathogens *Erwina carotovora I* and *Erwina carotovora II*. The remarkable antimicrobial activity of the endophytic bacteria *S. maltophilia* agrees with Messiha *et al*. (2007) who reported that *S. maltophilia* can significantly inhibit potato brown rot disease caused by *Ralstonia solancrearum* in Egyptian clay soil. Furthermore, Elhalag *et al.* (2016) clarified the efficiency of *S. maltophilia* in controlling the wilt caused by *Ralstonia solancrearum*. The biocontrol activity of *S. maltophilia* was ascribed to the impact of alkaline serine proteolytic enzyme in addition to the induction of host systemic acquired resistance.

Conclusion

Endophytic bacteria can release a wide array of extracellular bioactive metabolites with high capability to inhibit the growth of various bacterial and fungal species thus they can be used to manage different plant diseases. The present study revealed that the three endophytic bacterial strains *S. maltophilia* (H8), *B. subtilis* (H18) and *P. aeruginosa* (H40) demonstrated broad spectrum antimicrobial activities against various phytopathogens. Further investigations are recommended to identify metabolites with antifungal and antibacterial activities from endophytic bacteria and to evaluate their antimicrobial effectiveness against various phytopathogens *in vivo* study.

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التأثير المضاد لبعض أنواع البكتيريا النامية داخل النباتات ضد بعض مسببات الأمراض النباتية

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ان البكتيريا المعزولة من داخل النباتات قد لاقت الكثير من الاهتمام فى الفترة الاخيرة وذلك لعلاقتها الوثيقة والغير الضارة بالنباتات حيث تقوم تلك البكتيربا بإنتاج العديد من المركبات النشطة حيويا والتى تلعب دورا مهما فى التحكم البيولوجى للكثير من الكائنات الممرضة فى النباتات. فى هذه الدراسة تم عزل مجموعة متنوعة من البكتيريا من داخل العديد من النباتات التى تم جمعها من محافظة الفيوم - مصر. حيث تم تقدير التأثير المضاد للعزلات البكتيرية ضد بعض الكائنات الممرضة للنبات. تم تسجيل نشاط قوى مضاد للفطريات للعزلة البكتيرية (H8) ضد فطرى *Rhizoctonia solani* و *Pythium ultimum* وايضا تم تسجيل نشاط مضاد للعزلة البكتيرية (H18) ضد بكتيريا *Erwinia carotovora* و فطر *Rhizoctonia solani* . أما العزلة البكتيرية (H40) فقد أظهرت نشاط بارز مثبط ضد بكتيريا *Erwinia carotovora* و فطر *Fusarium solani*. أيضا تم تعريف العزلات البكتيرية بإستخدام تقنية 16S ribosomal DNA كالأتى: *Bacillus Stenotrophomonas maltophilia* *subtilis* - *Pseudomonas aeruginosa* وقد خلصت هذه الدراسة الى انه يمكن استخدام هذه العزلات البكتيرية كعوامل للتحكم البيولوجى فى العديد من الكائنات الممسببة للأمراض فى النباتات.