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MOLECULAR AND PHYSIOLOGICAL IDENTIFICATION OF ENDOPHYTIC BACTERIAL ISOLATES OF TOMATO (*LYCOPERISCON ESCULENTUM*) AND THEIR *IN VITRO* ANTAGONISTIC ACTIVITY

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ABSTRACT

Twelve endophytic bacterial isolates were isolated from surface sterilized root, stem, and leaves of healthy tomato plants which was collected form vegetables farm at faculty of agriculture, Minia University. Identification of these isolates based on morphological, physiological and chemical characteristics as well as 16S rDNA gene sequence analysis demonstrated that they belonged to seven bacterial genera *viz.*, *Rhizobium, Agrobacterium, Bacillus, Enterobacter, Pantoea, Serratia,* and *Ensifer*. The results of the *in vitro* antagonism experiments revealed that three out of the twelve endophytic bacterial isolates could significantly reduce the mycelial growth of five of the major phytopathogenic fungi (*Fusarium solani, Fusarium semitictum, Macrophomina phasolenia, Rhizoctonia solani* and *Aspergillus niger*) by forming an inhibition zone. These three antagonistic isolates were identified as (*Bacillus subtilis* HMS10, *Bacillus subtilis* HMS11 and *Bacillus malacitensis* HMS12).

Key words: endophytes**,** phytopathogens, antagonism, tomato, 16S rDNA

INTRODUCTION

Tomato (*Lycopersicum esculentum* L.) is considered one of the most important economic vegetable crops in Egypt. It is among the most intensively treated crops with agrochemicals because of its high susceptibility to phytopathogens attack. Thus, biological control agents have emerged as an alternative approach for the control of tomato diseases, which are generally associated with diverse microorganisms. Endophytic bacteria are defined as those detected "from inside surface-disinfested plants or extracted from inside plants and have no visibly harmful effects on the plants (Brady et al., 2000). Endophytic bacteria have been observed in a wide range of different plant species included Tomato (Jha *et al.,* 2013 and Nandhini *et al.,* 2012); Rice (Okunishi *et al.,* 2005); Maize (Rijavec *et al.*, 2007); Cotton (McInroy and Kloepper, 1995) ; Potato (Krechel *et al.,* 2002) and Sugar Cane (James and Olivares, 1997). Endophytic bacteria seen as promising alternatives to replace chemical pesticides and fertilizers in sustainable and organic agriculture systems. A variety of endophytes have been reported to have antagonistic activities toward bacterial and fungal pathogens (Lodewyckx *et al.,* 2002 and Sessitsch *et al.*, 2004). They can antagonize pathogens by competing for niche and nutrients, by stimulating the defensive capacities of the host plant and by producing antibiotics, siderophore, lytic enzymes and fungal toxic compounds (Jha *et al.* 2013; Ongena *et al.,* 2007 and Compant *et al.,* 2005). The present study aimed to isolate and characterizes the endophytic bacteria associated with different parts of tomato plants using molecular and physiological analysis. Determination of their *in vitro* antagonistic activity against major phytopathogenic fungi such as *Fusarium solani, Fusarium semitictum, Macrophomina phasolenia, Rhizoctonia solani* and *Aspergillus niger* was also achieved.

MATERIALS AND METHODS

Isolation of endophytic bacteria from tomato plants

Endophytic bacterial strains were isolated from leaves, stems and roots of tomato plant (cv. Super Jackal) according to the procedure described by Bacon *et al*. (2002). Since one key to success in isolating and studying endophytic bacteria is surface sterility (Hallmann *et al.,* 1997), sterility checks were carried out for each sample to monitor the efficiency of the disinfestations procedure Gyaneshwar *et al.,* 2001). Different single colonies were isolated, purified and stored at 4°C.

Morphological and physiological characterization of the endophytic bacterial isolates

Cell Shape: The purified cultures, at log phase were observed microscopically for the cell morphological characteristics as described by Aneja, (2006).

Gram staining: Gram's staining was performed to determine stain ability of

the endophytic bacterial isolates according to Cowan and Steel (1985).

Motility test: The diffusion of colony was observed on semi-solid nutrient agar plates (0.2% agar) after 24 hours of incubation at 30^{o}C for motility determination as described by Elbeltagy *et al.,* (2000).

Antibiotic resistance: Intrinsic antibiotic resistances were determined by measuring the clear zones around antibiotic discs which were placed on nutrient agar (NA) plates inoculated with endophytic bacterial isolates after incubation at 28°C for 2 days (Clower and Hay, 1968). The following antibiotics (µg/ml): Cefepime 30; Cefoperzone 75; ancomycin 30; Erythromycin 15; Oxacillin 1; Aztreonam 30; Tetracycline 30 and Cefoxitin 30 were used in this experiment.

Biochemical tests

Oxidase test: The endophytic isolates were streaked on potato dextrose agar (PDA) medium and incubated at 30^{o}C in an inverted position for 48h. After incubation, oxidase test was carried out as described by Cappuccino and Sherman (1996).

Catalase activity: A loopful of 24h old culture of endophytic isolates maintained on NA slants were transferred to a glass test tube containing 0.5 ml distilled water and mixed thoroughly with 0.5 ml of 3 % hydrogen peroxide solution and observed for the presence of the effervescence which indicate Catalase activity as described by Aneja, (2006).

Indole production: After 48 h of incubation into glucose tryptone broth, indole production was detected by reddening of the alcohol layer within few minutes after adding and mixing 0.3 ml of Kovacs reagent (Seeley and Vandemark, 1981).

Gelatin hydrolysis: The activity of the gelatinase enzyme for hydrolyzing gelatin was tested by gelatin liquefaction as described by Aneja, (2006).

Starch hydrolysis: The endophytic isolates were streaked on NA plates containing 2 % insoluble starch and incubated at room temperature for studding amylase activity. Hydrolysis of starch was tested by flooding with iodine solution and observing the presence of clear zones surrounding the colonies which considered for positive reaction. (Aneja, 2006)

Molecular characterization of endophytic bacterial isolates

Isolation of genomic DNA: Total DNA was extracted from the twelve endophytic bacteria isolates according to GeneJET genomic DNA purification Kit [Mini] obtained from Thermo scientific.

Amplification and analysis of 16S rDNA gene: The 16S-rDNA gene fragment was amplified using universal eubacterial full-length primers Forward 5′-AGA GTT TGA TCC TGG CTC AG-3′ and Reverse 5′-ACG GCT ACC TTG TTA CGA CTT-3′ (Patel *et al.,* 2012). Amplification was carried out in a 25 µl mixture which were subjected to the

following optimized conditions: Initial denaturation at 94^{o}C for 3 minutes, denaturation at 94°C for 30 seconds, primer annealing at 57^{o}C for 30 seconds, chain extension at 72^{o}C for 2 minutes and a final extension at 72°C for 10 minutes. Denaturation, annealing and extension cycles were repeated for 30 cycles.

Sequencing of the purified DNA samples was performed at GATC Company (GATC Biotech Ltd. - The London BioScience Innovation Centre - London, United Kingdom) by using ABI 3730xl DNA sequencer. The obtained sequences were compared to sequences in the public database using Basic Local Alignment Search Tool (BLAST) on the National Center for biotechnology Information (NCBI) website (http://www.ncbi.nih.gov) in order to determine similarity to sequences in the Gene bank database (Shayne *et al.,* 2003).

In vitro antagonistic activity of endophytic bacteria against phytopathogens

Bacterial isolates were assayed for antifungal activities against five of the major phytopathogens of vegetables by Dual culture assay (Wang *et al.,* (2009). The phytopathogenic fungi, *Fusarium solani*, *Fusarium semitictum*, *Macrophomina phasolenia*, *Rhizoctonia solani* and *Aspergillus niger*, were kindly obtained from the Central Laboratory of Organic Agriculture, Agriculture Research Center (ARC). The inhibition of fungal growth was evaluated by the reduction percentage of mycelium expansion compared to control plates without bacteria following the formula of Whipps, (1987): (R1–R2)/R1×100. All *in vitro* antagonism assays were made in triplicate. The percentages of growth inhibition were categorized on a growth inhibition category (GIC) scale from 0 to 4, where 0 = no growth inhibition; 1 = 1-25% growth inhibition; 2 = 26-50% growth inhibition; 3 = 51-75% growth inhibition; 4 = 76-100% growth inhibition (Korsten and Jager 1995).

RESULTS AND DISSCUTION

Isolation of endophytic bacteria

Selected sample of tomato plants (roots, leaves and stems) were surface sterilized by ethanol and sodium hypochlorite as described in materials and Methods. After sterility checks, the recovered bacteria which were prepared from surface-disinfected tissue (Fig.1) were considered to be endophytes (Gyaneshwar *et al.,* 2001). Single colonies showing different morphological appearances on PDA plates were selected for making further characterization.

Overall, the observed number of endophytic bacterial isolates was more in roots than leaves and stems. Twelve putative endophytic bacterial strains were selected from roots (7 isolates), stems (2 isolates) and leaves (3 isolates) of tomato plant on PDA medium and stored at 4°C, for further characterization.

The higher percentage of endophytic bacterial populations in roots and its reduction in the stems and leaves was also reported by Lamb *et*

al., (1996). Likewise, although the endophytic bacteria are found in almost all parts of the plants including roots, stems, leaves, seeds, fruits, tubers, ovules and also inside legume nodules (Hallmann *et al.,* 1997; Benhizia *et al.,* 2004), the below-ground parts of plants have been reported to have the higher numbers of endophytes as against above-ground tissues (Rosenblueth and Martinez-Romero, 2004).

Microbiological and physiological Characterization of the endophytic bacterial isolates

After purification, some microbiological and physiological tests were used for characterizing the isolated endophytic bacteria as shown in Table (1). All cells of the pure bacterial isolates were rod shaped and motile. Motility is an important characteristic for endophytes. Although endophytic bacteria can follow water fluxes for passive movement, they also need to be able to move inside the plant since endophytes tend to colonize specific plant parts that do not always correspond to the port of entry in the plant (Taghavi *et al.,* 2009). Nine (HMS1 through HMS9) of the twelve bacterial isolates selected were Gram negative belonging to different genera, while three (HMS10, HMS11 and HMS12) were Gram positive belonging to *Bacillus* sp, This finding agree with some of the earlier reports which stated that gram negative most abundant endophytic bacteria on many different plants (Khan and Doty, 2009 and Taghavi *et al.,* 2009)

Biochemical tests

Data of some biochemical tests (Catalase and Oxidase production, Indole formation, Nitrate reaction, Gelatin hydrolysis and Starch hydrolysis) are shown in Table (1). All of the tested isolates revealed positive results for catalase and oxidase which are involved in the protection of bacterial cells against plant reactive oxygen species (Fouts *et al.,* 2008) and considered as an important aspect required by the bacteria for avoiding cellular toxicity (Mbai *et al.,* 2013).

After 24 hr. of incubation with tryptophan, nine of the isolates were positive for indole formation demonstrating a significant amount of IAA production. The production of IAA (the primary auxins in the majority of plant species as a plant growth promoter) in the presence of tryptophan by most of the present isolates reveals their capabilities for tryptophan utilization as a precursor for growth and IAA production. The production of plant growth-promoting molecules like IAA is an important contribution of endophytic microorganisms (Spaepen et al., 2007) which can stimulate both rapid responses such as cell elongation and long-term responses such as cell division and differentiation of plants tissues (Taghavi *et al.,* 2009).

The nitrate reduction test was performed to determine the ability of the endophytic bacterial isolates to reduce nitrates into nitrogen gas. All isolates except that named HMS7 could reduce nitrate. Nitrate reduction is a critical feature of endophytes, as it

gives time for plants to absorb readily available nitrogen before it can be converted to free nitrogen gas by other denitrifying bacteria that could be present in the host plant or in the soil/rhizosphere (Mbai *et al.,* 2013). Nitrate reduction process is performed primarily by heterotrophic bacteria (Carlson and Ingraham, 1983).

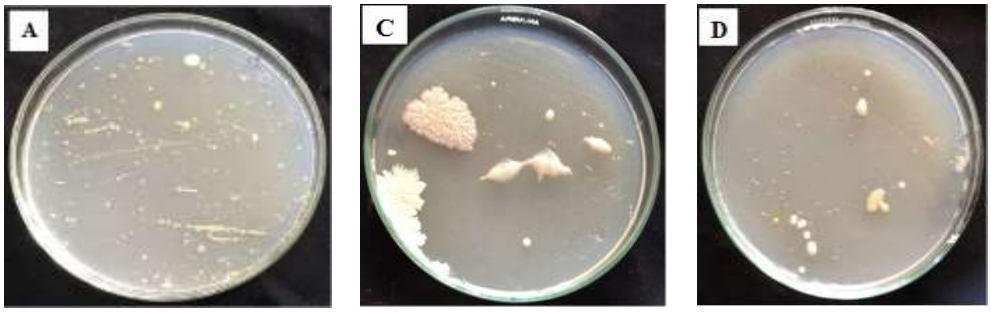

Fig (1): Endophytic bacterial isolates which were prepared from surface-disinfected roots (A), stems (B) and leaves (C) of tomato on PDA medium.

Table (1): Microbiological and physiological profiles of the endophytic bacterial isolates.

Isolates	Cell shape	Gram reaction	Motility	Catalase	Oxidase	Indole formation	Nitrate reaction test	Gelatin hydrolysis	Starch hydrolysis
HMS1	Rod	-	+	+	+	+	+	-	+
HMS2	"	-	+	+	+	+	+	+	-
HMS3	"	-	+	+	+	-	+	-	+
HMS4	"	-	+	+	+	+	+	-	-
HMS5	"	-	+	+	+	-	+	-	-
HMS6	"	-	+	+	+	+	+	-	+
HMS7	"	-	+	+	+	+	-	-	-
HMS8	"	-	+	+	+	+	+	-	+
HMS9	"	-	+	+	+	-	+	-	+
HMS10	"	+	+	+	+	+	+	+	+
HMS11	"	+	+	+	+	+	+	+	+
HMS12	"	+	+	+	+	+	+	+	+

Enzymatic activities

The degradation of starch and gelatin is an important process in terms of energy storage. The enzymatic activity of the endophytic bacterial isolates was studied in relation to gelatinase and amylase as shown in Table 1. Four of the endophytic bacterial isolates (HMS2, HMS10, HMS11 and HMS12) were able to produce gelatinase into nutrient gelatin deep tubes while the other eight

isolates were not able to produce gelatinase.

Amylase production and activity was indicated by the clear zones formation in starch agar media as a result of growing some isolates (HMS1, HMS3, HMS6, HMS8, HMS9, HMS10, HMS11 and HMS12) on the other hand; the isolates (HMS2, HMS4, HMS5 and HMS7) were not able to produce amylase. Similar results were reported by (Rajan 2012) when studied the amylase and gelatinase activities in the endophytic isolates of tomato plants.

Antibiotic resistance test

The Intrinsic antibiotic resistance of the twelve endophytic bacterial isolates was verified on NA media supplemented with one of eight antibiotics Fig (2) and Table (2)

Generally, the results indicated that all isolates were highly sensitive to Tetracycline 30 however; they were sensitive to Cefepime 30 except isolate HMS9 which exhibited resistance to this antibiotic. Two isolates (HMS 10, and HMS11) were sensitive to all antibiotics, whereas, isolate HMS12 was sensitive to all antibiotics except Aztreonam 30. Regarding Cefoxitin 30, all isolates were resistant except isolates HMS2, HMS8, HMS10, HMS11 and HMS12 that showed sensitivity to this antibiotic. Concerning the other antibiotic, all of the isolates revealed a considerable diversity of resistance as shown in Table (2).

Similarly the results recorded by Patel *et al.*, (2012) showed that all tested endophytic bacteria of tomato exhibited variable aspect in the resistance characters against different tested antibiotics. The Intrinsic antibiotic resistance is a mechanism in microbes which helps it to tide over stress situations. The wide variation of the intrinsic antibiotic resistance reveal the genetic difference concerning the presence of genes which are responsible for the synthesis of enzyme systems that detoxify the antibiotic and proteins that inhibit the cellular transfer of the antibiotic (Hayes and Wolf, 1990)..

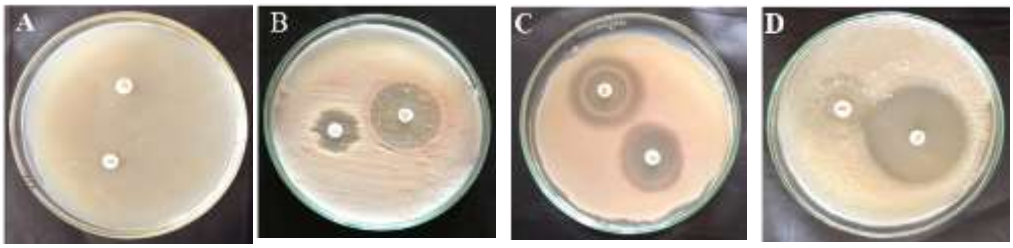

Fig (2): Effect of antibiotics on growth of endophytic bacterial isolates. (A) Cefoperzone 75 and Vancomycin 30 on HMS1, (B) Erythromycin 15 and Oxacillin 1 on HMS10, (C) Tetracycline 30 and Cefoxitin 30 on HMS12 and (D) Cefepime 30 and Aztreonam 30 on HMS4

Table (2): Growth of endophytic bacterial isolates on the presence of different antibiotics.

Isolates	Cefepime 30		Cefoperzone 75		Vancomycin 30		Erythromycin 15		Oxacillin 1		Aztreonam 30		Tetracycline 30		Cefoxitin 30	
	D/Z	R/S	D/Z	R/S	D/Z	R/S	D/Z	R/S	D/Z	R/S	D/Z	R/S	D/Z	R/S	D/Z	R/S
HMS1	29	S^{++}	0	R	0	R	0	R	0	R	0	R	52	S^{++}	0	R
HMS2	34	S^{++}	10	S^{+}	0	R	0	R	0	R	28	S^{++}	25	S^{++}	10	S^{+}
HMS3	18	S^{+}	0	R	8	S	22	S^{++}	0	R	22	S^{++}	32	S^{++}	0	R
HMS4	26	S^{++}	12	S^{+}	0	R	15	S^{+}	3	S	0	R	30	S^{++}	0	R
HMS5	30	S^{++}	16	S^{+}	0	R	0	R	0	R	31	S^{++}	30	S^{++}	0	R
HMS6	29	S^{++}	17	S^{+}	0	R	0	R	0	R	27	S^{++}	30	S^{++}	0	R
HMS7	16	S^{+}	0	R	1	S^{+}	39	S^{++}	0	R	0	R	49	S^{++}	0	R
HMS8	18	S^{+}	10	S	23	S^{++}	32	S^{++}	22	S^{++}	0	R	40	S^{++}	10	S^{+}
HMS9	0	R	0	R	0	R	12	S^{+}	13	S^{+}	0	R	23	S^{++}	0	R
HMS10	22	S^{++}	15	S^{+}	21	S^{++}	40	S^{++}	13	S^{+}	10	S^{+}	35	S^{++}	30	S^{++}
HMS11	33	S^{++}	21	S^{++}	24	S^{++}	24	S^{++}	20	S^{++}	18	S^{+}	47	S^{++}	29	S^{++}
HMS12	18	S^{+}	20	S^{++}	22	S^{++}	22	S^{++}	19	S^{+}	0	R	50	S^{++}	26	S^{++}

Abbreviations: Resistant (R); Weakly Sensitive (S); Sensitive (S^{+}); Highly Sensitive(S^{++}); Diameter (mm) of Zone around Antibiotic (D/Z)

Molecular characterization of the endophytic bacterial isolates of tomato.

Recently, one of the molecular approaches for detecting and classifying bacteria rely on the PCR amplification and sequence analysis of the 16S rRNA gene. PCR using isolated endophytic bacterial genomic DNA as template and universal bacterial primers for 16s rDNA, produced one fragment of 1500 bp (Fig. 3). The amplified fragments of the twelve endophytic bacterial isolates were subjected to nucleotide using the same primers and the sequences compared with the NCBI Genbank database. The nucleotide sequence data have been deposited in the NCBI GenBank under the accession numbers shown in Table (3).

According to the percentages of homology of 16S rDNA sequence analysis with the Closest NCBI strain, the twelve strains isolated in the present work were recognized to different genera and/or species (Table 3). Except isolate HMS5 which showed only 92% of homology with *Serratia marcescens* strain RS8101 HQ123487, and isolate HMS3 which showed 97% of homology with *Pantoea sp*. GrF KC311261, all of the other isolates showed high percentages of homology (99-100%) suggesting that they were the same species as shown in Table (3). Three isolates (HMS10, HMS11 and HMS12) were closest to *Bacillus subtilis* strain MSEB 67 (homology 100%), *Bacillus subtilis* strain JPS1-2 (homology 99%) and *Bacillus malacitensis* strain F-61 (homology 99%), respectively. All of them have been found earlier as

endophytes (Bai *et al.,* 2002; Berg *et al.,* 2005; Zinniel *et al.,* 2002, Vega *et al.,* 2005 and Bulgari *et al.,* 2009). These results suggest using Sequence analysis of the ubiquitous 16S rDNA as a tool for classification, detection, and evaluation of the microbial evolutionary relatedness as described by Ngoma *et al.* (2013). Similarly, Clarridge (2004) demonstrated the advantage of the 16S rDNA gene sequence for better identification of poorly described and rarely isolated strains.

***In vitro* antagonistic activity of endophytic bacteria**

The efficiency of present endophytic bacterial isolates were screened for *in vitro* growth inhibition of five of the major phytopathogenic fungi (*Fusarium solani, Fusarium semitictum, Macrophomina phasolenia, Rhizoctonia solani* and *Aspergillus niger*) on PDA media by dual culture assay and the results are presented in Table (4). The results of the *in vitro* screening revealed no antagonistic effects of the first nine isolates (HMS1 through HMS9) against mycelium growth of all tested phytopathogens (Fig. 4). However, the other three isolates (HMS10, HMS11 and HMS12) significantly reduced the mycelial growth of all tested phytopathogens by forming an inhibition zone (Table 4 and Fig. 5). The traditional microbiological testes and 16S rDNA sequence analysis showed that these endophytic bacterial isolates were Gram positive belonging to *Bacillus* sp.

These endophytic bacterial isolates strongly inhibited the growth of *R. solani* by 78.5, 78.2 and 74.3 % respectively, which belonged to growth inhibition categories (GIC) of 4, 4 and 3, respectively (Fig.5). In addition, isolates HMS10 and HMS12 showed a good degree of antagonistic activity against *F. solani* (29.3% and 32.8%, respectively) with GIC 2, while the third isolate (HMS11) exhibited poor antagonistic activity (23.6%) against this phytopathogen (Fig. 5). The three isolates viz., HMS10 (67. 2 %), HMS11 (66.8%) and HMS12 (71.5 %), exhibited moderate degree of mycelia growth inhibition of *M. phaseolina* and belonged to GIC 3 (Fig. 5). On the other hand, the three isolates viz., HMS10, HMS11 and HMS12 exhibited moderate antagonistic activity ranging from 62.3% to 67.5% against *A. niger* (Fig.5). Similarly, the same three isolates (HMS10, HMS11 and HMS12) showed a moderate inhibition (52.0, 49.2 and 56.3%, respectively), belonging to GIC 3, against *F. semitictum* (Fig. 5).

Similar results were reported on the endophytic bacteria in tomato plant. Amaresan *et al.,* (2012) found that six endophtic bacterial isolates belong to *Bacillus* genus exhibited antagonistic activity against *Sclerotium rolfsii, Fusarium oxysporum, Colletotrichum capsici* and *Pythium sp.* On the other hand, Munif *et al.* (2012) reported that endophytic bacterial isolates *Bacillus sp* and *Bacillus subtlis* of tomato inhibited *in vitro* the mycelia growth

of *Rhizoctonia solani* and *Fuzarium oxysporium*. In dual culture assay of 72 endophytic bacteria strains of tomato, 49 strains could inhibit *Botrytis cinerea* (tomato grey mould disease) in varying degrees (78 % in dual culture assay and 100 % using fermentation filtrate) according to Yang *et al.* (2011). Patel *et al.*, (2012) recorded antifungal activity of the endophytic isolates of tomato against *Fusarium oxysporium, Alternaria sp., Trichoderma sp.* and *Rhizoctonia solani* in plate assay. The widely recognized mechanisms of biocontrol mediated by endophytes are competition for an ecological niche or a substrate, production of inhibitory allelochemicals, and induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens and/or abiotic stresses.

Table 3 Identification of endophytic bacteria isolated from tomato (*Lycopresicum esculentum*) based on 16S rDNA sequence

Isolate	Identified as	Accession number	% Similarity	Closest NCBI strain and accession No.
HMS1	*Rhizobium sp.*	KT587347	99	*Rhizobium sp.* HJX3 KP979534
HMS2	*Enterobacter cloacae*	KT587348	100	*Enterobacter cloacae* strain VITDAJ KP305912
HMS3	*Pantoea sp.*	KT750022	97	*Pantoea sp*. GrF KC311261
HMS4	*Pantoea ananatis*	KT750023	99	*Pantoea ananatis* ITCC<IND>:B0055 JF756691
HMS5	*Serratia marcescens*	KT750024	92	*Serratia marcescens* strain RS8101 HQ123487
HMS6	*Enterobacter sp.*	KT750025	99	*Enterobacter sp.* UIWRF0482 KR190045
HMS7	*Agrobacterium tumefaciens*	KT750026	99	*Agrobacterium tumefaciens* strain R6-364 JQ659820
HMS8	*Agrobacterium sp.*	KT750027	99	*Agrobacterium sp.* HJX27 KP979558
HMS9	*Ensifer adhaerens*	KT750028	99	*Ensifer adhaerens* KT321683
HMS10	*Bacillus subtilis*	KT750029	100	*Bacillus subtilis* strain MSEB 67 KP261080
HMS11	*Bacillus subtilis*	KT750030	99	*Bacillus subtilis* strain JPS1-2 JQ308564
HMS12	*Bacillus malacitensis*	KT750031	99	*Bacillus malacitensis* strain F-61 KT027712

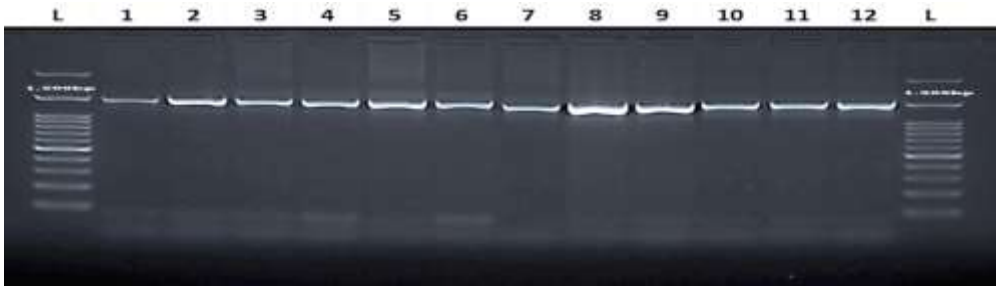

Fig (3): Electrophoresis patterns of 16S rDNA gene of endophytic bacteria. L: 100 bp Ladder marker and lanes 1 through 12 refer to endophytic isolates.

Figure (4): positive control showed no inhibition zone appear between isolate 2 toward *A. niger* (A), isolate 3 toward *R. solani* (B) and isolate 1 toward *M. phasolina* (C) and isolate 4 toward *F.* solani (D).

Table (4): Antagonistic activity of endophytic bacterial isolates against phytopathogenic fungi

Isolates	Phytopathogens				
	M. phasolenia	*R. solani*	*A. niger*	*F. solani*	*F. semitictum*
HMS1	-	-	-	-	-
HMS2	-	-	-	-	-
HMS3	-	-	-	-	-
HMS4	-	-	-	-	-
HMS5	-	-	-	-	-
HMS6	-	-	-	-	-
HMS7	-	-	-	-	-
HMS8	-	-	-	-	-
HMS9	-	-	-	-	-
HMS10	67.2	78.5	62.3	29.3	52.0
HMS11	66.8	74.3	67.4	23.6	49.2
HMS12	71.5	78.2	67.5	32.8	56.3
LSD(0.05)	1.56	1.28	1.05	1.72	1.68

*Values are the mean of 3 replicates; the formula for PIRG is as follows: PIRG (%) = [(R1 – R2)/R1] x 100 where, R1 is the farthest radial distance (measured in millimeters) grown by test fungus after 4 days of incubation in the direction of the antagonist (a control value), and R2 is the distance of fungal growth from the point of inoculation to the colony margin in the direction of the antagonist.

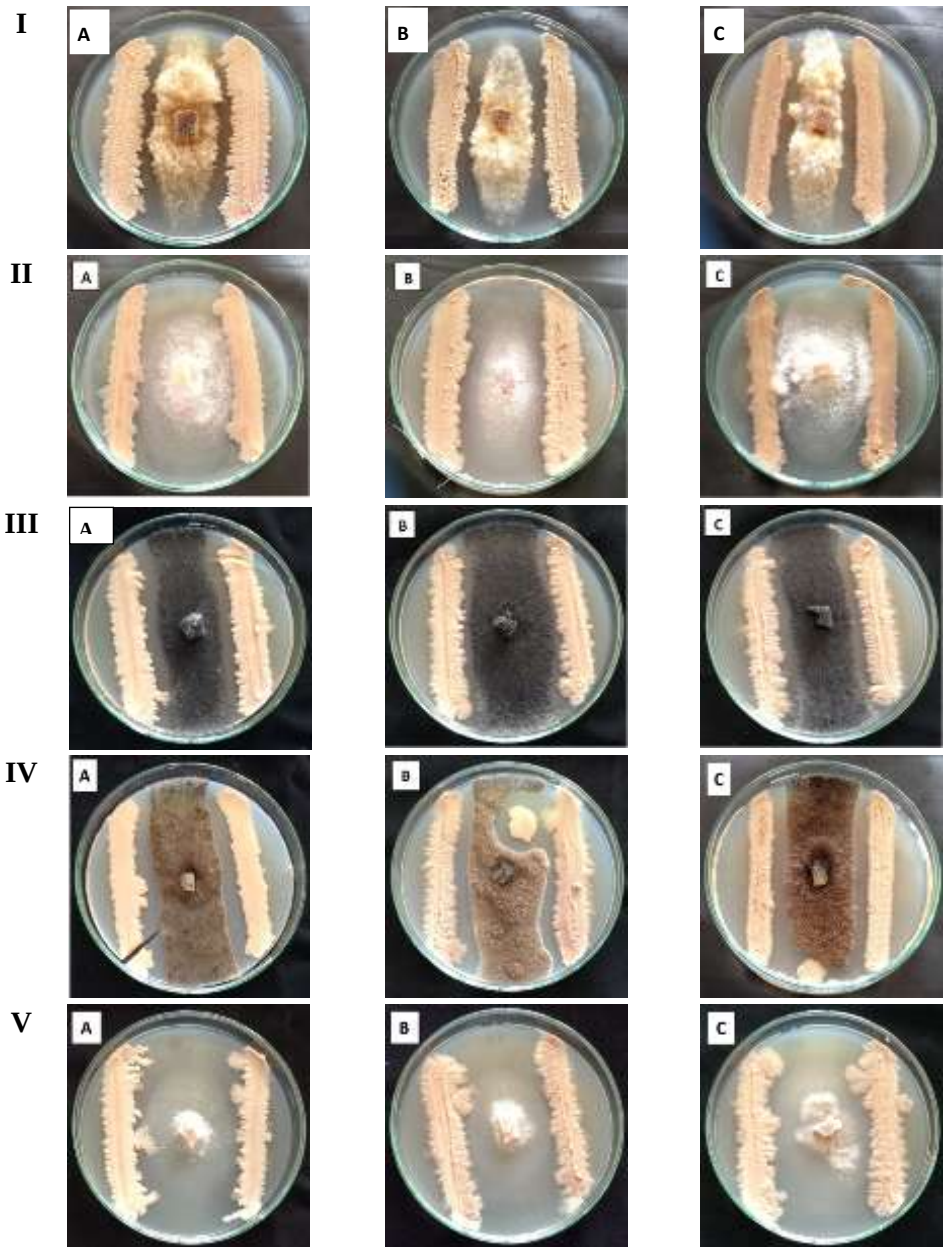

Fig. (5): Inhibition effect of endophytic bacterial isolates (A, 10; B, 11 and C, 12) against: (I) *R. solani*, (II) *F. solani*, (III) *M. phasolenia*, (IV) *A. niger and* (V) *F. semitictum*.

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التعريف الجزيئى والفسيولوجى لعزلات بكتيريا اندوفيتك الطماطم ونشاطها التضادى فى المعمل

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لقد تم اخذ أثنى عشر عزلة من بكتريا الاندوفيتك من الجذور والسيقان والاوراق المعقمة سطحيا لنباتات الطماطم التى جمعت من مزرعة الخضر بكلية الزراعة جامعة المنيا. ولقد تم تعريف هذه العزلات على اساس الخصائص المورفولوجية، الفسيولوجية و الكيماوية وكذلك تحليل تتابع جين 16s rDNA والتي اظهرت انهم ينتمون الى سبع اجناس بكتيرية (*Bacillus* ، *Agrobacterium*، *Rhizobium*، *Ensifer*، *Serratia*، *Panteao*، *Enterobacter*). وقد كشفت نتائج اختبار التضاد في المعمل ان ثلاث من اصل أثنتا عشرة عزلة من عزلات بكتيريا الاندوفيتك يمكن ان تقلل الى حد كبير نمو خمسة فطريات نباتية ممرضة ورئيسية هى (*Fusarium solani, Fusarium semitictum, Macrophomina phasolenia, Rhizoctonia solani* , *Aspergillus niger*) من خلال تكوين منطقة تثبيط لنمو ميسليوم الفطر ولقد تم تعريف هذه العزلات الثلاثة على انها (*Bacillus subtilis HMS10* ، *Bacillus subtilis HMS11* ، *Bacillus malacitensis HMS12*).