MOLECULAR IDENTIFICATION AND PLANT GROWTH PROMOTING ACTIVITIES OF ENDOPHYTIC *PANTOEA* SP. ISOLATED FROM *Zygophyllum album* MEDICINAL PLANT

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Plants are associated with a diverse community of microorganisms. The microorganisms residing within the plants or endophytes are unique in their adaptations to the environment of host plants. Endophytic bacteria have been isolated from a large diversity of plants as reviewed by Rosenblueth and Martínez-Romero (2006). Endophytic bacteria are found in roots, stems, leaves, seeds, fruits and tubers (Sturz *et al*., 2000). In most plants, roots have the higher numbers of endophytes compared with above-ground tissues (Rosenblueth and Martinez-Romero, 2004). Recent estimates suggest that the earth planet contains about 300,000 species of plants, the vast majority of which contain endophytes (Smith *et al*., 2008). Based on observations of the distribution of plant growth promoting endophytes in nature, it was found that a plant without endophytes would be less able to cope with pathogens and more susceptible to environmental stress conditions (Timmusk *et al*., 2011). More than 200 genera from 16 phyla of bacterial species have been reported to be associated with endophytes (Golinska *et al*., 2015). Most of these species belong to the phyla *Actinobacteria*, *Proteobacteria* and *Firmicutes* (Golinska *et al*., 2015). The diversity of endophytic bacteria ranges from gram-positive to gram-negative bacteria, such as *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Bacillus*, *Burkholderia, Enterobacter, Erwinia, Flavobacterium, Klebsiella, Pseudomonas*, *Rhizobium Serratia* and *Xanthomonas* (Sun *et al*., 2013; Santoyo *et al*., 2016).

Since endophytes start their journey as rhizosphere bacteria, it is assumable that they may retain their attributes inside plant. It seems that, their mechanisms of benefit are related to rhizosphere bacteria because most of endophytes can be cultured and can survive outside host in rhizosphere (Yadav and Yadav, 2017). However, endophytic bacteria may have an advantage over bacteria inhabiting the rhizosphere, since living within a plant's tissues represents an opportunity to always be in ¨contact¨ with the plant's cells and therefore, to more readily exert a direct beneficial effect (Santoyo *et al*., 2016). Endophytes can stimulate plant growth by providing antimicrobial metabolites (Pinheiro *et al*., 2013), phosphate solubilizing compounds (Li *et al*., 2016) and nitrogen fixing abilities (Knoth *et al*., 2014). In addition, endophytes influence plant growth through production of phytohormones (Khan *et al*., 2014) and siderophores (Devi *et al*., 2016).

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Zygophyllaceae is a widespread family of approximately 27 genera and 285 species; it consists of trees, shrubs and herbs, mostly restricted to arid and semi-arid areas in the tropics and subtropics regions (Beier, 2003). *Zygophyllum* is the largest genus of the family with about 80 species. *Z. album* L. and *Z. simplex* L. are two of nine species which are widespread in the deserts and salt marshes in Egypt and Sinai Peninsula (Hussein *et al*., 2011). *Z. album* L is an important medicinal plant largely used in traditional medicine (Mnafgui *et al*., 2016). Although medicinal plants provide an enormous bioresource of potential use in modern medicine and agriculture, yet their microbiome is largely unknown (Köberl *et al*., 2013). Thus, isolation and characterization of endophytes with diverse properties from unexplored sources will have much applications to manipulate plant growth promotion (Patten and Glick, 2002; Sergeeva *et al*., 2007). Therefore, the objectives of this study were isolation and identification of endophytic bacteria from *Z. album*, *in-vitro* screening of the isolated endophytes for their plant growth promoting properties, and evaluation of their effects on plant growth of wheat plants under greenhouse conditions.

MATERIALS AND METHODS

Plant sampling and isolation of endophytic bacteria

Zygophyllum album plants were collected from the Eastern Desert in Upper Egypt (26° 17’ 53.8” N; 32° 46’ 25.0” E) and were kept with the attached soil rhizosphere at 4°C. Endophytic bacteria were isolated as previously described by Kuan *et al*. (2016). Briefly, healthy roots were surface-sterilized with 70% ethanol for 5 min, followed by 1% sodium hypochlorite for 2 min and then washed five times with sterilized distilled water. The last washing water was plated onto Luria-Bertani (LB) agar and nutrient agar plates to check the sterilization efficiency and aseptically smashed with mortar and pestle to isolate the endophytic bacteria on Burk’s N-free medium (Wilson and Knight, 1952).

Molecular identification of endophytic bacteria

Genomic DNA of bacterial cells was isolated and purified using GeneJet™ Genomic DNA purification Kit (Thermo Scientific, MA, USA). The procedures were carried out according to manufacture instructions. Two primers, 27f-CM (5’-AGAGTTTGATCMTGGCTCAG-3’), and 1492r primer (5’-TACCTTGTTACGACTT-3’) (Frank *et al*., 2008) were used to amplify a near-full length, approximately 1500 bp fragment of 16S rRNA. PCR was performed using the standard reaction mixture (25 µl) containing: 1× PCR buffer, 1.5 mM $MgCl_2$, 5% dimethyl sulfoxide, 200 mM of each dNTPs, 15 pmol of each primer, 1 U of Taq polymerase enzyme (Promega Corporation, WI, USA) and 50 ng of DNA template. PCR reaction conditions were: an initial denaturation at 95°C for 3 min, 35 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 45 second, and

extension at 72°C for 1.5 minute. PCR products were separated on 1% agarose gel and stained by using ethidium bromide for visualization of DNA fragments. The 1500 bp 16S rDNA fragments were purified using QIAquick PCR purification kit (Qiagen, NRW, Germany) according to the manufacturer's instructions and sequenced using primers 27f-CM and 1492r (Frank *et al*., 2008) at Macrogen Inc., South Korea. Sequence reads were edited and assembled using the DNASTAR software (Lasergene, WI, USA). Sequence similarity searches were performed at the National Center for Biotechnology Information (NCBI) server using BLASTN (http://www.ncbi.nlm.nih.gov/blast).

The sequences were aligned using Clustal W version 1.8 (Altschul *et al*., 1997) and subjected to phylogenetic analysis. 16S rRNA phylogenetic tree was constructed using the Neighbor-Joining (NJ) method (Saitou and Nei, 1987) in MEGA software version 7 (Kumar *et al*., 2016) and 1000 bootstrap replication to assess branching confidence. The evolutionary distances were computed using the p-distance method (Kimura, 1980).

Biolog identification and metabolic profiling

Biochemical identification for isolates NGB-W48 and NGB-W53, including the carbohydrate fermentation patterns and chemical sensitivity tests were determined using the GEN III Biolog bacterial identification system kit (Biolog, CA, USA), following the manufacturer's instructions (www.biolog.com). The GEN III MicroPlate™ test panel provides a "Phenotypic Fingerprint" of microorganisms which can then be used to identify them to the species level. The test panel contains 71 carbon sources and 23 chemical sensitivity assays. Each strain was assessed on duplicate Biolog GEN III plates to confirm the results. Microplates were analyzed at 24 and 48 hr. on a Biolog microstation plate reader using MicroLog3 software.

In-vitro screening of plant growth promoting properties

Phosphate solubilization

Bacterial isolates were screened for solubilization of tricalcium phosphate quantitatively in a liquid medium as described by King (1932). Briefly, bacterial isolates were inoculated in a 25 mL Pikovskaya's broth medium and incubated for 96 hr. at 28°C. Bacterial cultures were centrifuged at 15,000 rpm for 30 min. The supernatant (1 mL) was mixed with 10 mL of chloromolibidic acid. Cholorostannous acid (0.25 mL) was added and the volume was completed to 50 mL with distilled water. The absorbance of the developing blue color was read at 600 nm. The amount of solubilized phosphate was detected from the standard curve of a pure substance of KH_2PO_4 (Sigma-Aldrich, MO, USA).

Indole acetic acid (IAA) production

For detection and quantification of IAA production, bacterial colonies were

inoculated into LB medium containing 0.5 mg $_{L}$-tryptophan/mL according to Rahman *et al*. (2010). Approximately 2 mL of culture was centrifuged at 15,000 rpm for 10 min, and a 1 mL aliquot of the supernatant was mixed with 2 mL of Salkowski's reagent as described by Gordon and Weber, (1951). IAA production was observed as the development of a pink-red color, and the absorbance was measured at 530 nm using a spectrophotometer (Thermo Scientific, MA, USA). The concentration of IAA was determined using a standard curve prepared from pure IAA (Sigma-Aldrich, MO, USA) solutions.

Siderophore production

Bacterial isolates were assayed for siderophores production quantitatively using Chrome azurol S (CAS)-liquid assay, in which 0.1 ml of culture supernatant was mixed with 0.1 ml of CAS reagent (Payne, 1994). Absorbance was measured at 630 nm against a reference consisting of 0.1 ml of uninoculated broth and 0.1 ml of CAS reagent. Siderophore content in the aliquot was calculated by using the following formula:

$$\% \text{ siderophore units} = Ar - As/Ar \times 100$$

where, Ar = absorbance of the reference at 630 nm (CAS reagent) and As = absorbance of sample at 630 nm.

Effect of endophytic inoculation on wheat growth

A pot experiment was conducted in the greenhouse of National Gene Bank, Agricultural Research Center, Giza to evaluate the effects of single and co-inoculations with isolates NGB-W48 and NGB-W53 on different growth parameters of wheat plants. Seeds of wheat (Misr 1) were surface sterilized as described by Turan *et al.* (2012). Each pot (13 cm diameter) was filled with 2 kg of autoclaved sandy soil. The soil was analyzed according to Page *et al*. (1982). The main physical and chemical properties of the soil used are presented in Table (1). All treatments irrigated with sterilized tab water and received the recommended dose of super phosphate (15.5% P_2O_5) and potassium sulfate (48.5% K_2O) at the rate of 0.4 g/pot and 0.2 g/pot, respectively. All pots received half of the recommended N dose of ammonium sulfate (20.5%N) at a rate of 0.6 g/pot (60 KgN/ feddan), as recommended by Ministry of Agriculture and Land Reclamation, Egypt (www.caae-eg.com). Pots were arranged in a complete randomized block design with three replicates. For inoculation treatments, seeds were soaked for 2 hr. in the bacterial suspension of 10^8-10^9 cells/mL. Plants were grown under controlled conditions: at 24°C for 12 hr. (light) and 12°C for 12 hr (dark). After 45 days, plants were uprooted and shoot height, root length, shoot N-content as well as shoot, root fresh and dry weights were recorded. Data were subjected to analysis of variance using MSTAT analysis software (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Currently, a considerable worldwide research has focused on the explora-

tion of varied agro-ecological niches for the existence of native beneficial microorganisms (El-Sayed *et al*., 2014). Medicinal plants growing in severe environments, such as deserts, are unique niche where plant associated microbiome induced their own mechanisms, permitting the plant to tolerate stress conditions (Rout, 2014).

Molecular identification of endophytic bacteria

Two endophytic bacteria (NGB-W48 and NGB-W53) were isolated and purified from the roots of *Z. album* plant using N-free medium. Nearly full length of 16S rRNA sequences (1402-1406 bp) was obtained from the two isolates. Based on the 16S rRNA sequence homology, isolates NGB-W48 and NGB-W53 were identified as *Pantoea* sp. with high similarity of 99% to *P. agglomerans* (Table 2). The nucleotide sequences of *Pantoea* sp. strains NGB-W48 and NGB-W53 have been deposited in the GenBank/EMBL/DDBJ under the accession number LC379256 and LC379257, respectively. The NJ-phylogenetic tree based on 16S rRNA sequences (Fig. 1) showed that, strains NGB-W48 and NGB-W53 were clearly grouped with the endophytic bacterium *P. agglomerans* strain Mz23 (Access. No. MF289156.) which was isolated from maize plants, supported by high bootstrapping value (93%). Also, they formed a sister monophyletic lineage with *P. septica* strain IHBB 1545 (Access. No. KF475883) which was isolated from the roots of tea plants and *P. septica* type strain LMG 5345^{T} [Access No. NR_116752, (Rezzonico *et al*., 2009)], supported by 83% bootstrapping value.

Members of the genus *Pantoea* have been isolated from various environmental habitats such as soils, water, foods, plants, humans and other animals (Walterson and Stavrinides, 2015). Some *Pantoea* species are known to interact with plants and may confer beneficial or deleterious effects to their hosts (Dutkiewicz *et al*., 2016a,b). For example, some isolates are antibiotic producers, and have been developed into commercial biocontrol products for the management of plant diseases (Lim *et al*., 2014; Mikiciński *et al*., 2016). Other isolates possess nitrogen fixation and plant growth-promoting capabilities, which are currently being explored for agricultural applications (Dastager *et al*., 2009; Rodrigues *et al*., 2016). In accordance with our results, a non-pathogenic rhizobacterium *P. agglomerans* isolated from Teosinte (maize ancestor) induces salt tolerance by triggering expression of salt resistance genes in modern maize (Gond *et al*., 2015). Similarly, Panwar *et al*. (2016) isolated *P. dispersa* strain PSB3 from the mungbean rhizosphere, which possessed different levels of plant growth promoting traits and could effectively increase the growth of mungbean plants under salt stress conditions. Recently, *P. alhagi* was described as a novel endophytic bacterium species that was isolated from the surface-sterilized leaves of *Alhagi sparsifolia* Shap, and effectively

promoted the growth of wheat and enhanced its resistance to drought stress (Chen *et al*., 2017).

Biolog identification and biochemical characterization

Although there is no identification has been provided by Biolog analysis, but the top-ranked results support the identification of strains NGB-W48 and NGB-W53 as *P. agglomerans* (Table 2). Though Biolog system was initially introduced for bacterial identification, but currently it is mostly used in assessing the metabolic diversity of isolates than in purpose of identification. The characteristics of biochemical reactions obtained from GEN III plates for strains NGB-W48 and NGB-W53 are described in Table (3). The two strains had very similar phenotypic profiles and could use the same tested substrates, except for N-acetyl-β-D-mannosamine (sugar substrate) and L-pyroglutamic acid (amino acid substrate), which have been used only in case of strain NGB-W53. Interestingly, strains NGB-W48 and NGB-W53 had two sugar substrate mismatches (D-salicin and D-sorbitol) compared to the Biolog reference database for the most closely matched species.

Plant growth promoting potential

In-vitro plant growth promotion traits of *Pantoea* sp. strains NGB-W48 and NGB-W53 are described in Table (4). The endophytic strains NGB-W48 and NGB-W53 possessed many plant growth promoting activities, including the ability of IAA production, siderophore production and inorganic phosphate solubilization. Production of IAA by endophytic bacteria plays a crucial role in plant growth and development (Khan *et al*., 2016). There are various types of bacteria which are documented for the production of IAA, exploit different IAA biosynthesis pathways and a single bacterial strain sometimes encompassing more than one pathway (Duca *et al*., 2014). Earlier, Dastager *et al*. (2009) reported the production of IAA by *Pantoea* sp strain NII-186 isolated from soil rhizosphere of Western Ghat in India, which produced 59 µg/mL in presence of $_{L}$-tryptophan (100 µg/mL), the main precursor for IAA synthesis. A similar level of IAA (69.4 µg/mL) in culture medium supplemented with 1000 µg/mL of $_{L}$-tryptophan, was produced by *Pantoea* sp. strain KRZ5 that was isolated from the rhizosphere of sugarcane (Rodrigues *et al*., 2016). Our results showed that, strains NGB-W48 and NGB-W53 recorded high levels of IAA (164 and 127 µg/mL, respectively) with the supplement of 500 µg/mL of $_{L}$-tryptophan; suggesting the ability of these endophytes to stimulate root development and promote plant growth. Data analysis showed that increased IAA concentration significantly accomplished with increased tryptophan levels. Interestingly, the two strains possessed the capability to produce IAA in the absence of $_{L}$-tryptophan (76.6 and 65.2 µg/mL, respectively), indicating the potentiality of these strains to have $_{L}$-tryptophan independent pathway for auxin synthesis. Siderophores also play an essential role in the microbial interactions,

enhancing the growth of plants and yield of agricultural crops in iron-limiting conditions. Our results showed that, strains NGB-W48 and NGB-W53 produced 42.3 and 34.2% siderophore unit, respectively. In consistent with our data, identification of many siderophore- producing strains belonging to the genus *Pantoea* has been previously reported (Dastager *et al*., 2009; Panwar *et al*., 2016). Phosphate (P) is a major essential macronutrient for biological growth and development however, a large portion of inorganic phosphates become immobilized after application as fertilizer, resulting in the inaccessibility of the phosphate to plants. Therefore, the capability of some bacterial species to solubilize insoluble or insufficiently soluble mineral phosphates is very important and are considered as a promising tool for biofertilizers application (Alori *et al*., 2017). This study indicated that, the amount of solubilized phosphate by strains NGB-W48 and NGB- 53 (187 and 190 µg/mL, respectively) after 96 hr. of incubation, was dramatically higher than those presented in other reports. For instance, Dastager *et al*. (2009) found that, the phosphate solubilizing ability of *Pantoea* sp. strain NII-186 was increased with prolonged incubation and it solubilized 80 µg/mL of tricalcium phosphate up to 96 hr. Bacterial strains from the genus *Pantoea* are generally regarded as good phosphate solubilizers (Shariati *et al*., 2017). For example, *P. agglomerans* strain P5 is one of the important strains that hydrolyze inorganic and organic phosphate compounds effectively by the conversion of insoluble forms of phosphate into soluble forms (Malboobi *et al*., 2009) and it is commercially produced as a phosphate biofertilizer named PhosphoBarvar®-2.

Plant growth promotion in wheat

Plant growth promotion in response to plant growth promoting endophytes has been frequently reviewed for different crops under different agro-climatic conditions (Rosenblueth and Martínez-Romero, 2006; Kandel *et al*., 2017). Inoculation of strains NGB-W48 and NGB-W53 along with 50% reduced N-fertilizer dose (60 kg N/feddan), were tested against the un-inoculated control to evaluate their plant growth promoting potential in wheat (Table 5 and Fig. 2). All inoculated wheat plants showed significant increases in respect to different estimated growth parameters over the uninoculated control. Co-inoculation of NGB-W48 with NGB-W53 strains generally enhanced the plant growth and shoot N-content compared to single strain inoculation. All inoculated plants had significant increases (ANOVA, $p<0.05$) in the shoot length ranged from 43.8- 45.4 cm/plant, compared to the uninoculated control (31.4 cm/plant). The highest root length was observed for the co-inoculated plants (16.2 cm/plant) followed by singly NGB-W53 inoculated ones (15.5 cm/plant) with significant increases ($p<0.05$) over the uninoculated control (12.1 cm/plant). Similarly, the co-inoculated or singly-inoculated treatments dramatically increased shoot fresh weight (2.91-3.22 g/plant) and dry weight (0.68-0.80 g/plant) compared to the

uninoculated control which gave 1.58 g/plant and 0.38 g/plant, respectively. Similarly, significantly higher root fresh weight (0.85-0.91 g/plant) and dry weight (0.26-0.28 g/plant) were recorded in all inoculated plants relative to the uninoculated control which gave 0.39 g/plant and 0.13 g/plant, respectively. In terms of shoot N-uptake, inoculated treatments yielded shoot N- contents (13.1-15.1 mg/plant) which were significantly higher than the uninoculated control (4.1 mg/plant). Although there was no significant difference between singly-inoculated or co-inoculated treatments, but the maximum shoot N-content was estimated in the co-inoculated plants. These results are in agreement with previous findings published by other workers who showed that, endophytic microorganisms associated with medicinal plants have the potential to improve plant growth and biomass yield (Devi *et al*., 2016; Li *et al*., 2016). For instance, the positive effect on the growth of wheat plants was observed after inoculation with *Paenibacillus* strain 130 that was isolated from medicinal plant *Lonicera japonica* (Zhao *et al*., 2015). Recently, Hassan (2017) found that, the growth promotion of maize plants after co-inoculation with *Bacillus cereus* and *Bacillus subtilis* strains that were also isolated from *Teucrium polium* L. medicinal plant, collected from Sinai Peninsula in Egypt. Our results are also in consistent with other numerous reports described the potentiality of many species of the genus *Pantoea* to promote the growth of many crops under greenhouse conditions or field trials including mungbean (Panwar *et al*., 2016), maize (Rodrigues *et al*., 2016), potato (Malboobi *et al*., 2009), sugarcane (Quecine *et al*., 2012) and wheat (Chen *et al*., 2017).

SUMMARY

The present study revealed that *Z. album* medicinal plant which naturally inhabitant arid conditions, is an ecological niche for promising growth promoting bacterial endophytes (*Pantoea* sp. strains NGB-W48 and NGB-W53). These endophytes displayed multiple plant growth promoting traits (phosphate solubilization, IAA and siderophore production), without symptomatic injury. Inoculation of wheat plants with strains NGB-W48 and NGB-W53 along-with 50% reduced N- fertilizer dose, dramatically stimulated plant growth and increased the accumulated N in plant shoots compared to the uninoculated plants. This study indicates that bacterial endophytes isolated from medicinal plants possessing a vital role to improve plant growth and could be used as inoculants to enhance soil fertility and establish a sustainable crop production system.

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Table (1): Physical and chemical properties of sandy soil used in the plant inoculation assay.

Property	Value	Property	Value
Particle size distribution (%)		Soluble anions (meq/L)	
Sand	90.10	CO_3^{2}	0
Silt	3.90	HCO_3^{-}	0.88
Clay	6.00	Cl^{-}	0.59
Texture grade	Sandy	SO_4^{-2}	1.67
$CaCo_3$ (%)	1.61	Total N (%)	0.021
Saturation percent S.P (%)	21.50	Total Soluble-N (mg kg^{-1})	16.3
pH	7.82	Available-P (mg kg^{-1})	6.71
E.C. (dS m^{-1} at 25°C)	0.32	Available-K (mg kg^{-1})	52.1
		Organic matter (%)	0.23
Soluble cations (meq/L)		DTPA extractable (ppm)	
Ca^{+2}	0.54	Fe	1.62
Mg^{+2}	0.33	Mn	0.31
Na^{+}	1.62	Zn	0.42
K^{+}	0.65	Cu	0.18

Table (2): Bacterial identification using Biolog GEN III microplate™ and 16S rRNA sequence analysis.

Bacterial isolate	Biolog GEN III*				16S rRNA sequence analysis				
	Homology	PROB	SIM	DIST	Access No.	Seq. (bp)	Homology to the reference strains		Identity (%)
NGB-W48	*P. agglomerans*	-	0.624	5.138	LC379256	1,402	*P. agglomerans* Mz23	(MF289156)	99
NGB-W53	*P. agglomerans*	-	0.707	4.669	LC379257	1,406	*P. agglomerans* Mz23	(MF289156)	99

No ID call as a final result was provided and the displayed biologic results are the top-ranked ID choices. (Prob) probability, (Sim) similarity, (Dist) distance.

Table (3): Biochemical analysis of *Pantoea* sp. strains NGB-W48 and NGB-W53 using Biolog GEN III microplate™.

Property	Strain NGB-W48	Strain NGB-W53	Property	Strain NGB-W48	Strain NGB-W53
Dextrin	+	+	L-Galactonic Acid Lactone	+	+
D-Maltose	+	+	D-Gluconic Acid	+	+
D-Trehalose	+	+	D-Glucuronic Acid	+	+
D-Cellobiose	+	+	Glucuronamide	+	+
Gentiobiose	-	-	Mucic Acid	+	+
Sucrose	+	+	Quinic Acid	+	+
D-Turanose	-	-	D-Saccharic Acid	+	+
Stachyose	-	-	p-Hydroxy-Phenylacetic Acid	-	-
D-Raffinose	-	-	Methyl Pyruvate	+	+
α-D-Lactose	-	-	D-Lactic Acid Methyl Ester	-	-
D-Melibiose	-	-	L-Lactic Acid	+	+
β-Methyl-D-Glucoside	+	+	Citric Acid	-	-
D-Salicin*	-	-	α-Keto-Glutaric Acid	-	-
N-Acetyl-D-Glucosamine	+	+	D-Malic Acid	+	+
N-Acetyl-β-D-Mannosamine	-	+	L-Malic Acid	+	+
N-Acetyl-D-Galactosamine	-	-	Bromo-Succinic Acid	+	+
N-Acetyl Neuraminic acid	-	-	Tween 40	+	+
α-D-Glucose	+	+	γ-Amino-Butryric Acid	+	+
D-Mannose	+	+	α-Hydroxy-Butryric Acid	-	-
D-Fructose	+	+	β-Hydroxy-D, L-Butryric Acid	-	-
D-Galactose	+	+	α-Keto-Butryric Acid	-	-
3-Methyl Glucose	+	+	Acetoacetic Acid	+	+
D-Fucose	+	+	Propionic Acid	-	-
L-Fucose	+	+	Acetic Acid	+	+
L-Rhamnose	+	+	Formic Acid	+	+
Inosine	+	+	pH 5	+	+
D-Sorbitol**	+	+	pH 6	+	+
D-Mannitol	+	+	4% NaCl	+	+
D-Arabitol	+	+	8% NaCl	-	-
myo-Inositol	+	+	1% Sodium Lactate	+	+
Glycerol	+	+	Fusidic Acid	+	+
D-Glucose- 6-PO_4	+	+	Troleandomycin	+	+
D-Fructose-6-PO_4	+	+	Rifamycin SV	+	+
D-Aspartic Acid	-	-	Minocycline	-	-
D-Serine	-	-	Lincomycin	+	+
Gelatin	-	-	Guanidine HCl	+	+
Glycyl-L-Proline	+	+	Niaproof 4	+	+
L-Alanine	+	+	Vancomycin	+	+
L-Arginine	+	+	Tetrazolium Violet	+	+

Table (3): Cont'

L-Aspartic Acid	+	+	Tetrazolium Blue	+	+
L-Glutamic Acid	+	+	Nalidixic Acid	-	-
L-Histidine	+	+	Lithium Chloride	+	+
L-Pyroglutamic Acid	-	+	Potassium Tellurite	-	-
L-Serine	+	+	Aztreonam	+	+
Pectin	+	+	Sodium Butyrate	-	-
D-Galacturonic Acid	+	+	Sodium Bromate	-	-

* The test result was negative for strains NGB-W48 and NGB-53, while the Biolog reference database record was positive for the same test.

** The test result was positive for strains NGB-W48 and NGB-53, while the Biolog reference database record was negative for the same test.

Table (4): *In vitro* plant growth promotion characteristics of *Pantoea* sp. strains NGB-W48 and NGB-W53.

Strain	P- solubilization (µg mL^{-1})	Siderophore production (%)	IAA production (µg mL^{-1})	
			without tryptophan	with tryptophan
NGB-W48	187 ± 4.7	42.3 ±1.08	76.6 ± 3.1	164 ± 5.1
NGB-W53	190 ± 3.8	34.2 ±0.61	65.2 ± 2.7	127 ± 4.5

(P) Phosphate, (IAA) indole acetic acid. IAA was tested with (500 µg tryptophan /mL) and without the addition of precursor. Data are average values of three replicates, (±) standard deviation (SD)

Table (5): Effect of *Pantoea* sp. strains NGB-W48 and NGB-W53 on different growth parameters of wheat plants under greenhouse conditions.

Treatment	Shoot height cm/plant	Root length cm/plant	Fresh weight (gm/plant)		Dry weight (gm/plant)		Shoot N-content (mg/plant)
			Shoot	Root	Shoot	Root	
NGB-W48	45.4^{a}	$14.3^{a,b}$	$3.12^{a,b}$	0.85^{a}	$0.76^{a,b}$	0.26^{a}	13.6^{a}
NGB-W53	43.8^{a}	15.5^{a}	2.91^{b}	0.91^{a}	0.68^{b}	0.28^{a}	13.1^{a}
Co-inoculation	44.7^{a}	16.2^{a}	3.22^{a}	0.86^{a}	0.80^{a}	0.28^{a}	15.1^{a}
Control	31.4^{b}	12.1^{b}	1.58^{c}	0.39^{b}	0.38^{c}	0.13^{b}	4.1^{b}

Means followed by the same letter are not significantly different at 5% level.

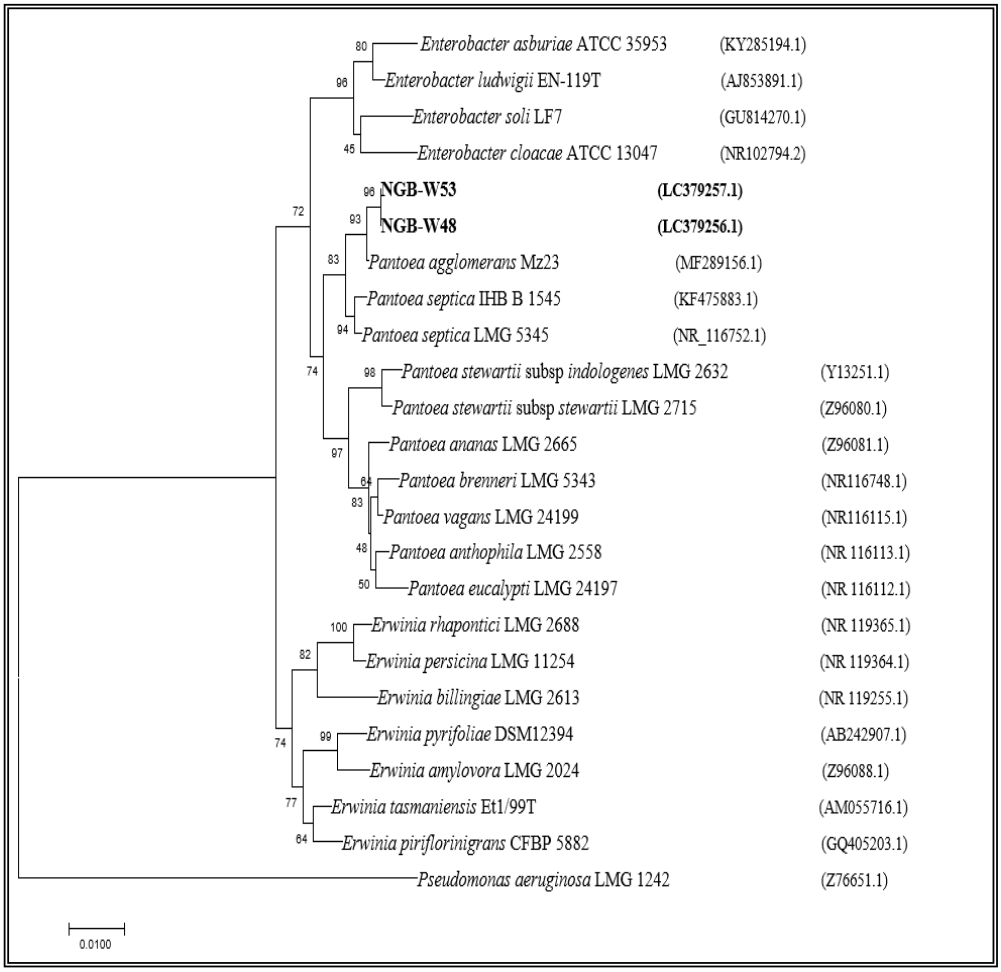

Fig. (1): NJ phylogenetic tree based on the 16S rRNA sequences showing the relationships between strains NGB-W48 and NGB-W53 and GenBank reference strains using the p-distance model. Bootstrap values (using 1000 replicates) are indicated at the branching points.

Fig. (2): Response of wheat plants growth to single and co-inoculation of *Pantoea sp*. strains NGB-W48 and NGB-W53 under greenhouse conditions. Plant treatments from left to right in the photograph as the following: control treatment (uninoculated), co-inoculated plants *of strain NGB-W48* with *NGB-W53*, singly inoculated plants with strain NGB-W48, and strain NGB-W53, respectively. Bar= 10 cm.