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Bacterial Endophyte *Sphingomonas* sp. LK11 Produces Gibberellins and IAA and Promotes Tomato Plant Growth

Abdul Latif Khan[1], Muhammad Waqas[2], Sang-Mo Kang[2], Ahmed Al-Harrasi[1]*, Javid Hussain[1], Ahmed Al-Rawahi[1], Salima Al-Khiziri[1], Ihsan Ullah[2], Liaqat Ali[1], Hee-Young Jung[2], and In-Jung Lee[2]*

[1]*Department of Biological Sciences and Chemistry, University of Nizwa, Nizwa Oman*
[2]*School of Applied Biosciences, Kyungpook National University, Daegu 702-701, Republic of Korea*

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Plant growth promoting endophytic bacteria have been identified as potential growth regulators of crops. Endophytic bacterium, *Sphingomonas* sp. LK11, was isolated from the leaves of *Tephrosia apollinea*. The pure culture of *Sphingomonas* sp. LK11 was subjected to advance chromatographic and spectroscopic techniques to extract and isolate gibberellins (GAs). Deuterated standards of [17, 17-$^2H_2$]-GA_4, [17, 17-$^2H_2$]-GA_9 and [17, 17-$^2H_2$]-GA_{20} were used to quantify the bacterial GAs. The analysis of the culture broth of *Sphingomonas* sp. LK11 revealed the existence of physiologically active gibberellins (GA_4: 2.97 ± 0.11 ng/ml) and inactive GA_9 (0.98 ± 0.15 ng/ml) and GA_{20} (2.41 ± 0.23). The endophyte also produced indole acetic acid (11.23 ± 0.93 µM/ml). Tomato plants inoculated with endophytic *Sphingomonas* sp. LK11 showed significantly increased growth attributes (shoot length, chlorophyll contents, shoot, and root dry weights) compared to the control. This indicated that such phyto-hormones-producing strains could help in increasing crop growth.

Keywords: *Solanum lycopersicum*, *Sphingomonas* sp. LK11, endophytism, gibberellins, indole acetic acid

Introduction

Endophytes, which live within host plant tissues without causing diseases, are prominent sources of bioactive secondary metabolites. Various classes of chemical constituents, including flavonoids, peptides, alkaloids, steroids, phenolics, terpenoids, lignans, and volatiles (Brader *et al*., 2014) have been reported. A previous study by Schulz and Boyle (2005) showed that 51% of biologically active metabolites originate from endophytes as compared to only 38% of novel substances originating from other soil microflora. Approximately 20,000 biologically active compounds have been reported which influence the performance and survival of other organisms (Brader *et al*., 2014). In the past two decades, many novel bioactive compounds with antimicrobial, insecticidal, cytotoxic, and anticancer properties have been successfully isolated and characterized from endophytic fungi (Strobel *et al*., 2004; Aly *et al*., 2010; Supaphon *et al*., 2013). However, endophytic bacteria have only been recently acknowledged in this regard, and their niche is poorly understood (Zin *et al*., 2007; Ryan *et al*., 2008; Bascom-Slack *et al*., 2009; Qin *et al*., 2011; Bhore *et al*., 2013; Christina *et al*., 2013; Brader *et al*., 2014).

In addition to being a reservoir of bioactive secondary metabolites, endophytic fungi have recently been known to produce plant growth regulators (Redman *et al*., 2011; Higginbotham *et al*., 2013). Such regulators not only increase plant growth and development but also improve plant health by increasing the tolerance against diverse array of environmental stresses (Higginbotham *et al*., 2013; Hilbert *et al*., 2013; Jasim *et al*., 2013). Plant growth regulators such as indole 3-acetic acid (IAA) and gibberellins (GAs) can stimulate rapid responses of cell elongation, cell division, and differentiation in plants (Redman *et al*., 2011; Davičre and Achard, 2013). Some of the strains of rhizobacteria, viz., *Rhizobium phaseoli* (Atzhorn *et al*., 1998), *Acetobacter diazotrophicus*, and *Herbaspirillum seropedicae* (Bastian *et al*., 1998), *Bacillus pumilus* and *B. licheniformis* (Gutierrez-Manero *et al*., 2001), *B. cereus*, *B. macroides*, and *B. pumilus* (Joo *et al*., 2004), *Azotobacter chroococcum* SE370 (Verma *et al*., 2001), and *Burkholderia cepacia* SE4 (Kang *et al*., 2014), have been known to produce GAs. Some strains of bacteria also produce IAA, which can also extend growth-promoting effects during symbiosis (Barazani and Friedman, 1999; Verma *et al*., 2001; Lee *et al*., 2004). Genera such as *Bacillus*, *Microbacterium*, *Methylophaga*, *Agromyces*, and *Paenibacillus* have been found to produce IAA (Lata *et al*., 2006; Khan and Doty, 2009; Hussain and Hasnain, 2011; Bal *et al*., 2013; Naveed *et al*., 2013; Nagata *et al*., 2014; Weyens *et al*., 2014). Some endophytic fungi have also been recently reported to secrete GAs and IAA in their pure culture (Redman *et al*., 2011; Ansari *et al*., 2013). However, endophytic bacteria associated with plants have not been thoroughly studied, especially for gibberellin production. In present study, it was aimed to assess the potential of *Sphingomonas* sp. LK11 for production of GAs and IAA and effects on tomato (*Solanum lycopersicum*) plants.

*For correspondence. (I.J. Lee) E-mail: ijlee@knu.ac.kr / (A. Al-Harrasi) E-mail: aharrasi@unizwa.edu.om

Material and Methods

Bacterial endophyte isolation and cultivation

The leaves of *Tephrosia apollinea* (Papilionaceae) plants, growing in the wild mountains of Jabal Al-Akhdar (23°04′ 22.00″N; 57°40′07.00″E), Sultanate of Oman, were collected. *T. apollinea* (Del.) is a perennial shrublet distributed in Africa. It is abundant in Egypt's Nile Valley, along the coast of the Red Sea and in all Egyptian deserts and eastern Oman. *T. apollinea* is used to make indigo dyes, however, its shoot/leaves are toxic to goats and sheep because of the presence of semiglabrin, semiglabrinol, and apollineanin. This plant grows in arid areas with extreme water deficient conditions (Ψ= - 2.21 hPa).

A total of 32 leaf samples from 15 plants were randomly collected and stored for a short period in polythene zip-bags for transportation purpose to cold storage. The samples were thoroughly washed in running tap water in the laboratory. Endophytic microbes were isolated according to the method described by Jasim *et al.* (2013). All 32 leaf samples were then sliced (average size, 2.0 mm), surface sterilized with 2.5% sodium hypochlorite (30 min in a shaking incubator at 120 rpm), and washed with autoclaved distilled water (DW) to wash away the contaminants and surface microflora. The surface sterilized leaves were carefully spread on petri-plates containing Hagem media (0.5% glucose, 0.05% KH_2PO_4, 0.05% $MgSO_4{\cdot}7H_2O$, 0.05% NH_4Cl, 0.1% $FeCl_3$, and 1.5% agar; pH 5.6 ± 0.2) supplemented with 80 ppm fungicide to suppress fungal endophytes (Sheng *et al.*, 2008). The sterilized leaves were also imprinted on separate Hagem plates to ensure the effectiveness of surface sterilization (Jasim *et al.*, 2013). Newly emerged bacterial spots or layers from the leaves were isolated and grown on nutrient agar (NA) medium under sterile conditions.

The isolated strains were cultured in 50 ml nutrient broth [NB composition gm/L, peptic digest of animal tissue 5.00, sodium chloride 5.00, beef extract 1.50, yeast extract 1.50, final pH (at 25°C), 7.4 ± 0.2] with or without tryptophan and incubated at 28°C for five days in a shaking incubator at 200 rpm. The supernatant and the cell pellets were partitioned by centrifugation at 2,500×*g* at 4°C for 15 min and the supernatant was filtered through 0.45-µm filter papers.

Endophyte identification by PCR

On the basis of initial phytohormonal screening, bacterial isolate LK11 was identified on the basis of partial 16S ribosomal RNA sequence through standard procedures (Sambrook and Russel, 2001). The 16S rRNA was amplified by PCR using the 27F primer (5′-AGAGTTTGATC (AC) TGGCT CAG-3′) and 1492R primer (5′-CGG(CT)TACCTTGTTA CGACTT-3′), which complemented the 5′ end and 3′ end of the prokaryotic 16S rRNA, respectively. The amplification reaction was performed as described previously (Adachi *et al.*, 1996).

The BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST) was used to compare the sequence homology of nucleotides. The closely related sequences obtained were aligned through CLUSTALW using MEGA version 4 software, and the maximum parsimony tree was constructed using the same software. The bootstrap replications (1K) were used as a statistical support for the nodes in the phylogenetic tree.

Quantification of gibberellins

To characterize GAs secreted in the culture filtrate (CF) of *Sphingomonas* sp. LK11, it was cultivated in NB for 5 days at 28°C (200 rpm) in 500-ml Erlenmeyer flasks (150 ml liquid medium) as described previously (Kang *et al.*, 2014). The known GA-producing strain of *B. cepacia* SE4 was also grown under the same conditions as described for *Sphingomonas* sp. LK11. The CF was used to extract and purify GAs as described by Lee *et al.* (1998). The CF (pH 2.5) was briefly partitioned with ethyl-acetate (EtOAc) and the organic layer was vacuum dried. 60% methanol (MeOH) was added while the pH was adjusted up to 8.0±0.3 by adding 2 N NH_4OH. Before column chromatography, deuterated GA internal standards ([17, 17-$^2H_2$] GA_1, GA_3, GA_4, GA_7, and GA_{20}) were added to the CF. The quantification of GAs was performed according to the method described by Lee *et al.* (1998). The extracts were passed through a Davisil C18 column (90–130 µm; Alltech, USA). The eluent was reduced

Table 1. GC/MS-SIM analysis of HPLC fractions of a pure culture filtrate of *Sphingomonas* sp. LK11

HPLC fraction No./RT	RT[a]	Sample/Standard	GAs[b]	KRI[c]	*m/z* (%, relative intensity of base peak)[d]		
23~26	24.44	sample	GA_1	2674	506 (100)	448 (20)	313 (17)
		standard	[$^2H_2$] GA_1	2674	508 (100)	450 (19)	315 (14)
23~26	24.93	sample	GA_3	2692	504 (100)	489 (8)	347 (10)
		standard	[$^2H_2$] GA_3	2692	506 (100)	491 (7)	349 (8)
34~35	24.31	sample	GA_4	2506	284 (100)	225 (80)	289 (70)
		standard	[$^2H_2$] GA_4	2506	286 (100)	227 (76)	291 (71)
21-24	22.69	sample	GA_7	2514	222 (100)	416 (18)	384 (13)
		standard	[$^2H_2$] GA_7	2514	224 (100)	418 (18)	386 (12)
37~38	23.49	sample	GA_9	2305	298 (100)	270 (78)	227 (48)
		standard	[$^2H_2$] GA_9	2305	300 (100)	272 (77)	229 (48)
24~26	23.91	sample	GA_{20}	2485	418 (100)	375 (45)	403 (14)
		standard	[$^2H_2$] GA_{20}	2485	420 (100)	377 (45)	405 (13)

[a] RT, Retention time (in min); [b] GAs, Gibberellins; [c] KRI, Kovats retention indices; [d] Identified as methyl ester trimethylsilyl ether derivatives by comparison with reference spectra and KRI data as elucidated by Gaskin and MacMillan (1991). Gibberellins are identified with three ions and quantified by first ion with comparison of labelled standards. About 50 ml of CF extract of *Sphingomonas* sp. LK11 resulted in various HPLC fractions.

to near dryness at 40°C in vacuum. The sample was then dried onto celite and loaded onto a SiO_2 partitioning column (deactivated with 20% water) to separate the GAs from polar impurities. GAs were eluted with 80 ml of 95:5 (v/v) ethyl acetate (EtOAc): hexane saturated with formic acid. This solution was dried at 40°C in vacuum, re-dissolved in 4 ml of EtOAc, and partitioned three times against 4 ml of 0.1 M phosphate buffer (pH 8.0). Drop-wise addition of 2 N NaOH was required during the first partitioning to neutralize residual formic acid. One-gram polyvinylpolypyrrolidone (PVPP) was added to the combined aqueous phases and slurred for 1 h. The pH was reduced to 2.5 using 6 N HCl. The extract was partitioned three times against equal volumes of EtOAc. The combined EtOAc fraction was dried in vacuum, and the residue was dissolved in 3 ml of 100% MeOH. This solution was dried on a Savant Automatic Environmental Speedvac (AES 2000, Spain). The extract of fungal CF was subjected to HPLC using a 3.9 × 300 m Bondapak C18 column (Waters Corp., USA) and eluted at 1.0 ml/min with the following gradient: 0 to 5 min, isocratic 28% MeOH in 1% aqueous acetic acid; 5 to 35 min, linear gradient from 28% to 86% MeOH; 35 to 36 min, 86% to 100% MeOH; 36 to 40 min, isocratic 100% MeOH. Forty-eight fractions of 1.0 ml each were collected (Table 1). The fractions were then prepared for gas chromatography/mass spectrometry (GC/ MS) with selected ion monitoring (SIM) system (6890N Network GC System, and 5973 Network Mass Selective Detector; Agilent Technologies, USA). For each GA, 1 µl of sample was injected in GC/MS (Table 1). Full-scan mode (the first trial) and three major ions among the supplemented GAs internal standards ([17, 17-$^2H_2$] GA_1, GA_3, GA_4, GA_7, and GA_{20}) and the bacterial GAs were monitored simultaneously. The detected bacterial CF GAs (GA_4, GA_9, and GA_{20}) were calculated from the peak area ratios. The retention time was determined using hydrocarbon standards to calculate the KRI (Kovats retention index) value (Table 1). The data was calculated in nanograms per milliliter (for CF); the analyses were repeated three times.

Quantification of indole acetic acid

Salkowski reagent (Patten and Glick 2002) was used for IAA detection and quantification. *Sphingomonas* sp. LK11 was grown in 2000 ml NB with and without 0.5 g/L tryptophan (precursor of IAA) in darkness (Mergeay *et al*., 1985) for 3 days at 28°C at 110 rpm. Bacterial culture suspensions were centrifuged (30 min at 3220 × g) and 0.2 ml of the supernatant was mixed with 1 ml Salkowski's reagent (50 ml 35% $HClO_4$, 1 ml 0.5 M $FeCl_3$). After 30 min, a pink color was developed, which indicated IAA production. The absorbance of pink color was read at 530 nm using a spectrophotometer. The IAA concentration was determined using a calibration curve of pure IAA as a standard following the linear regression analysis.

Bioassay of bacterial endophyte in tomato plants

Due to slow growth of *Tephrosia apollinea*, it is difficult to evaluate the bioactivity of *Sphingomonas* sp. LK11 in host plant in short span of time. Therefore tomato was selected for bioassay as a model plant, which could quickly reveal the activity of detected IAA and GAs produced by the selected strain. The bacteria culture suspension was incubated for 3 days at 30°C on a shaking incubator at 200 rpm to an estimated cell density of 10^8 CFU/ml. All materials, including seeds, pots, soil, DW, and the boxes were sterilized prior to the experiment. Tomato (*Solanum lycopersicum* cv.) seeds (Seminis Korea Co., Korea) were surface sterilized with NaOCl (5%) for 10 min and thoroughly rinsed with autoclaved distilled water. Plastic pots, plastic boxes, DW, and horticulture soil were sterilized thrice by autoclaving at 121°C at 15 psi for 15 min. Seeds were sown in plastic pots containing horticulture soil (peat moss (13–18%), perlite (7–11%), coco-peat (63–68%) and zeolite (6–8%), while the macro-nutrients present were as follows: NH_4^+ ~90 mg/L, NO_3^- ~205 mg/L, P_2O_5 ~350 mg/L, and K_2O ~100 mg/L) under controlled greenhouse conditions at temperatures of 30 ± 2°C. Tomato plants were treated with 50 ml × 2 in split application of bacterial culture suspension at the time of seedling transfer to pots and 2 weeks after sowing. The growth attributes, i.e., shoot length, shoot and root fresh weight, shoot and root dry weight, and chlorophyll contents were recorded after 21 days of the first treatment. The experiment comprised three treatments and four replicates. Each replicate comprised 24 plants. Distilled water and NB media were used as negative and positive controls, respectively, during the experiment. The chlorophyll contents of fully expanded leaves were analyzed using a chlorophyll meter (Minolta Co. Ltd, Japan).

Statistical analysis

The data were analyzed statistically for standard deviation and error by using GraphPad Prism (Ver 5.0; USA). The mean values were compared using Duncan's multiple range tests at $P<0.05$ (SAS, USA).

Results and Discussion

Endophyte isolation and initial phytohormonal screening

A total of 13 bacterial strains were isolated from the 32 leaf samples. These bacterial strains were evaluated for morphological trait analysis such as colony shape, height above the medium, base color, growth rate and pattern, margin characteristics, and surface texture (Tehler, 1995) revealed the same morphotypes. However, all strains were initially screened for the detection of phytohormones production like IAA and GAs (data not shown) in their CF. Endophytic bacterial strain LK11 was selected for further plant growth promotion bioassays as it showed the presence of GAs and IAA among all isolated strains.

Identification of endophytes

After the confirmation of phytohormones producing ability, the endophyte isolate LK11 was identified by PCR amplification and sequencing of the 16S rRNA gene region. Phylogenetic analysis of the bacterial strain was performed by using MEGA 5.1 following the maximum parsimony (MP) method. A consensus tree was constructed from 13 (12 references and 1 clone) aligned 16S rRNA gene sequence with

Table 2. **Gibberellin secretion potential of *Sphingomonas* sp. LK11**

Treatments	GA_4 (ng/ml)	GA_9 (ng/ml)	GA_7 (ng/ml)
Burkholderia cepacia SE4	2.56±0.06[b]	0.41±0.1[b]	0.21+0.11[b]
Sphingomonas sp. LK11	2.97+0.11[a]	0.98+0.15[a]	2.41+0.23[a]

Comparison of *Sphingomonas* sp. LK11 with known GA-producing *B. cepacia* SE4. *Burkholderia cepacia* SE4 was grown and analyzed for GA production in parallel with *Sphingomonas* sp. LK11. The purpose of this experiment was to compare and evaluate the GA ability of both bacterial strains.
In a column, means represented by a different letter(s) are significantly different at the 5% level by DMRT.

1,000 bootstrap replications. Results of BLASTn search of the National Center of Biotechnology Information (NCBI) revealed that endophytic bacteria have 100% sequence homology with *Sphingomonas*. In the MP dendrogram, LK11 formed 100% bootstrap support with *Sphingomonas*. Therefore, on the basis of sequence similarity and phylogenetic analysis, the isolate was identified as a strain of *Sphingomonas* sp. LK11. The sequence data was submitted to GenBank and with the accession number, KF515708.

Gibberellins and IAA production by endophytes

The CF of bacterial strain was subjected to chromatography and GC/MS SIM analysis for the quantification of GAs. The wild type of previously identified GAs producing strain of *B. cepacia* SE4 was also subjected to GA analysis, which is known for active GA production (Kang *et al*., 2014). The results showed that the CF of *Sphingomonas* sp. LK11 contained GAs. The results of GC/MS SIM analysis indicated the presence of GA ion signals in correlation with [$^2H_2$] GA standards (Table 1). The GA produced (GA_4, GA_9, and GA_{20}) were detected in the CF (Table 2). The GAs produced by *Sphingomonas* sp. LK11 was significantly higher than that by *B. cepacia* SE4. However, other bioactive GAs such as GA_3 and GA_7 were not found in *Sphingomonas* sp. LK11, compared to *B. cepacia* SE4 (Joo *et al*., 2009). In addition to GAs, the *Sphingomonas* sp. LK11 also produced IAA. On addition of tryptophan as a precursor in the growing media, the culture of bacterial endophytes revealed 11.23 ± 0. 93 µM/ml of IAA after 3 days of incubation.

GC MS/SIM is an established method to identify targeted novel secondary metabolites and the results were confirmed when no GAs were detected in the microbe-free culture broth. The repetition of the experiment and correlation with deuterated GA standards confirmed the findings. Many fungal/endophytes species for example *Gibberella fujikuroi* (Bömke *et al*., 2008), *Fusarium sacchari*, *Fusarium konzum*, *Fusarium subglutinans* (Troncoso *et al*., 2010), *Phoma herbarum*, *Chrysosporium pseudomerdarium*, and *Scolecobasidium tshawytschae* (Hamayun *et al*., 2009) have been known to produce GAs. The detection of different bioactive and inactive GAs clearly suggest the existence of a GA gene cluster as reported for *Gibberella fujikuroi*, *Phaeospheria* sp. L487, and *Sphaceloma* sp. (Bömke *et al*., 2008).

Fig. 1. **Effects of *Sphingomonas* sp. LK11 inoculation on the growth of tomato plants.** *Sphingomonas* sp. LK11 was isolated from semi-arid-inhibited *Tephrosia apollinea* leaves growing in the wild of Jabal Al-Akhadar, Oman, and was found to be capable of producing GA_4, GA_9, and GA_{20} and IAA.

Although endophytic bacteria are not known for GA production and are less familiar for IAA, there are a few rhizobacteria which can produce GAs (Bottini *et al*., 2004). Rhizobacteria, e.g., *Rhizobium phaseoli*, produced gibberellin-like hormones such as GA_9 and GA_{20}, while IAA was also detected during its growth in the medium (Atzhorn *et al*., 1998). Similarly, *Acetobacter diazotrophicus* produced GA_1 and GA_3 during its growth in the culture media. The bacterial GAs were simultaneously monitored with deuterated GA standards using GC/MS techniques (Bastian *et al*., 1998). Other strains such as *Bacillus pumilus, B. licheniformis* (Gutierrez-Manero *et al*., 2001), *B. cereus*, *B. macroides*, and *B. pumilus* (Joo *et al*., 2004) were reported to produce GAs or GA-like compounds. Joo *et al*. (2004) isolated *Bacillus cereus*, *B. macroides*, and *B. pumilus* and for the first time observed the production of GA_5, GA_8, GA_{34}, GA_{44}, and GA_{53} by these bacterial species. The plant growth promoting rhizobacteria (PGPR) were also evaluated for growth promotion in red pepper; they not only enhanced different plant growth parameters but also increased the endogenous gibberellin level. In addition, *Azotobacter chroococcum* (Verma *et al*., 2001), and *B. cepacia* SE4 (Kang *et al*., 2014) have been reported to produce gibberellins.

Bioassay of *Sphingomonas* sp. LK11 and growth promotion in tomato plants

Sphingomonas sp. has been recently identified in the degradation of persistent metabolites in the environment (Yu *et al*., 2013). *Sphingomonas* sp. was confirmed to contain genes

Table 3. **Effect of *Sphingomonas* sp. LK11 culture application on the growth attributes of tomato plants**

Treatments	Shoot length (cm/plant)	Shoot dry weight (g)	Root dry weight (g)
Distilled Water (DW)	16.49±1.07[c]	0.49±0.07[b]	0.27±0.05[b]
Nutrient broth (NB)	19.51±1.22[b]	0.53±0.06[b]	0.34±0.06[b]
Sphingomonas sp. LK11	27.47±1.69[a]	0.73±0.03[a]	0.47±0.15[a]

In a column, treatment means represented by a different letter are significantly different at the 5% level by DMRT. A 50 × 2 ml of bacterial culture suspension in split application was applied to tomato seedlings. The tomato plant seedlings were allowed to grow for 21 days after the first treatment and then analyzed for various growth attributes.

responsible for carbazole degradation (Kilbane *et al*., 2002). Additionally, it has the capability to regulate certain class of pesticides such as dibenzo-ρ-dioxins and remediate heavy metals (Yu *et al*., 2013). However, the role of *Sphingomonas* sp. in crop growth regulations is poorly known (Islam *et al*., 2013; Thepsukhon *et al*., 2013; Castanheira *et al*., 2014). We performed the bioassay of *Sphingomonas* sp. LK11 in tomato plants to study the effect of bacterial culture suspension on its growth attributes. Inoculation of *Sphingomonas* sp. to tomato plants significantly enhanced the growth attributes (shoot length, shoot fresh/dry biomass, root fresh/dry biomass, and chlorophyll contents), compared to NB media and distilled water (DW) controls (Fig. 1). Our results indicated that *Sphingomonas* sp. LK11 application resulted in 66.58% and 40.87% higher shoot length in tomato plants, in comparison to DW and NB, respectively. The *Sphingomonas* sp. LK11 treated plants showed 48.97% and 37.13% higher dry weights than DW and NB, respectively (Table 3). Similarly, the endophytic bacterial application to tomato plants also increased (14.46%) the chlorophyll contents, compared to the controls.

The beneficial effects of rhizobacteria has been demonstrated in many agricultural crop species such as wheat, soybean, leaf mustered, tomato, bell pepper, mungbean, and rice (Ahemad and Kibret, 2014). The role of endophytic bacteria is well known in plant growth promotion caused by factors such as, nitrogen fixation, auxin, phosphorus solubilization, hydrogen cyanide, exopolysaccharide, and siderophore production; however, little is known in this regard caused by phytohormone production (Naveed *et al*., 2013). This study demonstrated that *Sphingomonas* sp. LK11 producing GAs and IAA can cause growth promotion in tomato plants. IAA is an important plant hormone, which affect plant growth promotion in number of ways. The general beneficial effects on plant growth promotion mainly included cell division, its elongation, and differentiation of cells and tissues (Duca *et al*., 2014; Tivendale *et al*., 2014). The influence of IAA varies with the respect to the plant organ and growth stages e.g. below the soil it improves xylem and phloem formation in roots, and then initiates lateral and adventitious roots formation (Duca *et al*., 2014; Selvakumar *et al*., 2014; Tivendale *et al*., 2014). While above the soil it increases the harvesting of sunlight by improving photosynthesis mechanism, biosynthesis of various kind of pigments, metabolites formation, initiation and development of flower, fruit and leaves (Duca *et al*., 2014; Selvakumar *et al*., 2014). However, IAA production by microbes in certain amount can only promotes host plant growth, exceeding than that can exert negative effect which is usually the property of pathogenic microbes (Spaepen *et al*., 2007). The IAA producing quantity (11.23 ± 0. 93 µM/ml) of *Sphingomonas* sp. in this study seems to be very reasonable for effective plant growth promotion of tomato. The perfect required amount of IAA then increase surface area and length of roots, loose cell wall and release exudates. It enhances two-way traffic for uptake of nutrients towards plant and down ward promotes host plant associated microbial growth (Ahemad and Kibret, 2014). Previously, Yanni *et al*. (2001) observed that inoculation of rice seedlings with rhizobacteria (*Rhizobium* strains) increased seedling vigor, root length, shoot length, and yield of rice plants, while the bacteria simultaneously produced IAA, as well. Gibberellins are also considered as the most important phytohormone to increase the agriculture and horticulture productivity in number of ways, including initiation of early flowering, improvement of crop yield and bigger fruit size (Albermann *et al*., 2013). Similarly among the cyclic diterpenoid GAs; GA_4 is considered as the most bioactive precursor for GA_3. The GA_4 also exhibit extended stability than GA_3 and GA_7 during culture fermentation (Albermann *et al*., 2013). The bioactive GA_4 production by *Sphingomonas* sp. LK11 and then growth promotion results of tomato plant is in harmony with those of Xu *et al*. (1998). In the study of Xu *et al*. (1998), application of GA_4 enhanced the stolon elongation and increased the endogenous GA_1 level. Cerny-Koening *et al*. (2004) reported the positive effect of GA_4 application in the absence of far red light on accelerated anthesis and stem elongation in *Petunia*, which are in coincidence with the current study results. Similarly, Kang *et al*. (2014) and Verma *et al*. (2001) demonstrated that rhizobacteria like *B. cepacia* SE4, and *Azotobacter chroococcum* produced GAs which improved the growth of crop plants. This study on tomato plant growth promotion by *Sphingomonas* sp. LK11 due to GA and IAA production is also consistent with a previous study on phytostimulatory effects by bacterial endophytes attributed to their phytohormone-producing ability by Jasim *et al*. (2013) and a review by Gaiero *et al*. (2013). Further studies incorporating broader field trials and biochemical levels are required.

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