INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–1025/2018/20–6–1289–1297
DOI: 10.17957/IJAB/15.0627
http://www.fspublishers.org

Full Length Article

Isolation and Identification of PGPR Strain and its Effect on Soybean Growth and Soil Bacterial Community Composition

Mingchao Ma[1,2], Xin Jiang[1,3], Qingfeng Wang[1,3], Dawei Guan[1,3], Li Li[1,3], Marc Ongena[2*] and Jun Li[1,3*]
[1]*Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China*
[2]*Microbial Processes and Interactions Research Unit, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés, 2, Gembloux, Belgium*
[3]*Laboratory of Quality & Safety Risk Assessment for Microbial Products, Ministry of Agriculture, Beijing 100081, China*
[*]For correspondence: lijun01@caas.cn; marc.ongena@ulg.ac.be

Abstract

Plant growth-promoting rhizobacteria (PGPR) are considered environmentally sound option to reduce chemical fertilizer inputs, improve soil quality and increase crop yields. The objective of this study was to isolate an effective PGPR strain and investigate its effects on soybean growth and soil bacterial community composition. A total of 163 bacterial isolates were obtained from rhizospheres of plants in four provinces of China. According to capacities for mineral potassium and phosphate solubilization, the best strain (designated 3016) was selected and identified as *Paenibacillus mucilaginosus* based on biochemical characterization and phylogenetic analysis. Moreover, strain 3016 showed a higher capacity for nitrogen fixation and phytohormone production than commercial strains. In a field experiment, *P. mucilaginosus* 3016 was used as an inoculant for seed dressing survived in the soybean rhizosphere as revealed by a species-specific PCR method. Inoculation significantly improved symbiotic nodulation, soybean growth parameters, nutrient contents and yields. The number of nodules was increased by 31.8% for the inoculation treatment compared with CK. Soybean height, pods and seeds per plant and dry weight of nodules were also significantly higher for the inoculation, as well as nutrient contents. Regarding yields, the highest of 3191.4 kg hm^{-2} was obtained under inoculation regime. Moreover, numerous bacterial classes and genera, which were associated with symbiotic nitrogen-fixation, plant growth promotion, biological control and soil catalase activity improvement, were also overrepresented in the inoculation treatment. Some taxa with negative impacts on soil quality decreased. In conclusion, inoculation with *P. mucilaginosus* 3016 had beneficial effects on both soybean growth and soil quality, and is a potential candidate for developing commercial inoculants of PGPR to be used as a bio-fertilizer.

Keywords: PGPR; Bio-fertilizer; Illumina MiSeq; *Paenibacillus mucilaginosus*; Soybean

Introduction

Potassium (K) and phosphorus (P) are major macronutrients essential for plant growth and are abundant in soil (Watanabe *et al*., 2015). However, the levels of available K (AK) and available P (AP) in soil has dropped significantly and are often present mainly as minerals, rocks and other deposits, which are not directly available to plants (Basak and Biswas, 2012; Hamilton *et al*., 2017). Supplementation of AK and AP in soil principally depends on chemical fertilizer inputs; however, AK is easily lost by leaching, runoff and erosion (Sheng and He, 2006), and much of the inorganic P is rapidly converted into unavailable forms of low solubility (Singh and Kapoor, 1998). Additionally, intensive use of chemical fertilizers has resulted in considerable environmental pollution and soil salinization (Liu *et al*., 2016).

Microbial inoculants used as bio-fertilizers have been investigated in attempts to reduce chemical inputs, to improve soil quality and sustainability, and to increase crop production (Li *et al*., 2007; Nosratabad *et al*., 2017). For many years, plant growth-promoting rhizobacteria (PGPR) have been widely used as commercial inoculants and have proven to be environmentally sound options for increasing crop yields through various mechanisms, including K and P solubilization, nitrogen (N) fixation, plant hormone production, pathogen suppression, resistance to abiotic stresses and reducing ethylene concentration in the rhizosphere (Nosheen *et al*., 2016; Khosravi and Zarei, 2017; Stamenov *et al*., 2018). However, some PGPR strains in bio-fertilizers are senescent, degenerative and cultivar specific (Figueiredo *et al*., 2010), which results in inconsistent product quality and effects. Moreover, the search for effective and functional strains has not kept step with their production and application, which may restrict future development. Therefore, it is important to search for multifunctional PGPR strains associated with a large range of plant species (Gao *et al*., 2015). In addition, after being inoculated, the PGPR strains need to cope with the challenge

To cite this paper: Ma, M., X. Jiang, Q. Wang, D. Guan, L. Li, M. Ongena and J. Li, 2018. Isolation and identification of PGPR strain and its effect on soybean growth and soil bacterial community composition. *Int. J. Agric. Biol*., 20: 1289–1297

of living environment changes and interactions with local microbial communities (Gomez *et al*., 2010). The survivals of strains in rhizosphere soil are crucial for their application effects. In this work, we have selected and characterized a new *Paenibacillus* isolate based on its potential to favor K- and P-solubilization and N fixation in vitro. Additional traits related to bio-stimulation were further examined in this rhizobacterium and its survival in rhizosphere soil was investigated. A field experiment was conducted in order to evaluate its positive impact on soybean growth and soil bacterial community structure under inoculation conditions.

Materials and Methods

Isolation of K- and P-solubilizing Bacteria

The K- and P-solubilizing bacterial strains were isolated from rhizosphere soil of peanut, cotton, tobacco and lettuce, in Shandong, Shanxi, Sichuan and Gansu Provinces, China. Plants with roots were pulled out of soil and shaken lightly. The soil adhering to roots was carefully collected as a rhizosphere sample. Each sample (5 g) was added into 50 mL of sterile water and shaken at 200 rpm for 30 min. The appropriate dilution was plated onto Aleksandrov agar medium (Hu *et al*., 2010) plates with a modification of potassium-feldspar powder (<100 mesh) and tricalcium phosphate [$Ca_3(PO_4)_2$] as the sole K and P sources. Plates were incubated at 30ºC for 5–7 d until a single colony appeared. Colonies that showed large clear zones of K- and P-solubilization were screened and this resulted in a total of 163 isolates with potential K-and P-solubilization capacity.

Quantitative estimations were performed in a flask containing 50 mL of modified Aleksandrov medium [potassium-feldspar powder and $Ca_3(PO_4)_2$ as sole K and P sources, 1% w/v], which was then inoculated with each isolate (10%, v/v) in triplicate. *Paenibacillus mucilaginosus* AS1.153 strain (formerly named *Bacillus mucilaginosus*), widely used as an inoculant of K bio-fertilizer in China (Hu *et al*., 2006), was the reference strain. The un-inoculated modified Aleksandrov medium served as a control (Control-1). After incubation in a shaker (200 rpm) at 30ºC for 7 d, the culture solution was spun down at 10,000 *g* for 5 min. The soluble P in the supernatant was measured using a UV-visible spectrophotometer 2550 (Shimadzu, Tokyo, Japan) according to Fankem *et al*. (2006), and K concentration was assayed using an atomic absorption spectrophotometer AA-6300 (Shimadzu, Tokyo, Japan) as documented by Monib *et al*. (1984). Each treatment was performed in triplicate. A bacterial strain designated 3016, which showed the best capacity for K-and P-solubilization, was obtained and kept on Aleksandrov medium at 4ºC for further study.

Determination of N-fixation Capability

Three methods were used to assay the N-fixation capability of strain 3016: nitrogenase activity, ^{15}N natural abundance method and assessment of N concentration in liquid culture. The reference strain was made with *Azotobacter chroococcum* ACCC11103, which is widely used in bio-fertilizers as an N-fixing bacterium. The un-inoculated medium served as a control (Control-2). Each treatment had three replicates. For comparison with the reference strain, two media were used: Aleksandrov and ACCC55 N-free media (Gao *et al*., 2013).

Nitrogenase activities of strains were determined by acetylene reduction assay according to Piromyou *et al*. (2011). Strains were inoculated into 10-mL capped test tubes containing 2 mL of liquid medium. Acetylene gas was injected at a final concentration of 10% (v/v). After incubation at 30 ºC for 72 h, ethylene content was measured by gas chromatograph Agilent 6850 (Wilmington, DE, USA). The N concentration in liquid culture was also determined. The strains were inoculated into flasks containing 50 mL of medium (10%, v/v) and incubated in a shaker (200 rpm) at 30ºC for 7 d. The N concentration in liquid culture was assayed using the Kjeldahl method (Chromy *et al*., 2015). The ^{15}N natural abundance method was used to confirm the N-fixing capability. The strains were inoculated into 100-mL anaerobic bottles containing 20 mL of medium. The $^{15}N_2$ gas was injected at a final concentration of 10% (v/v); the bottles were then sealed and shaken at 200 rpm for 5 d at 30ºC. The culture solutions were centrifuged at 10,000 *g* for 30 min to collect the bacteria. The ^{15}N natural abundance was measured using a Finnigan MAT Delta S mass spectrometer (Bremen, Germany) coupled to an elemental analyzer (Vernaison, France), according to Galiana *et al*. (2002).

Quantification of Phytohormone Production

Strain 3016 was inoculated into 20 mL of Aleksandrov medium and incubated at 30ºC for 7 d. The culture solution was spun down at 10,000 *g* for 5 min. Three representative phytohormones – indole-3-acetic acid (IAA), abscisic acid (ABA) and gibberellic acid (GA_3) – were determined by liquid chromatography–tandem mass spectrometry (Agilent) according to Hou *et al*. (2008) and Masciarelli *et al*. (2014).

Identification of Strain 3016

Strain 3016 was characterized based on physiological and biochemical tests, including methyl red test, Voges–Proskauer reactions, nitrate reduction, milk peptonization and enzymatic activities (Claus and Berkeley, 1986). In addition, phylogenetic analyses of 16S rRNA and *gyrB* genes were performed. DNA was extracted according to Prakamhang *et al*. (2009). The 16S rRNA and *gyrB* genes were amplified as recommended by Ovreås *et al*. (1997) and Hu *et al*. (2010), respectively. After purification of PCR products, the nucleotide sequences were determined commercially by Sangon Biotech (Shanghai, China) and deposited in GenBank database (JF810849 and JF810824, respectively). The acquired sequences were then compared with the most closely related sequences in GenBank using

BLAST. Phylogenetic trees were reconstructed using MEGA 5.1 (Saitou and Nei, 1987).

Preparation of Soybean Seed Dressing and Experimental Design

In the field experiment, strain 3016 was used as inoculant for seed dressing. Pure bacterial culture of strain 3016, grown in solid ACCC55 medium in Roux flask at 30°C for 5 d, were gently scraped from the surface of medium using sterile water and a bamboo stick, and then immediately adjusted to an approximate concentration of 5×10^8 CFU mL^{-1} of bacterial suspension. In the process of seed dressing, each 100 g of soybean seeds was inoculated using 10 mL of bacterial suspension and dried in the shade for immediate use.

The field experiment with a wheat–soybean crop rotation was established in Tai'an City, Shandong Province, China (35°96′N, 117°02′E) in 2013. Samples were collected from two treatments: seed without inoculant (CK) and seed dressing with strain 3016 (inoculation). There were three replicate plots per treatment. Each field plot was 15 m^2 and surrounded by a 1-m wide unplanted buffer zone to minimize possible influence from adjacent plots. Soybean was planted at a rate of 250 seeds per plot with four rows spaced 0.6 m apart. The experiment field was managed in the manner usual for this region.

Sampling, Soybean Growth Parameters and Soil Chemical Properties

At the flower and legume stage, taproot nodules were collected from soybean seedlings in each plot. The quantity and dry weight of nodules per plant were measured. At the soybean mature stage, soybean height and numbers of branches, pods, empty pods and seeds per plant were recorded. After harvest, nutrient contents in soybean seeds and stems were assessed and soybean yields were determined. For use in determining nutrient contents, stem and seed samples were dried at 70˚C for 48 h and finely ground. The N, P and K concentrations were determined using Kjeldahl, sodium carbonate fusion and sodium hydroxide molten flame photometric methods, respectively.

Rhizosphere soils were collected after harvest in 2016. Eight soybean plants and their roots were removed from the middle of each plot and shaken gently. The remaining adhering soil was carefully collected and mixed thoroughly as a single rhizosphere sample. Each soil sample was divided into two parts: one for soil microbial community analysis and one for soil chemical properties. Soil pH, organic matter (OM), available N (AN), AP and AK were determined after drying at room temperature and sieving through a 2.0 mm sieve, according to previously documented methods (Strickland and Sollins, 1987; Hart *et al.,* 1994; Hadas and Portnoy, 1997).

Survival of Strain 3016 in Soybean Rhizosphere

A species-specific PCR detection technique, previously developed for rapid identification of *P. mucilaginosus* from either bacterial strains or complex soil samples (Wang *et al*., 2011; Ma *et al*., 2014), was used to confirm the survival of strain 3016 in rhizosphere soil after inoculation. The three rhizosphere soil samples of CK and inoculation treatments were mixed, separately. Soil DNA was extracted using a MOBIO PowerSoil DNA Isolation Kit (Carlsbad, CA, USA). A 10-fold dilution series was made, and used as templates for PCR. The PCR protocol used a species-specific primer pair, orf06701-F (5′-ATG GAG GAA ACA TGG GGT GA-3′) and orf06701-R (5′-TCA GGA ATG AAG GCC CCC TT-3′), that specifically amplified a 333-bp amplicon from *P. mucilaginosus* (Wang *et al*., 2011). Comparison of the same dilution level was carried out to estimate the survival of strain 3016 in soybean rhizosphere.

Impacts on Soil Bacterial Community Composition

Soil bacterial communities for different treatments were evaluated using a high-throughput sequencing approach. Total soil DNA was extracted, and for each soil sample, six replicate extractions were combined to obtain sufficient and homogeneous DNA (Ding *et al.,* 2016). The DNA concentration and quality were evaluated and the V4 region of the 16S rRNA gene sequence was amplified using the 515F/806R primer set (Peiffer *et al*., 2013). Illumina MiSeq Sequencing was carried out according to Caporaso *et al.* (2012). The sequences were accessed in NCBI database with the number SRX2248212. The qPCR detection system Applied Biosystems 7500 (Foster City, CA, USA) was used to quantify the abundance of 16S rRNA gene with the 515F/806R primer set. The reaction mixture and amplification conditions were performed as recommended by Lauber *et al*. (2013).

Bioinformatics and Statistical Analyses

The pyrosequencing reads and chimeric sequences were processed using Mothur v1.32 (Schloss *et al*., 2011) and USEARCH software (Edgar *et al*., 2011), respectively. Operational taxonomic units were identified using a cut-off of 97% similarity. Alpha diversity analyses (including the Shannon and Simpson indices, ACE and Chao1) were calculated using Mothur (v1.32). Analysis of variance was performed on all experimental data using SPSS (V.19). In all tests, $P < 0.05$ was considered significant, according to Tukey's multiple comparison.

Results

Selection and Characterization of a *Paenibacillus* Isolate by Virtue of Plant Growth-Promoting Characters

Based on in vitro tests, a strain (designated 3016) displaying good performance for N fixation as well as for K- and P-solubilization was selected from a collection of 163 isolates collected from the rhizosphere of plants in four provinces in

China. The concentrations of K and P in liquid culture inoculated with strain 3016 were significantly higher than for the control and commercial strain *P. mucilaginosus* AS1.153 used as reference (Table 1). The N-fixation activities of strain 3016 are detailed in Table 2. The N concentrations in the culture of strain 3016 on Aleksandrov medium and ACCC55 medium were both significantly higher than that of the control. Moreover, ^{15}N abundances in both media inoculated with strain 3016 were 15.45 and 36.95% higher than the control, respectively providing strong evidence of N fixation by strain 3016. However, no nitrogenase activity was detected for strain 3016 under laboratory conditions, but *A. chroococcum* ACCC11103 showed positive nitrogenase activity (8.64 and 8.91 nmol C_2H_4 mg^{-1} protein h^{-1}, respectively) in both media. In addition, strain 3016 produced IAA, ABA and GA_3 contents of 25.65, 0.12 and 3.82 μg mL^{-1}, respectively, indicating positive plant growth-promoting characteristics.

Phenotypic and biochemical analyses, as well as the phylogeny of 16S rRNA and *gyrB* genes, were applied to preliminarily identify strain 3016. It could utilize D-glucose, mannitol, lactose, sucrose, fucose, inositol, maltose and ribose as sole carbon sources, but not sorbitol, galactose, fructose, arabinose, D-raffinose, D-xylose and cellobiose. The methyl red test, Voges–Proskauer reaction and nitrate reduction gave negative results; and litmus fading and milk peptonization were positive. There were positive results for activities of amylase, alkaline phosphatase, lecithin hydrolase, pyrazinamidase and catalase, but negative results for lipase, urase, acetate, oxidase and phenylalanine deaminase. In addition, phylogenetic analysis of 16S rRNA placed strain 3016 within genus *Paenibacillus*, and it clustered with *P. mucilaginosus* STRAIN VKPM B-7519^T in a defined group with a similarity of 98.0%. Furthermore, an approximately 1200-bp *gyrB* gene was amplified and sequenced. A phylogenetic tree (Fig. 1) indicated that strain 3016 clustered with *P. mucilaginosus* STRAIN VKPM B-7519^T in a defined group with over 97.9% sequence similarity, confirming the results of phylogenetic analysis of 16S rRNA. Thus, strain 3016 was preliminarily identified as *P. mucilaginosus* 3016, based on physiological and biochemical characterizations and phylogenetic analyses.

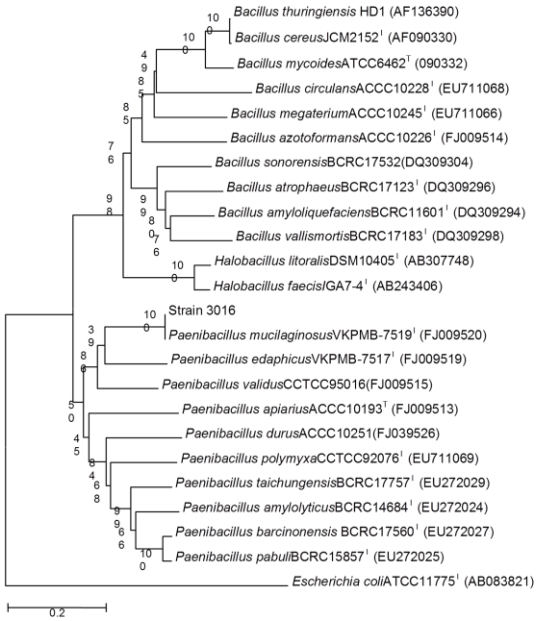

Fig. 1: Phylogenetic tree based on comparative analysis of the *gyrB* gene sequence. *Escherichia coli* ATCC 11775^T was used as the outgroup. Bootstrap values (%) are indicated at the nodes. The scale bars represent 0.2 substitutions per site

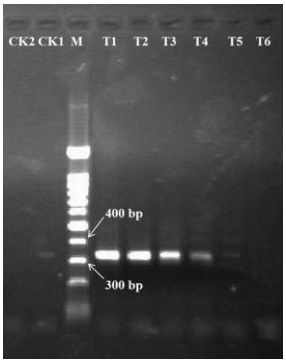

Fig. 2: Amplification products of serial dilutions of rhizosphere soil samples for two treatments. *Lanes T1–T6*: 10^0, 10^1, 10^2, 10^3, 10^4 and 10^5 dilution of DNA extracted under inoculation treatment, respectively; *lanes CK1* and *CK2*: 10^0 and 10^1 dilution of DNA extracted under CK treatment, respectively. *Lane M*: 2-Log ladder marker, 0.1–10 kb

Survival of Strain 3016 in Soybean Rhizosphere

The survival of strain 3016 in the soybean rhizosphere was revealed by a species-specific PCR method. The PCR products of rhizosphere soil samples for two treatments are shown in Fig. 2. An expected 333-bp band was successfully amplified from 10^0, 10^1, 10^2 and 10^3 dilutions of DNA extracted from the inoculation treatment, with negative results for 10^4 and 10^5 dilutions. The expected band was also observed in 10^0 dilution of DNA extracted under CK treatment, but not in 10^1 dilution. These results indicated that *P. mucilaginosus* strains were overrepresented for inoculation treatment compared with CK, due to the survival of *P. mucilaginosus* 3016 in the soybean rhizosphere.

Positive Effects of Strain 3016 on Soybean Growth and Yield in the Field Experiment

The plant growth-promoting potential of strain 3016 was further tested under field conditions. The field experiment has been in operation for 4 years and strain 3016 was used as an inoculant for seed dressing. Soybean growth parameters at different growth stages as well as yields are shown in Table

Table 1: Quantitative estimations of soluble K and P in liquid culture for strain 3016 and the reference strain

Strain	K concentration in culture (mg L^{-1})	P concentration in culture (mg L^{-1})
Control-1	1.71±0.11 a	2.50±0.16 a
AS1.153	4.15±0.10 b	6.36±0.22 b
3016	6.01±0.12 c	10.17±0.17 c

Values are means ± standard deviations (n = 3). Values within the same column followed by different letters indicate significant differences ($P < 0.05$) according to Tukey's multiple comparison

Strain: Control-1, the un-inoculated Aleksandrov medium; AS1.153, Aleksandrov medium inoculated with *P. mucilaginosus* AS1.153; 3016, Aleksandrov medium inoculated with strain 3016

Table 2: N-fixation capacities of strain 3016 and the reference strain

Strain	N concentration in culture (%)		N^{15} natural abundance (%)		Nitrogenase activity/nmol(C_2H_4) (mg^{-1} h^{-1})	
	Medium 1	Medium 2	Medium 1	Medium 2	Medium 1	Medium 2
Control-2	0.44±0.01 a	0.46±0.02 a	0.3676±0.0021 a	0.3691±0.0018 a	Null	Null
ACCC11103	0.62±0.03 c	0.63±0.02 c	0.4418±0.0086 c	0.5724±0.0099 c	8.64±0.55	8.91±0.41
3016	0.49±0.01 b	0.51±0.01 b	0.4244±0.0066 b	0.5055±0.017 b	Null	Null

Values are means ± standard deviations (n = 3). Values within the same column followed by different letters indicate significant differences ($P < 0.05$) according to Tukey's multiple comparison

Strain: Control-2, the un-inoculated medium; ACCC11103, medium inoculated with *A. chroococcum* ACCC11103; 3016, medium inoculated with strain 3016

Medium 1: Aleksandrov medium; Medium 2: ACCC55 medium

Table 3: Soybean growth characteristics and yields for different treatments

Treatment	Height (cm)	Branches/plant	Pods/plant	Empty pods/ plant	Seeds/plant	Weight of 1000-seed (g)	Nodules/plant	Dry weight of nodules/plant (g)	Yields (kg hm^{-2})
CK	69.9±1.4 a	4.1±0.6 a	43.3±3.1 a	3.4±0.9 a	100.5±3.6 a	238.4±1.8 a	164.8±9.5 a	0.57±0.03 a	2846.3±114.5 a
Inoculation	72.5±1.0 a	4.7±0.6 a	59.7±1.5 b	2.8±0.3 a	113.7±4.5 b	239.5±2.6 a	217.2±15.0 b	0.77±0.06 b	3191.4±90.0 b

Values are means ± standard deviations (n = 3). Values within the same column followed by different letters indicate significant differences ($P < 0.05$) according to Tukey's multiple comparison

Treatment: CK, seed without inoculant; Inoculation, seed dressing with *P. mucilaginosus* 3016

3. Compared with CK, the inoculation treatment significantly increased the quantity of pods, seeds and nodules per plant, nodule dry weight and soybean yields. The K and N contents in soybean seeds, as well as K and P contents in soybean stem, were significantly higher for the inoculation treatment than for CK (Table 4).

Inoculation Correlated with Enhanced K, and P Availability and with Shifts in the Rhizosphere Bacterial Community

Inoculation with *P. mucilaginosus* 3016 increased rhizosphere soil nutrients (Table 5). Compared with CK, the concentrations of AK, AP and OM for the inoculation treatment were significantly increased by 12.89, 8.60 and 9.38%, respectively. Furthermore, the inoculation treatment increased the numbers of 16S rRNA gene copies in 1 g of soil from 5.16×10^9 to 3.53×10^{10}, with a decline in bacterial richness and diversity ($P > 0.05$). Bacterial community compositions, as relative abundances of dominant phyla and classes, are shown in Fig. 3. Proteobacteria was the dominant phylum in all soil samples, followed by Acidobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia and Firmicutes. The inoculation treatment significantly increased the relative abundance of Actinobacteria. Furthermore, the dominant classes present were Alphaproteobacteria, Acidobacteria, Gammaproteobacteria, Betaproteobacteria and Deltaproteobacteria. The inoculation treatment had positive effects on the relative abundances of Actinobacteria, Thermoleophilia and Bacilli, which were significantly higher than these in CK. At the genus level, *Bradyrhizobium*, *Haliangium*, *Cupriavidus*, *Methylibium*, *Steroidobacter*, *Ochrobactrum*, *Streptomyces*, *Paenibacillus* and *Bacillus* were overrepresented for the inoculation treatment, with a significantly decreased abundance of *Mycobacterium* and *Rhodanobacter* (Table 6).

Discussion

Due to the plant growth-promoting characters, *P. mucilaginosus* has attracted considerable attention and is consequently widely used as a bio-fertilizer in China (Wu *et al*., 2010). In this study, strain 3016 of *P. mucilaginosus* was obtained, with notably superior K- and P-solubilizing capability compared to commercial strain *P. mucilaginosus* AS1.153. This makes *P. mucilaginosus* 3016 a candidate as a microbial inoculant by suppling nutrient elements and enhancing chemical fertilizer use efficiency. Although there is general agreement on K- and P-solubilizing capability of *P. mucilaginosus* (Basak and Biswas, 2009; Liu *et al*., 2011), its capacity for N fixation is less well understood and even inconsistent (Ma *et al*., 2014). In this study, the ^{15}N natural abundance results were the strongest confirmation for N fixation of *P. mucilaginosus* 3016, consistent with the

Table 4: Soybean nutrient contents for different treatments

Treatment	Soybean seed			Stem (leave)		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
CK	6.30±0.08 a	0.74±0.01 a	1.55±0.05 a	0.85±0.01 b	0.34±0.05 a	0.94±0.02 a
Inoculation	6.56±0.03 b	0.74±0.02 a	1.70±0.04 b	0.83±0.05 b	0.46±0.03 b	1.05±0.02 b

Values are means ± standard deviations (n = 3). Values within the same column followed by different letters indicate significant differences ($P < 0.05$) according to Tukey's multiple comparison

Treatment: CK, seed without inoculant; Inoculation, seed dressing with *P. mucilaginosus* 3016

Table 5: Soil chemical properties for different treatments

Treatment	pH	AP (mg kg^{-1})	AK (mg kg^{-1})	AN (mg kg^{-1})	OM (g kg^{-1})
CK	5.7±0.29 a	21.29±0.51 a	76.31±3.26 a	111.29±2.39 a	20.05±0.49 a
Inoculation	5.9±0.18 a	23.12±0.75 b	86.14±3.14 b	114.12±3.88 a	21.93±0.64 b

Values are means ± standard deviations (n = 3). Values within the same column followed by different letters indicate significant differences ($P < 0.05$) according to Tukey's multiple comparison

Treatment: CK: seed without inoculant; Inoculation: seed dressing with *P. mucilaginosus* 3016

Table 6: Relative abundances of phylogenetic genera with significant differences between two treatments

Phylum	Class	Order	Family	Genus	CK (%)	Inoculation (%)
Proteobacteria	Alphaproteobacteria	*Rhizobiales*	Bradyrhizobiaceae	*Bradyrhizobium;*	1.65±0.40 a	2.71±0.22 b
			Brucellaceae	*Ochrobactrum*	0.07±0.01 a	0.12±0.01 b
	Deltaproteobacteria	*Myxococcales*	Haliangiaceae	*Haliangium;*	0.88±0.06 a	1.22±0.12 b
	Betaproteobacteria	*Burkholderiales*	Oxalobacteraceae	*Cupriavidus*	0.36±0.08 a	0.57±0.05 b
			Comamonadaceae	*Methylibium*	0.26±0.03 a	0.35±0.01 b
	Gammaproteobacteria	*Pseudomonadales*	Pseudomonadaceae	*Pseudomonas*	0.31±0.03 a	0.46±0.02 b
		Xanthomonadales	Sinobacteraceae	*Steroidobacter*	0.27±0.02 a	0.42±0.01 b
			Xanthomonadaceae	*Rhodanobacter*	0.21±0.02 b	0.12±0.01 a
Acidobacteria	Acidobacteriia	*Acidobacteriales*	Koribacteraceae	*Candidatus*	1.31±0.14 b	0.93±0.06 a
Actinobacteria	Actinobacteria	*Actinomycetales*	Mycobacteriaceae	*Mycobacterium*	0.27±0.03 b	0.13±0.01 a
			Streptomycetaceae	*Streptomyces*	0.16±0.01 a	0.31±0.01 b
			Pseudonocardiaceae	*Pseudonocardia*	0.05±0.01 a	0.10±0.02 b
Firmicutes	Bacilli	*Bacillales*	Paenibacillaceae	*Paenibacillus*	0.08±0.01 a	0.17±0.04 b
			Bacillaceae	*Bacillus*	0.08±0.02 a	0.16±0.01 b

Values within the same row followed by different letters indicate significant differences ($P < 0.05$) according to Tukey's multiple comparison. At least one group's relative abundance is more than0.1% of the total sequences

Treatment: CK: seed without inoculant; Inoculation: seed dressing with *P. mucilaginosus* 3016

assessment of N concentration in liquid culture; however, neither nitrogenase activity under laboratory conditions in this study nor the *nifH* gene in chromosomes in our previous study (Ma *et al*., 2012) were detected in *P. mucilaginosus* 3016, which agreed well with results for the N-fixing *P. mucilaginosus* KNP414 (Lu *et al*., 2013). Further research will focus on the "unknown" genes to demonstrate the mechanisms involved in N fixation in *P. mucilaginosus* 3016 (Ma *et al*., 2014). In addition, strain 3016 possesses capacities to produce plant growth-promoting substances: IAA, ABA and GA_3. These phytohormones are positively correlated with various physiological processes in germination, seedling growth, root growth, plant colonization and bacterial establishment, probably due to their effects on plant metabolism and morphology and so improving mineral and water absorption in rhizosphere soil (Perrig *et al*., 2007).

With regards to phylogenetic analyses of *P. mucilaginosus,* it was validly published as the name *B. mucilaginosus* in 1998, and reclassified as *P. mucilaginosus* in 2010 (Hu *et al*., 2010). The classical method to identify *P. mucilaginosus* was according to phenotypic, physiological and biochemical characteristics, phylogenetic analysis of 16S rRNA, or DNA–DNA hybridization (Wu *et al.*, 2010). However, multiple strains in *Bacillus circulans* and *P. mucilaginosus* have similar morphological characteristics and functions, making it impossible to distinguish them from closely related species or strains (He *et al*., 2003; Nastasijevic, 2006). Moreover, the 16S rRNA gene is used for standard determination of bacteria at genus level with a 97% threshold value for a species (Wayne *et al*., 1987), but it is difficult to differentiate *Bacillus pumilus*, *Bacillus megaterium*, *P. mucilaginosus* and *Paenibacillus edaphicus* (Cao *et al*., 2008). The *gyrB* gene can be used to identify bacteria at the species or subspecies level (Srinivasan *et al.*, 2013), due to its faster evolution. In this study, multiphase approaches such as phenotypic and biochemical analyses, and phylogeny of 16S rRNA and *gyrB* genes, were applied to preliminarily identify the multifunctional strain 3016 as *P. mucilaginosus.*

Inoculation with *P. mucilaginosus* 3016 benefited soybean growth and yield, probably due to improved symbiotic N fixation by *Bradyrhizobium*. The quantity and dry weight of nodules were significantly higher for inoculation, indicating a beneficial effect of *P. mucilaginosus* 3016 on symbiotic nodulation. Studies by Linu *et al.* (2009)

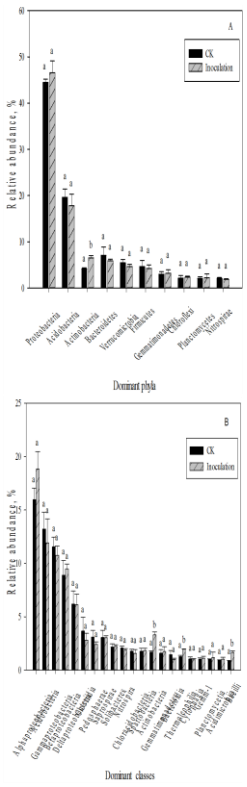

Fig. 3: Relative abundances of dominant phyla (A) and classes (B) for two treatments. Different letters above columns indicate significant differences ($P < 0.05$) according to Tukey's multiple comparison. At least one group's relative abundance is more than 1% of the total sequences

showed that inoculation with P-solubilizing bacteria promoted nodulation, increased biomass and yield, and improved nutrient element uptake by cowpea. Co-inoculation with *Bradyrhizobium* and PGPR microorganisms significantly altered plant growth parameters and significantly improved nodulation (Masciarelli *et al.*, 2014). Moreover, exopolysaccharides and IAA also play important roles in symbiotic nodulation (Janczarek *et al*., 2009), and were found to be positively correlated with crop yield (Dhami and Prasad, 2010). In addition, the inoculation significantly increased the nutrient content in soybean seeds and stems, probably due to increased AK and AP in soil and enhanced symbiotic N-fixation of *Bradyrhizobium*, thus supplying an abundant N source.

Inoculation with *P. mucilaginosus* was beneficial to soil nutrient levels, in agreement with previous results (Liu *et al*., 2011). The AK and AP concentrations in rhizosphere soil significantly increased for inoculation, probably resulting from K- and P-solubilizing by *P. mucilaginosus* 3016. Moreover, inoculation also resulted in accumulated soil OM. As mentioned above, inoculation stimulated the soybean growth and yield, in turn, the amount of soybean residues and decaying roots would also increase, leading to increased OM as residue decomposed over time (Geisseler and Scow, 2014). In addition, inoculation increased 16S rRNA gene copy numbers but decreased bacterial richness and diversity to a certain extent, which may be explained by the selectivity and enrichment of root exudates favoring specific microorganisms.

The phyla Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia and Firmicutes were dominant in the bacterial community and accounted for more than 85% of the total bacteria, which were in agreement with the law of "*species abundance distributions*" indicating a few very abundant species and many rare species in an ecological community (McGill *et al*., 2007). The inoculation treatment showed higher abundance of Proteobacteria, which may indicate better disease-suppressive activity as discussed by Mendes *et al*. (2011). Actinobacteria abundance significantly increased for the inoculation, probably due to higher soil nutrient levels – this is a copiotrophic group with fast growth rates, relies on more labile C sources and is more likely to increase in abundance with increased nutrient input (Zeng *et al.*, 2016). Moreover, classes Actinobacteria, Thermoleophilia and Bacilli had significantly higher abundances for the inoculation than for CK. Actinobacteria and Thermoleophilia are known to function in OM degradation in soil (Zhou *et al.*, 2015). In addition, inoculation showed positive effects on many beneficial genera, including *Bradyrhizobium*, *Haliangium*, *Cupriavidus*, *Pseudomonas*, *Methylibium*, *Steroidobacter*, *Ochrobactrum*, *Streptomyces*, *Paenibacillus* and *Bacillus*. The benefits of *Bradyrhizobium* for soybean growth cannot be overemphasized. As a common soil-dwelling microorganism, *Bradyrhizobium* can develop a symbiotic relationship with soybean plants (which are leguminous) in which they fix N into forms readily available for soybean to use (Saharan and Nehra, 2011). Multiple species in the genera *Pseudomonas*, *Streptomyces* and *Bacillus* are considered as biological control agents (Figueiredo *et al.*, 2010); especially *Streptomyces*, which is the largest antibiotic-producing genus in the microbial world discovered so far (Watve *et al*., 2001). *Haliangium* can also produce antifungal substances that act against phytopathogens (Kundim *et al*., 2003). Moreover, genera *Bacillus* and *Paenibacillus* within class Bacilli are used as PGPR in sustainable agriculture due to three ecological functions: improving available nutrients in soil, antagonism against pathogens and stimulation of host defense and hormones Govindasamy *et al.*, 2010). Abundance of *Steroidobacter*, members of which play important roles in the improvement of soil catalase activity (Sakai *et al*., 2014), also increased in response to the inoculation. However, genera with negative impacts on soil quality, such as *Rhodanobacter* and *Mycobacterium*, decreased significantly for the inoculation. *Rhodanobacter* is likely involved in the denitrifying process that leads to N losses (Green *et al*., 2012) and some *Mycobacterium* species are opportunistic pathogens (Collins and Franzblau, 1997).

Conclusion

A multifunctional plant growth-promoting bacterial strain 3016 was isolated and identified as *P. mucilaginosus*. It showed a higher capacity for K-and P-solubilizing than the most common strain used as a commercial inoculant in China, as well as N fixation and phytohormone production. Inoculation with *P. mucilaginosus* 3016 had beneficial effects on soybean growth, symbiotic nodulation and soybean yields, and shifted the soil bacterial community composition toward a better status. *P. mucilaginosus* 3016 is a potential candidate for commercial inoculant to be used as bio-fertilizer.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (41573066 and 31200388), the National Key Basic Research Program of China (973 Program: 2015CB150506), the Foundation for Safety of Agricultural Products by the Ministry of Agriculture, China (GJFP201801202), the National Key R&D Program of China (2016YFF0201801) and the Fundamental Research Funds for Central Non-profit Scientific Institution (No. 1610132017010). We wish to thank Guoliang Zhu of the Tai'an Academy of Agricultural Sciences for fieldwork assistance. We also thank the University of Liège-Gembloux Agro-Bio Tech and more specifically the research platform Agriculture Is Life for the funding of the scientific stay in Belgium that made this paper possible.

References

Basak, B.B. and D.R. Biswas, 2012. Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop. *Biol. Fert. Soils*, 46: 641–648

Basak, B.B. and D.R. Biswas, 2009. Influence of potassium solubilizing microorganism (*Bacillus mucilaginosus*) and waste mica on potassium uptake dynamics by sudan grass (*Sorghum vulgare* Pers.) grown under two Alfisols. *Plant Soil*, 317: 235–255

Cao, F., D. Shen, J. Li, D. Guan, X. Jiang, L. Li, R. Feng, X. Yang, H. Chen and Y. Ge, 2008. Multiplex-PCR approach to identify *Bacillus* species applied in microbial fertilizers. *Acta Microbiol. Sin*., 48: 651–656

Caporaso, J.G., C.L. Lauber, W.A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S.M. Owens, J. Betley, L. Fraser, M. Bauer and R. Rormley, 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J*., 6: 1621

Chromy, V., B. Vinklárková, L. Šprongl and M. Bittová, 2015. The Kjeldahl method as a primary reference procedure for total protein in certified reference materials used in clinical chemistry. I. A review of Kjeldahl methods adopted by laboratory medicine. *Crit. Rev. Anal. Chem*., 45: 106–111

Claus, D. and R.C.W. Berkeley, 1986. *Genus Bacillus Cohn 1872*, pp: 1105–1140. Murray, R.G.E., P.H.A. Sneath, M.E. Sharpe and J.G. Holt (eds.). Bergey's Manual of Systematic Bacteriology, Springer

Collins, L.A. and S.G. Franzblau, 1997. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents C*., 41: 1004–1009

Dhami, N. and B.N. Prasad, 2010. Increase in root nodulation and crop yield of soybean by native *Bradyrhizobium japonicum* strains. *Bot. Orient. J. Plant. Sci*., 6: 1–3

Ding, J., X. Jiang, M. Ma, B. Zhou, D. Guan, B. Zhao, J. Zhou, F. Cao, L. Li and J. Li, 2016. Effect of 35 years inorganic fertilizer and manure amendment on structure of bacterial and archaeal communities in black soil of northeast China. *Appl. Soil Ecol*., 105: 187–195

Edgar, R.C., B.J. Haas, J.C. Clemente, C. Quince and R. Knight, 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, 27: 2194–2200

Fankem, H., D. Nwaga, A. Deubel, L. Dieng, W. Merbach and F.X. Etoa, 2006. Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeis guineensis*) rhizosphere in Cameroon. *Afr. J. Biotechnol*., 5: 2450–2460

Figueiredo, M.D.V.B., L. Seldin, F.F. de Araujo and R.D.L.R. Mariano, 2010. Plant growth promoting rhizobacteria: fundamentals and applications, *In: Plant Growth and Health Promoting Bacteria*, pp: 21–43. Springer Berlin Heidelberg, Germany

Galiana, A., P. Balle, A. Kanga and A.M. Domenach, 2002. Nitrogen fixation estimated by the 15 N natural abundance method in *Acacia mangium* Willd. inoculated with *Bradyrhizobium* sp. and grown in silvicultural conditions. *Soil Biol. Biochem*., 34: 251–262

Gao, M., J. Zhou, E. Wang, C. Qian, X.U. Jing and J. Sun, 2015. Multiphasic characterization of a plant growth promoting bacterial strain, *Burkholderia* sp. 7016 and its effect on tomato growth in the field. *J. Integr. Agric.*, 14: 1855–1863

Gao, M., H. Yang, J. Zhao, J. Liu, Y. Sun, Y. Wang and J. Sun, 2013. *Paenibacillus brassicae* sp. nov., isolated from cabbage rhizosphere in Beijing, China. *Antonie. Van. Leeuwenhoek*, 103: 647–653

Geisseler, D. and K.M. Scow, 2014. Long-term effects of mineral fertilizers on soil microorganisms-A review. *Soil Biol. Biochem*., 75: 54–63

Gomez, J.P., G.A. Bravo, R.T. Brumfield, J.G. Tello and C.D. Cadena, 2010. A phylogenetic approach to disentangling the role of competition and habitat filtering in community assembly of Neotropical forest birds. *J. Anim. Ecol.*, 79: 1181–1192

Govindasamy, V., M. Senthilkumar, V. Magheshwaran, U. Kumar, P. Bose, V. Sharma and K. Annapurna, 2010. *Bacillus* and *Paenibacillus* spp.: potential PGPR for sustainable agriculture, *In: Plant Growth and Health Promoting Bacteria*, pp: 333–364. Springer

Green, S.J., O. Prakash, P. Jasrotia, W.A. Overholt, E. Cardenas, D. Hubbard, J.M. Tiedje, D.B. Watson, C.W. Schadt and S.C. Brooks, 2012. Denitrifying bacteria from the genus *Rhodanobacter* dominate bacterial communities in the highly contaminated subsurface of a nuclear legacy waste site. *Appl. Environ. Microbiol.*, 78: 1039–1047

Hadas, A. and R. Portnoy, 1997. Rates of decomposition in soil and release of available nitrogen from cattle manure and municipal waste composts. *Compost Sci. Util.*, 5: 48–54

Hamilton, H.A., E. Brod, O. Hanserud, B.M. Danielüller, H. Brattebø and T.K. Haraldsen, 2017. Recycling potential of secondary phosphorus resources as assessed by integrating substance flow analysis and plant-availability. *Sci. Total Envion*., 575: 1546–1555

Hart, S.C., J.M. Stark., E.A. Davidson and M.K. Firestone, 1994. Nitrogen mineralization, immobilization, and nitrification. Methods Soil Anal. Part 2. *Microbiol. Biochem.*, 985–1018

He, L.Y., Y.X. Yin and W.Y. Huang, 2003. Characterization and Phylogenetic Analysis of a Strain of Silicate Baterium. *Acta Microbiol. Sin. Chin. Ed*., 43: 162–168

Hou, S., J. Zhu, M. Ding and G. Lv, 2008. Simultaneous determination of gibberellic acid, indole-3-acetic acid and abscisic acid in wheat extracts by solid-phase extraction and liquid chromatography-electrospray tandem mass spectrometry. *Talanta*, 76: 798–802

Hu, X.F., S.X. Li, J.G. Wu, J.F. Wang, Q.L. Fang and J.S. Chen, 2010. Transfer of *Bacillus mucilaginosus* and *Bacillus edaphicus* to the genus *Paenibacillus* as *Paenibacillus mucilaginosus* comb. nov. and *Paenibacillus edaphicus* comb. nov. *Int. J. Syst. Evol. Microbiol*., 60: 8–14

Hu, X., J. Chen and J. Guo, 2006. Two phosphate-and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World J. Microbiol. Biotechnol.*, 22: 983–990

Janczarek, M., J. Jaroszuk-Ściseł and A. Skorupska, 2009. Multiple copies of rosR and pssA genes enhance exopolysaccharide production, symbiotic competitiveness and clover nodulation in *Rhizobium leguminosarum* bv. trifolii. *Anton Leeuw. Int. J.G.*, 96: 471–486

Khosravi, A. and M.R.A. Zarei, 2017. Influence of biofertilizers and phosphate sources on the phosphorus uptake of lettuce and chemical forms of phosphorus in Soil. *Commun. Soil. Sci. Plant. Anal.,* 48: 2701–2714

Kundim, B.A., Y. Itou, Y. Sakagami, R. Fudou, T. Iizuka, S. Yamanaka and M. Ojika, 2003. New haliangicin isomers, potent antifungal metabolites produced by a marine myxobacterium. *J. Antibiot. (Tokyo)*, 56: 630–638

Lauber, C.L., K.S. Ramirez, Z. Aanderud, J. Lennon and N. Fierer, 2013. Temporal variability in soil microbial communities across land-use types. *ISME J*., 7: 1641–1650

Li, X., Z. Wu, W. Li, R. Yan, L. Li, Y. Li and M. Li, 2007. Growth promoting effect of a transgenic *Bacillus mucilaginosus* on tobacco planting. *Appl. Microbiol. Biotechnol*., 74: 1120–1125

Linu, M.S., J. Stephen and M.S. Jisha, 2009. Phosphate solubilizing *Gluconacetobacter* sp., *Burkholderia* sp. and their potential interaction with cowpea (*Vigna unguiculata* (L.) Walp.). *Int. J. Agric. Res.*, 4: 79–87

Liu, H., D. Chen, R. Zhang, X. Hang, R. Li and Q. Shen, 2016. Amino acids hydrolyzed from animal carcasses are a good additive for the production of bio-organic fertilizer. *Front. Microbiol.*, 7: 1290

Liu, H.U.I., X.Q. WU, J.H. Ren and J.R. Ye, 2011. Isolation and identification of phosphobacteria in poplar rhizosphere from different regions of China. *Pedosphere*, 21: 90–97

Lu, J.J., J.F. Wang and X.F. Hu, 2013. Genome sequence of growth-improving *Paenibacillus mucilaginosus* strain KNP414. *Genom. Announc*., 1: 13

Ma, M., X. Jiang, L. Li and J. Li, 2014. Function and genomics of *Paenibacillus mucilaginosus*. *Chin. Bull. Life Sci.*, 26: 1038–1045

Ma, M., Z. Wang, L. Li, X. Jiang, D. Guan, F. Cao, H. Chen, X. Wang, D. Shen and B. Du, 2012. Complete genome sequence of *Paenibacillus mucilaginosus* 3016, a bacterium functional as microbial fertilizer. *J. Bacteriol*., 194: 2777–2778

Masciarelli, O., A. Llanes and V. Luna, 2014. A new PGPR co-inoculated with *Bradyrhizobium japonicum* enhances soybean nodulation. *Microbiol. Res*., 169: 609–615

McGill, B.J., R.S. Etienne, J.S. Gray, D. Alonso, M.J. Anderson, H.K. Benecha, M. Dornelas, B.J. Enquist, J.L. Green and F. He, 2007. Species abundance distributions: moving beyond single prediction theories to integration within an ecological framework. *Ecol. Lett.*, 10: 995–1015

Mendes, R., M. Kruijt, I. de Bruijn, E. Dekkers, M. van der Voort, J.H.M. Schneider, Y.M. Piceno, T.Z. DeSantis, G.L. Andersen, P.A.H.M. Bakker and J.M. Raaijmakers, 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science*, 332: 1097–1100

Monib, M., M.K. Zahra, S.I. Abdel el-Al and A. Heggo, 1984. Role of silicate bacteria in releasing K and Si from biotite and orthoclase. *In*: *Soil biology and Conservation of the Biosphere*, pp: 733–743. Szegi, J. (ed.). Budapest: Akademiai Kiado. ISBN 9630537001

Nastasijevic, B., 2006. *Influence of Silicate Substrate on Growth and Production of Macrocapsules of Certain Strains of Bacillus Circulans*. Mikrobiol

Nosheen, A., A. Bano, H. Yasmin, R. Keyani, R. Habib, S.T.A. Shah and R. Naz, 2016. Protein quantity and quality of safflower seed improved by NP fertilizer and Rhizobacteria (*Azospirillum* and *Azotobacter* spp.). *Front. Plant Sci.*, 7: 104

Nosratabad, A.R.F., H. Etesami and S. Shariati, 2017. Integrated use of organic fertilizer and bacterial inoculant improves phosphorus use efficiency in wheat (*Triticum aestivum* L.) fertilized with triple superphosphate. *Rhizosphere*, 3: 109–111

Ovreås, L., L. Forney, F.L. Daae and V. Torsvik, 1997. Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Appl. Environ. Microbiol*., 63: 3367–3373

Peiffer, J.A., A. Spor, O. Koren, Z. Jin, S.G. Tringe, J.L. Dangl, E.S. Buckler and R.E. Ley, 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proc. Natl. Acad. Sci.*, 110: 6548–6553

Perrig, D., M.L. Boiero, O.A. Masciarelli, C. Penna, O.A. Ruiz, F.D. Cassán and M.V. Luna, 2007. Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. *Appl. Microbiol. Biotechnol.*, 75: 1143–1150

Piromyou, P., B. Buranabanyat, P. Tantasawat, P. Tittabutr, N. Boonkerd and N. Teaumroong, 2011. Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand. *Eur. J. Soil Biol*., 47: 44–54

Prakamhang, J., K. Minamisawa, K. Teamtaisong, N. Boonkerd and N. Teaumroong, 2009. The communities of endophytic diazotrophic bacteria in cultivated rice (*Oryza sativa* L.). *Appl. Soil Ecol*., 42: 141–149

Saharan, B.S. and V. Nehra, 2011. Plant growth promoting rhizobacteria: a critical review. *Life Sci. Med. Res.*, 21: 30

Saitou, N. and M. Nei, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406–425

Sakai, M., A. Hosoda, K. Ogura and M. Ikenaga, 2014. The growth of *Steroidobacter agariperforans* sp. nov., a novel agar-degrading bacterium isolated from soil, is enhanced by the diffusible metabolites produced by bacteria belonging to *Rhizobiales*. *Microb. Environ*., 29: 89–95

Schloss, P.D., D. Gevers and S.L. Westcott, 2011. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PLoS One*, 6: e27310

Sheng, X.F. and L.Y. He, 2006. Solubilization of potassium-bearing minerals by a wild-type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Can. J. Microbiol*., 52: 66–72

Singh, S. and K.K. Kapoor, 1998. Effects of inoculation of phosphate-solubilizing microorganisms and an arbuscular mycorrhizal fungus on mungbean grown under natural soil conditions. *Mycorrhiza*, 7: 249–253

Srinivasan, S., J. Kim, S.R. Kang, W.H. Jheong and S.S. Lee, 2013. *Burkholderia humi* sp. nov., isolated from peat soil. *Curr. Microbiol*., 66: 300–305

Stamenov, A.D.R., S.S. Djuric and T.H. Jafari, 2018. Effect of Plant Growth Promoting Rhizobacteria on the Germination and Early Growth of Onion (*Allium Cepa*). *Int. J. Agric. Biol. Eng.*, 12: 80321

Strickland, T.C. and P. Sollins, 1987. Improved method for separating light- and heavy-fraction organic material from soil. *Soil Sci. Soc. Amer. J*., 51: 1390–1393

Wang, X., M. Ma, D. Guan, X. Jiang, L. Li, Y. Ding and J. Li, 2011. Rapid identification for *Paenibacillus mucilaginosus* by PCR. *Acta Microbiol. Sin*., 51: 1485–1493

Watanabe, T., M. Urayama, T. Shinano, R. Okada and M. Osaki, 2015. Application of ionomics to plant and soil in fields under long-term fertilizer trials. *SpringerPlus*, 4: 781

Watve, M.G., R. Tickoo, M.M. Jog and B.D. Bhole, 2001. How many antibiotics are produced by the genus Streptomyces? *Arch. Microbiol.*, 176: 386–390

Wayne, L.G., D.J. Brenner, R.R. Colwell, P.A.D. Grimont, O. Kandler, M.I. Krichevsky, L.H. Moore, W.E.C. Moore, R. Murray and E. Stackebrandt, 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Evol. Microbiol*., 37: 463–464

Wu, J.G., J.F. Wang, X.H. Zhang, S.S. Zhang, X.F. Hu and J.S. Chen, 2010. A gyrB-targeted PCR for rapid identification of *Paenibacillus mucilaginosus*. *Appl. Microbiol. Biotechnol*., 87: 739–747

Zeng, J., X. Liu, L. Song, X. Lin, H. Zhang, C. Shen and H. Chu, 2016. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biol. Biochem.*, 92: 41–49

Zhou, J., D. Guan, B. Zhou, B. Zhao, M. Ma, J. Qin, X. Jiang, S. Chen, F. Cao, D. Shen and J. Li, 2015. Influence of 34-years of fertilization on bacterial communities in an intensively cultivated black soil in northeast China. *Soil Biol. Biochem.*, 90: 42–51

(Received 13 September 2017; Accepted 20 January 2018)