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ISOLATION AND IDENTIFICATION OF RHIZOBACTERIA FROM MAIZE (*ZEA MAYS* L.) IN LUVISOLS AND DOCUMENTATION THEIR PLANT GROWTH PROMOTING TRAITS

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ABSTRACT

There is a growing interest in the issue of inoculation of rhizobacteria into the agricultural soil because this group of bacteria can increase productivity and quality of agriculturally important crops and contributes to the stability of agroecosystems. The aim of our work was to isolate and characterize of plant growth promoting traits (production of IAA, siderophores, phosphate solubilisation, antifungal activity) of rhizobacteria belonging to a group of plant growth promoting rhizobacteria (PGPR), from rhizosphere of maize (*Zea mays* L.) in luvisols. Quantitative representation of rhizobacteria of maize rhizosphere was 7.4 . 10^6 CFU.g^{-1} dry soil. A total of eleven species of maize rhizosphere where isolated and confirmed as PGPR *in vitro*. The all isolates showed positive indole-3-acetic acid (IAA) production ranging between 1.39 and 15.74 µg.ml^{-1}. Seven strains (63.6 %) has been shown with low and 1 strain with intermediate solubilisation index of phosphates and the positive production of siderophores showed 7 isolates (63, 6 %). Except for the isolate KmiJP17B089, all others inhibited the growth of *Sclerotinia sclerotiorum* and *Rhizoctonia solani* by more than 50 %. In the case of *Fusarium graminearum*, on the other hand, we observed a very low inhibitory activity. Three isolates which were the most active in observed traits were identified by the 16S rRNA gene sequencing and by BLAST alignment of NCBI database as *Bacillus altitudinis* strain KmiJP17B089, *Bacillus aryabhattai* strain KmiJP17B090 and *Bacillus megaterium* strain KmiJP17B091. These results suggest the possibility of *in vitro* testing of these *Bacillus* species as potential biological fertilizer to increase maize production.

Keywords: rhizobacteria, indole-3-acetic acid, siderophores, phosphate solubilization, antifungal activity, *Zea mays* L., *Bacillus* sp., luvisols

INTRODUCTION

Hiltner (1904) discovered that the rhizosphere is the layer of soil influenced by the root, and is much richer in bacteria than the surrounding bulk soil. The plant rhizosphere is determined by the synergistic relationship between the soil, plant root, and the microbes present and is influenced by the soil pH, texture, complexity and plant roots exudates mainly composed of sugars, amino acids and different nutrients **(Mendes *et al.*, 2013)**. The rhizosphere can be defined as a zone of soil that surrounds the plant root, is a hot spot for numerous organisms and is considered as one of the most complex ecosystem on Earth **(Raaijmakers *et al.*, 2009).** Plant growth promoting rhizobacteria are free-living soil bacteria that colonize the rhizosphere area of root and improve the ability of nutrients and in a variety of modes of action such as the production of phytohormones, phosphate solubilization and siderophore production or supression the phytopathogenic fungi **(Grobelak *et al.*, 2015).** Many bacterial genera such as *Pseudomonas*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Bradyrhizobium*, and *Xanthomonas* have been reported as potential phytohormones producing rhizobacteria **(Asari et al., 2016)** to support plant growth.

Maize (*Zea mays* L.) is the second most abundantly produced cereal in the world and one of the oldest crops. Inoculation of maize with various PGPR strains, however could result in significant increases in plant biomass, root and shoot length and uptake of essential plant nutrients. The use of plant growth-promoting rhizobacteria (PGPR) is a promising alternative method to external chemical inputs to improve crop yield in sustainable agricultural systems **(Yadav *et al.*, 2017).**

The aim of our study was isolation, characterization and identification of the most active rhizobacteria from maize and *in vitro* testing of their PGPR traits (phosphate solubilization, production of IAA, production of siderophores and antifungal activity) for potential in enhancing the growth of a maize crop.

MATERIAL AND METHODS

Sampling characteristics and isolation of bacterial isolates

Bacteria were isolated from the rhizosphere of maize that grown on the altitude of 199 m above sea level in University farm in the Slovak University of Agriculture (SUA) in Kolíňany (48°21´33´´N, 18°11´28´´E). According to FAO classification, the experiment was conducted on the luvisols (Chromic Luvisols). The soil type from the experimental field Kolíňany was haplic luvisols with low to medium humus content (1.91 %), very low total nitrogen content (0.19 %) and neutral pH/H_2O (7.32) soil reaction. Genotype KESSOS is a two-liner hybrid of maize, designed for grain, very well resistant to dry stress, evenly ripening with moderate initial growth. Samples (50 grams each) were collected from the upper 5 cm layer of the rhizosphere from 6 randomly roots of the plant in the phenological growth phase 15 (BBCH 15).

The quantification and isolation of rhizobacteria to pure culture was conducted following a standard microbiological procedure using the serial dilution of agar plate methods on Triptone soya agar (TSA) (Himedia Laboratories, India). The plates were prepared on three replicates and incubated at 28 ± 2°C, for 48 h. It is shown in colony-forming units (CFU) per g of dry soil. Subsequently, individual colonies were picked and streaked on Cetrimide agar (Himedia Laboratories, India) and Pikovskaya´s agar (Himedia Laboratories, India) plates for further purification and test PGPR traits (phosphate solubilization, production of IAA, production of siderophores and antifungal activity). In soil were observed active pH in H_2O, the total nitrogen content (Nt) by Kjeldahl method, and the total carbon content (Cox) by Tjurin method.

Phosphate solubilization

Bacterial phosphate solubilization ability was determined qualitatively on Pikovskya's (PVK) medium with 0,4% bromophenol blue solution **(Gupta *et al.*, 1994).** The plates were incubated at 28 ± 2 °C for 7–10 days and observed for the formation of a clear halo zone around the bacterial colonies, were considered

phosphate solubilizers. All assays were performed in triplicate and mean was calculated from halo diameter and colony diameter in millimeters (mm). From these measurements the Solubilisation index (SI) of phosphates was calculated determined by **Berraquero *et al.* (1976)** as the ratio of diameter of the bacterial colonies + halozone / diameter of the bacterial colonies. The all measurements were performed using a IUL Haloes digital caliper. Based on the solubilisation index, strains were classified according to **Marra *et al*. (2011)** as low (SI < 2.00), intermediate (2.00 ≤ SI < 4.00) or high (SI ≥ 4.00) for their ability to solubilise.

Production of Indole-3-acetic acid (IAA)

Bacterial cultures were grown in Pikovskaya´s medium with tryptophane (100 µg . ml^{-1}) and incubated at 28 ± 2°C for 7 days. IAA production was determined using a colorimetric method **(Gordon and Weber, 1951)** with Salkowski´s reagent**.** Development of pink colour indicated IAA production, and the absorbance of the solutions were observed at 530 nm. IAA-like auxins were expressed as milligram per liter over uninoculated control. The experiment was three replicates and mean was calculated.

Production of siderophores

Siderophore production was detected using Chrome Azurol Sulphonate (CAS) agar plates qualitatively according to **Schwyn** and **Neilands (1987).** The plates were incubated at 30 ± 2°C for 72h and the ability of bacterial cultures was confirmed by the formation of orange or yellow zone around the bacterial colonies. The experiment was three replicates and mean was calculated.

Antifungal activity

Screening of bacterial culture to inhibit the growth of phytopathogenic fungi *Fusarium graminearum* KMi-16H006, *Rhizoctonia solani* KMi-003-JM and *Sclerotinia sclerotiorum* KMi-003-JM were performed *in vitro*. Phytopathogenic fungi was obtained from Collection of Microorganisms of Department of Microbiology Slovak University of Agricultural in Nitra, and incubated on the Potato Dextrose agar (PDA) at 25 ± 2°C for 7 days. These genus were chosen because it has been proposed to have worldwide distribution as pathogenic agent and to cause considerable losses under field conditions. From bacterial cultures was prepared bacterial suspension in sterile ultrapure water and inoculated in the plates with PDA agar, into the centre of plates was placed mycelium of phytopathogenic fungi. The plates were incubated at 25 ± 2°C for 10 days. For the negative control, the same amount of sterile medium was not inoculated with bacterial suspension. The experiment was three replicates and mean was calculated. The inhibition of the growth of fungal mycelium was calculated according the **Sgroy *et al*. (2009).** Mycelia growth inhibition was calculated as I = (C - T/C) x 100, where I = mycelia growth inhibition in percentage, C = mycelia diameter in control, and T = mycelia diameter in bacteria-inoculated plates. Subsequently, we evaluated the antifungal activity as + + + strong inhibition (> 80 %), + + moderate inhibition (80 – 40 %), + low inhibition (< 40 %).

Characterization and identification of PGPR strains

Morphological, growth and biochemical studies of viable colonies of PGPR were performed using standard methods in soil microbiology **(Alef and Nannipieri, 1995).** Gram staining, sporulation, growth of anaerobic, acid from glucose, mobility, oxidase, catalase were used for characterization of the all 11 isolates of PGPR.

Strains for genetic analysis were inoculated on Triptone soya agar and incubated at 28 ± 2 °C. DNA was extracted from 24 hours old bacterial culture. Approximately 50 mg of bacterial culture was placed in 200 µl of PrepMan solution (Life technologies) and homogenized with glass beads on BeadBug homogenizer (Benchmark scientific). DNA sequences of 16S rRNA gene were amplified by using the universal primers. The forward primer 27 F (5´-AGAGTTTGATCCTGGCTCAG-3´) and reverse primer 1492R (5´-TACGGTTACCTTGTTACGACTT-3´) were used **(Lane *et al*., 1991).** Thirty µl of PCR mixture contained 3 µl 10X Dream Taq DNA buffer, 3 µl of 2 mM dNTP mix, 1 µl of $MgCl_2$, 1.2 µl each of the opposing amplification primers (10 mM), 0.1 µl Dream Taq DNA polymerase and 1 µl of genomic DNA. PCR amplification was performed in thermocycler MJ Mini (Biorad, USA). The amplification reaction was performed in a thermal-cycler (Eppendorf thermocycler) programmed for an initial cycle of 95 °C for 3 min, followed by 40 cycles of 95 °C for 30s, 56 °C for 30s, 72 °C for 90 sec, followed by a final extension at 72 °C for 10 min. PCR – amplified 16S rDNA was purified by enzymatic treatment and sequenced in MacroGen Company, South Korea. The similarity and homology of the 16S rRNA partial gene sequences was compared with available sequences by the BLAST algorithm **(Saitou and Nei, 1987)** in the NCBI (National Centre for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov). The DNA sequences were aligned and phylogenetic tree was constructed by PhyML software using Maximum like hood tree. A bootstrap analysis of 1000 replicates was carried out using Molecular Evolutionary Genetics Analysis (MEGA) 6.06 **(Tamura *et al*., 2011).**

RESLUTS AND DISCUSSION

Quantity of rhizobacteria

Quantitative representation of bacteria in the rhizosphere of maize was 7.4 . 10^6 $CFU.g^{-1}$ of dry soil. Despite the fact that important factors influencing the numerous abundance of microorganisms in the soil include in particular the plant species and the soil type, the determined numbers of rhizobacteria were of the order of (10^6) comparable to the numbers of rhizobacteria isolated e.g. from the rhizosphere of wheat **(Melnichuk *et al*., 2019**) from black soil in the Crimea, or maize in the tropical soils of West Africa **(Agbodjato *et al*., 2015).** The numbers of rhizobacteria isolated from chernozem maize in Novi Sad, Serbia, were twice as high **(Mrkovački *et al*., 2016).** However, this could be due to high doses of 250 kg NPK . ha^{-1} in autumn and 140 kg urea . ha^{-1} in the spring. Fertilization of agricultural soils increases the proportion of bacteria that have a significant antagonistic relationship to phytopathogens **(Charousová *et al*., 2016; Halenárová *et al*., 2016; Javoreková *et al*., 2019).** The determined low values of humus and nitrogen content in the tested soil, but for most microorganisms an acceptable neutral pH, also give rise to a significantly higher number of rhizobacteria after the application of the fertilizer.

Plant growth promoting traits of isolates

The all tested isolates (Table 1) based on the determined plant growth promoting traits supporting properties proved in at least one of them that we can call them PGPR. Based on these results we selected the 3 most active isolates for the next morphological, biochemical and genotypic characterization.

Table 1 Bacterial isolates and their PGPR traits

Strain	SI	IAA [$mg.L^{-1}$]	Sidero-phores	Antifungal activity [%]		
				Fusarium	*Rhizoctonia solani*	*Sclerotinia*
KmiJP17B085	1.9	3.65	NP	0 (+)	78.83 (++)	100.00 (+++)
KmiJP17B086	0	2.83	NP	71.43 (++)	100 (+++)	100.00 (+++)
KmiJP17B087	1.82	1.39	PP	35.71 (+)	38.89 (+)	100.00 (+++)
KmiJP17B088	1.74	2.71	PP	14.43 (+)	72.22 (++)	100.00 (+++)
KmiJP17B089	1.29	15.74	PP	14.28 (+)	100 (+++)	4.44 (+)
KmiJP17B090	1.65	10.56	PP	27.14 (+)	100 (+++)	100.00 (+++)
KmiJP17B091	2.96	3.13	NP	20.72 (+)	77.78 (++)	100.00 (+++)
KmiJP17B092	0	1.65	PP	47.86 (++)	100 (+++)	74.44 (11)
KmiJP17B093	1,.8	3.43	NP	41.43 (++)	100 (+++)	77.78 (++)
KmiJP17B094	1	2.58	PP	24.29 (+)	56.11 (++)	63.89 (++)
KmiJP17B095	0	4.08	PP	64.29 (++)	100 (+++)	100.00 (+++)

SI – solubilization index of phosphate, IAA – indole-3-acetic acid, NP - no production, PP – positive production, Antifungal activity: + + + strong inhibition (> 80 %), + + moderate inhibition (80 – 40 %), + low inhibition (< 40 %).

IAA production

Indole-3-acetic acid (IAA) is the physiologically most potent auxin in plant growth and development **(Vurukonda *et al*., 2016)** and supported to enhanced lateral root branching and development of root hairs **(Vacheron *et al*., 2013**). According to the date in Table 1, the all isolates (100 %) in our study produced of IAA, while **Silini - Cherif *et al.* (2016)** states that only 80% of the rhizosphere bacteria are capable of producing IAA. The amount of IAA produced by these

isolates ranged from 1.39 to 15.74 $\mu g.ml^{-1}$ in Pikovskaya´s broth with L-tryptophan as a precursor of IAA synthesis. The amino acids, especially tryptophan played a major role in the production of IAA by rhizobacteria **(De La Torre-Ruiz *et al*., 2016).** IAA production was comparable to the IAA values (0.5- 5 $\mu l.ml^{-1}$) reported by several authors (**De La Torre-Ruiz *et al*., 2016; Karnwal, 2017)** in the same tested dose of tryptophan 100 $\mu g.ml^{-1}$. Similarly, our results were in agreement with other research reports, 100 $mg.ml^{-1}$ L-tryptophan in cultivation medium is suitable for IAA production by rhizospheric bacteria *in vitro* studies. Only two isolates KmiJP17B089 (15.74 $\mu g.ml^{-1}$) and KmiJP17B090 (10.56 $\mu g.ml^{-1}$) produced relatively high levels of IAA. According to **Barazani and Friedmann (1999)** value IAA above 13.5 $\mu g.ml^{-1}$ which confirms that it is considered PGPR. Strains that produce a large amount of IAA, and acetamide indole, in soil increase the growth and yield of crops. It has been reported that plants, under certain constraints, are dependent mainly on exogenous sources of phytohormones including those synthesized by bacteria. Root exudates are a natural source of L-tryptophan for microorganisms of the rhizosphere. In addition to the tryptophan dose, production is dependent on species, culture condition, growth phase and substrate availability **(Silini - Cherif *et al*., 2016).**

Phosphate solubilisation

Phosphorus is the second most important nutrient after nitrogen, required by plants for growth. In the environment, most phosphorus is available in an insoluble form that cannot be directly utilized by plants. Various soil bacteria are capable of solubilising mineral phosphates into a plant utilizable form that represents a possible mechanism of plant growth promotion **(Ashrafuzzaman *et al*,. 2009).** Of the 11 isolates evaluated for the solubilization of phosphates, eight (72. 7 %) isolates showed a low solubilization index SI (< 2.00) and only 1 isolate KmiJPB091 (2.96 mm) showed medium solubilization index. Phosphate solubilization bacteria (PSB) can convert tricalcium phosphate in Pikovskaya´s agar plates in medium from insoluble to soluble form. **Kumar *et al*. (1999)** isolated bacteria from rhizosphere of maize and 33 % of bacterial isolates showed a medium solubilization index. Phosphate solubilization is mainly due to the production of microbial metabolites including organic acids which decreases the pH of the culture media **(Puente *et al*., 2004; Shahid *et al*., 2012).** The presence of P-solubilizing microbial population in soils may be considered a positive indicator of utilizing the microbes as biofertilizers for crop production and beneficial for sustainable agriculture. The application of these strains could prove to be highly beneficial in calcareous soils where phosphorus deficiency is attributed to the binding of phosphate with calcium **(Kaur *et al*., 2018).**

Siderophore production

Siderophores are low molecular weight iron-coordinating, organic compounds produced by most aerobic and facultative anaerobic microorganisms to combat low-iron stress **(Neilands and Leong, 1986).** The excretion of siderophores by rhizosphere bacteria may stimulate plant growth by improving Fe nutrition of the plants **(Masalha *et al*., 2000)** and protect the health of plants from several fungal or bacterial diseases. The majority of the isolated strains produced siderophores at highly variable rates **(Silini - Cherif *et al*., 2016).** Of the 11 isolates evaluated in our study for the production of siderophores, 7 isolates (63.6 %) showed a positive test as indicated by the formation of an orange halo zone on CAS agar media (Table 1). If we used a liquid medium, the production of siderophores could be even higher **(Tian *et al*., 2009).** However, soil samples had favourable pH values, and according to **Khan *et al. (*2018)** in neutral and alkaline pH soils the production of siderophores is increased, because the iron solubility is reduced. The production of siderophore is mainly characteristic for gram negative bacteria, as a rule, only 1.7 % of the total present are gram positive bacteria (Tian et al., 2009). However, in our case, all three identified isolates (*Bacillus* sp.) were gram positive (Table 2) and two of them confirmed the ability to produce siderophores (Table 1). Siderophores did not produce the isolate KmiJP17B091, identified as *Bacillus megaterium*, which may have been due to static conditions delayed and reduced the growth and the production of siderophore, compared with the incubation in stirred conditions (Santos et al., 2014). Only in the case of the KmiJP17B091 isolate identified as *Bacillus megaterium* have we not recorded the production of siderophores. Also according to **Silini - Cherif *et al*. (2016)** some species of *Bacillus* sp., caused to iron-limiting conditions. PGPR produces a range of siderophores that have a strong affinity for iron. Siderophore producing bacteria plays an important role in the biological control against certain phytopathogens. Bacteria produce siderophore and it is bind with the iron strongly and make it unavailable for the plant pathogens, therefore inhibiting the growth phytopathogens **(Beneduzi *et al*., 2012).** However, in our study we could not establish a relationship between siderophore production and antagonism to the plant pathogenic fungi (isolate KmiJP17B086) what may suggest that the detected antagonism probably was due to the synthesis of toxic compounds, such as antibiotics **(Ribeiro *et al*., 2012).**

Antifungal activity

Pathogenic microorganisms affect plant health and are a major threat to crop production. Many plant growth-promoting rhizobacteria (PGPR) residing in the rhizosphere of plants have been reported to enhance plant growth and inhibit plant pathogens by various mechanisms **(Kaur *et al*., 2018).** Maize is one of the crops that is attacked by a number of pests at all growth stages. Among the most common are microscopic fungi, especially representatives of the genus *Fusarium*. This was confirmed by our results, as we have recorded the lowest antifungal effect of PGPR isolates on *Fusarium graminearum*. For the other two fungi representatives tested, the inhibitory effect was greater than 60 %, but never reached 100 %. Our results confirmed good resistance of maize *to Rhizoctonia solani* and *Sclerotinia sclerotiorum,* which can be caused by precisely high efficiency (100 %) many PGPR against these pathogens. Similar results with low antifungal activity (max. 78 %) published **Ali *et al.* (2020). Zhen *et al.* (2011)** reported, that PGPR are able to inhibit the growth of mycelium of phytopathogenic fungi *in vitro* of the strains *Rhizoctonia solani, Fusarium oxysporum, Fusarium solani, and Physalospora piricola.* The inhibition rates against the different fungi ranged from 55.26 to 88.17. %. Of the 11 isolates tested for the antifungal activity of the three phythopathogens, 7 isolates (63.6 %) had more than 80 % growth inhibition of *Sclerotinia sclerotiorum,* 6 isolates (54. 5%) had more than 80 % growth inhibition of *Rhizoctonia solani*. only 4 isolates had more than 40 % growth inhibition of *Fusarium solani.* The highest antagonistic activity was shown by isolate KmiJP17B086 against all tested fungi (Table 2). Interestingly, this traits did not have arelated with the other PGPR traits studied, because it was this representative that was the lowest. Many PGPR which show good results *in vitro* fail to give the same results in the field, when applied as microbial inoculants due to the stress imposed by the sudden change in the environment **(Praveen Kumar *et al*. 2014).**

Table 2 Characterization of putative PGPR strain isolated from maize based on morphological, biochemical and sequencing of partial 16S rDNA sequences and BLAST alignment

Test	***Bacillus altitudinis* KmiJP17B089**	***Bacillus aryabhattai* KmiJP17B090**	***Bacillus megaterium* KmiJP17B091**
Morphological and biochemical characteristics			
Bacteria shape	**Long rod**	**Long rod**	**Long rod**
Gram reaction	**positive**	**positive**	**Positive**
Sporulation	**+**	**+**	**+**
Colony colour	**white**	**peach**	**white/cream**
Acid from glucose	**+**	**+**	**+**
Mobility	**+**	**+**	**+**
Oxidase	**+**	**+**	**+**
Catalase	**+**	**+**	**+**
Nitrate reduction	**-**	**+**	**-**
Genetic characteristics			
GenBank accession no.	**NR_042337.1**	**NR_115953.1**	**NR_116873.1**
E-value	**0.0**	**0.0**	**0.0**
SI	**100**	**99**	**99**
Homology with	***Bacillus altitudinis 41KF2b***	***Bacillus aryabhattai B8W22***	***Bacillus megaterium ATCC 1458***

SI - Similarity index 0 – 100 %

Identification and characterization of the bacterial isolates

Based on the results (Table 1) from the PGPR traits isolates KmiJP17B089, KmiJP17B090 and KmiJP17B091 were chosen for further morphological, biochemical and genotypic characterization (Table 2). The cells were Gram positive, rod shaped, motile and spore forming when observed under phase contrast microscope. In acid production from glucose, catalase and oxidase testes, the strain was positive, differed only in the colour of the colony and nitrate reduction. PGPR mainly belong to the genera *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Pseudomonas*, and *Serratias* **(Gupta *et al.*, 2015)** and among these, the species of *Bacillus* and *Pseudomonas* have perhaps been the most extensively studied **(Podile and Nehra, 2006)** and for their ability to produce beneficial substances **(Kejela *et al.*, 2016). Garbeva *et al.*** (2003) concluded that a majority of soil gram positive bacteria (95%) are members of the genus *Bacillus* (*B. mycoides*, *B. pumilus*, *B. megaterium*, *B. thuringiensis*, and *B. firmus*, etc.) similar to *Paenibacillus*. *Bacillus* sp. is the most abundant genus in rhizosphere, and due to their genetic and metabolic diversity, *Bacillus* sp. are well-adapted to a wide range of environmental conditions **(Saxena *et al.*, 2019).**

This was confirmed in our study, using genetic analysis and PCR to identify three species of the genus *Bacillus*. The nucleotide sequences for a section of the 16S rRNA gene from 3 selected isolates were subjected to BLAST analysis using NCBI database for identification at the genus level. All three isolates contained different nucleotide sequences for the 16S rRNA gene, indicating that they were different strains of genus *Bacillus* (Figure 1). KmiJP17B089 was most closely related to *Bacillus altitudinis* (similarity index 100 %), KmiJP17B090 to *Bacillus aryabhattai* (similarity index 99 %) and KmiJP17B091 to *Bacillus megaterium* with similarity index 99 % (Table 2). The presence of the species *B.megaterium was* not surprising, but the other two identified species (*B. altiniduides* and *B. aryabhattai)* are among the less frequently occurring or occurring in specific conditions with different activity. Our results indicate that the occurrence of *B. altitudinis* is not exclusive to high altitudes **(Shivaji *et al.*, 2006),** and to fruits **(Elbanna *et al.*, 2014)**, but also occurs in rhizosphere of maize in arable soil. However, that strain play important roles not only as PGPR, but caused soft rot disease of apple and pear in Egyptian markets **(Elbanna *et al.*, 2014)**. According to **Li *et al.* (2019),** pot experiments verify the biocontrol effect of *B. altitudinis* AMCC 101304 against potato common scab. **Sun *et al.* (2017)** isolated *B. altitudinis* and characterized it for high IAA production, while **Lu *et al.* (2017)** characterized it like a new potential biocontrol agent against phytopathogens fungi. *Bacillus aryabhattai* (strain B8W22) was initially, isolated from cryotubes used to collect air samples from the Earth's stratosphere at an altitude between 27 and 41 km **(Shivaji *et al.*, 2009)**, resulting in suggestions of a cosmic origin of this bacterium. Subsequently, the bacterium was isolated from rhizosphere soil in many parts of the world such as South Korea **(Lee *et al.*, 2012)**, India **(Pailan *et al.*, 2015)**, and Tibet **(Zhang *et al.*, 2016).** The plant growth promoting activity of *B. aryabhattai* was first reported by **Lee *et al.* (2012)** because promotes the growth of *Xanthium italicum*. More recently, it has been shown that zinc solubilizing strains of *B. aryabhattai* improved the growth of soybean and wheat plants by increasing the mobilization and bio-fortification of zinc **(Ramesh *et al.*, 2014).)**. *Bacillus aryabhattai* has been described by **Lee *et al.* (2011)** like a potential plant growth promoting bacteria with high index of phosphate solubilization and the produce of indole-3-acetic acids. In addition to being able to produce indolyl-3-acetic acid **(Zhang *et al.*, 2015)**, *Bacillus aryabhattai* strain SRB02, isolated from soybean rhizosphere, was found to produce high levels of IAA, gibberellins and jasmonic acid (JA) and are found to promote the growth of soybean and alleviate oxidative stress **(Park *et al.*, 2017).**

Representatives of *B. megaterium* are very active as PGPR because their ability to produce cytokinin **(Asari *et al*. 2017),** to fix atmospheric nitrogen **(Yousuf *et al.*, 2017**), have been reported as phosphorus solubilizers **(Panda *et al.*, 2016)**, to exhibit acid and alkaline phosphatase activity **(Ibarra-Galeana *et al.*, 2017)**, potassium solubilization **(Verma *et al.*, 2015)**, and have been reported as organic phosphorus mineralizing bacteria **(Tao *et al.*, 2008).** Bacillus megaterium was reported to enhance plant growth parameters like root and shoot dry weight and seed weight under field condition in wheat **(Mukhtar *et al.* 2017)**. *Bacillus megaterium* can suppress the growth of the phytopathogenic fungus *Sclerotinia sclerotiorum* **(Gao *et al.*, 2012).**

The members of the genus *Bacillus* hold tremendous potential for biotechnological applications due to their multifarious functional attributes. Their PGP activities in soil and spore forming ability makes them an ideal candidate for developing efficient biopesticide products from technological point of view **(Saxena *et al.*, 2019)**. In various countries around the world, such as China, Germany, India, Italy, Japan, Switzerland, USA, there are already commercially produced and applied products based on *Bacillus* species (*B. amyloliquefacines*, *B. cereus*, *B. megaterium*, *B. subtilis*, *B. thuringiensis*, *B. velezensis*) the main mechanism of action of which is their plant growth supporting traits, mainly P and Zn solubilization, auxin production, antibiotic production and Cry 1 and Cry 2 toxins production.

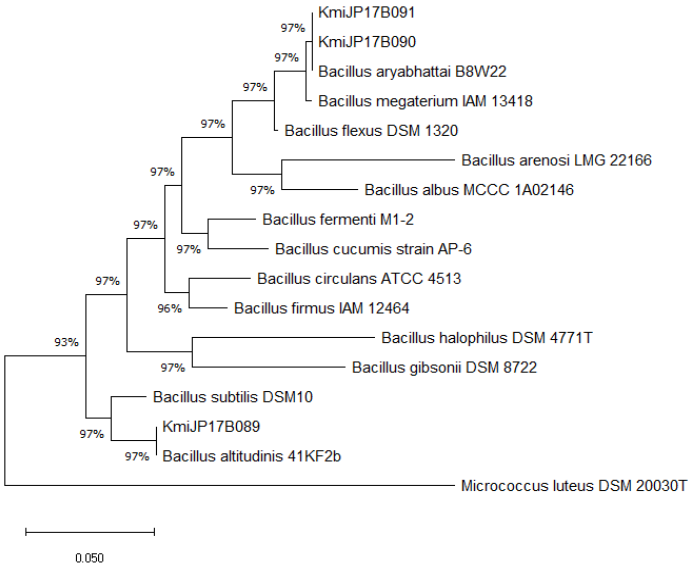

Figure 1 Phylogenetic tree based on a comparison of the 16S rDNA sequences of *Bacillus* sp. isolates and some of their closest phylogenetic relatives. The tree was created by the neighbourjoining method. The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications.

CONCLUSION

This study confirmed that 11 bacterial isolates from the maize rhizosphere demonstrated at least one of the four traits of PGPR (the production of indole-3-acetic acid, siderophores, the ability to solubilize phosphates and the antifungal activity against phytopathogenic fungi) evaluated using *in vitro* assays.. Bacterial strains, *B. altitudinis* strain KmiJP17B089, *B. aryabhatta*i strain KmiJP17B090 and *B. megaterium* strain KmiJP17B091 showed the best properties of PGRP and will therefore be further tested *in vivo* to promote the growth of maize without or with reduced use of industrial fertilizers and pesticides.

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REFERENCES

AGBODJATO, N. A., NOUMAVO, P. A., BABA-MOUSSA, F., SALAMI, H. A., SINA, H., SÈZAN, A., BANKOLÉ, A., ADJANOHOUN, A.,BABA-MOUSSA, L. 2015. Characterization of potential plant growth promoting rhizobacteria isolated from maize (*Zea mays* L.) in central and northern Benin (West Africa). *Applied and Environmental Soil Science*, 9, 1–9. http://dx.doi.org/10.1155/2015/901656.

ALEF, K., NANNIPIERI, P. 1995. Methods in applied soil microbiology and biochemistry. London: Academic Press. 576 p.

ALI S, HAMEED S, SHAHID M, IQBAL M, LAZAROVITS G, IMRAN A. 2020. Functional characterization of potential PGPR exhibiting broad-spectrum antifungal activity. *Microbiological Research*, 232(126389). http://dx.doi.org/10.1016/j.micres.2019.126389.

ASARI, S., TARKOWSKÁ, D., ROLČÍK, J., NOVÁK, O., PALMERO, D. V., BEJAI, S., & MEIJER, J. 2016. Analysis of plant growth-promoting properties of *Bacillus amyloliquefaciens* UCMB5113 using *Arabidopsis thaliana* as host plant. *Planta*, 245(1), 15–30. http://dx.doi.org/10.1007/s00425-016-2580-9.

ASHRAFUZZAMAN M., HOSSEN F.A., ISMAIL M.R., HOQUE M.A., ISLAM M.Z., SHAHIDULLAH S.M., MEON S. 2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology*, 8 (7), 1247–1252. https://doi.org/10.5897/AJB2009.000-9198.

BARAZANI, O., FRIEDMAN, J. 1999. Is IAA the major root growth factor secreted from plant-growth-mediating bacteria? *Journal of Chemical Ecology*, 25(10), 2397–2406. http://dx.doi.org/10.1023/a:1020890311499

BERRAQUERO, F.R., BAYA, A.M., CORMENZANA, A.R. 1976. Establishment of indices for the study of phosphate solubilization by soil bacteria. *Ars Pharmacéutica,* 17, 399-406.

BENEDUZI, A., AMBROSINI, A., PASSAGLIA, L. 2012. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and molecular biology*, 35 (4 suppl.) 1044-1051. https://www.scielo.br/pdf/gmb/v35n4s1/20.pdf.

CHAROUSOVÁ, I., MEDO, J., HALENÁROVÁ, E., MAKOVÁ, J., JAVOREKOVÁ, S. 2016. Effect of fertilization on biological activity of community of soil streptomycetes. *Journal of Central European Agriculture*, 17(4), 1134-1148. http://dx.doi.org/10.5513/JCEA01/17.4.1822.

DE LA TORRE-RUIZ N., RUIZ-VALDIVIEZO V.M., RINCON-MOLINA C.I., RODRIGUEZ-MENDIOLA M., ARIAS-CASTRO C., GUTIERREZ-MICELI, F.A., PALOMEQUE-DOMINGUEZ H., RINCON-ROSALES R. 2016. Effect of plant growth-promoting bacteria on the growth and fructan production of *Agave americana* L. *Brazilian Journal of Microbiology* 47 (3), 587–596. https://10.1016/j.bjm.2016.04.010.

ELBANNA, K., ELNAGGAR, S., BAKEER, A.2014. Characterization of *Bacillus altitudinisas* a new causative agent of bacterial soft rot. *Journal of Phytopathology*, 162(11-12), 712–722. http://dx.doi.org/10.1111/jph.12250.

GARBEVA, P., VAN VEEN, J.A., VAN ELSAS, J.D., 2003. Predominant *Bacillus* spp. in agricultural soil under different management regimes detected via PCR-DGGE. *Microbial Ecology*, 45(3), 302–316. http://dx.doi.org/10.1007/s00248-002-2034-8.

GAO, X., HAN, Q., CHEN, Y., QIN, H., HUANG, L., KANG, Z. 2012. Biological control of oilseed rape *Sclerotinia* stem rot by Bacillus subtilis strain Em7. *Biocontrol science and technology*, 2014, 24(1), 39-52. http://dx.doi.org/10.1080/09583157.2013.844223.

GORDON, S.A.; WEBER, R.P. 1951. Colorimetric estimation of indoleacetic acid. *Plant physiology*, 26 (1), 192-195.

GROBELAK, A., NAPORA, A., KACPRZAK, M. 2015. Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. *Ecological Engineering*, 84, 22-28. http://dx.doi.org/10.1016/j.ecoleng.2015.07.019.

GUPTA, R., SINGAL, R., SHANKAR, A., KUHAD, R.C., SAXENA, R.K. 1994. A modified plate assay for screening phosphate solubilizing microorganisms. *The Journal of General and Applied Microbiology*, 40(3), 255–60. http://dx.doi.org/10.2323/jgam.40.255.

GUPTA, G., PARIHAR, S.S., AHIRWAR, N.K., SNEHI, S.K., SINGH, V. 2015. Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. *Journal of Microbial and Biochemical Technology*, 2015, 7(2), 96–102. http://dx.doi.org/10.4172/1948-5948.1000188.

HALENÁROVÁ, E., MEDO, J., KOVÁCSOVÁ, S., CHAROUSOVÁ, I., MAKOVÁ, J., ELBL, J., ZÁHORA, J.,JAVOREKOVÁ, S. 2016. Effect of vermicompost on changes in the bacterial community in maize rhizosphere. *Journal of Central European Agriculture*, 17(4), 1033–1049. http://dx.doi.org/10.5513/jcea01/17.4.1808.

HILTNER, L. 1904. Uber neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderden berucksichtigung und Brache. *Arb. Dtsch. Landwirtsch. Gesellschaft*, 98, 59–78. https://ci.nii.ac.jp/naid/10014758256/en/

IBARRA-GALEANA, J.A., CASTRO-MARTINEZ, C., FIERRO-CORONADO, R.A., ARMENTA-BOJORQUEZ, A.D. AND MALDONADO-MENDOZA, I.E. 2017. Characterization of phosphate-solubilizing bacteria exhibiting the potential for growth promotion and phosphorus nutrition improvement in maize (Zea mays L.) in calcareous soils of Sinaloa, Mexico. *Annals of Microbiology*, 67(12), 801–811. http://dx.doi.org/10.1007/s13213-017-1308-9.

JAVOREKOVÁ, S., KOVÁCSOVÁ, S., MEDO, J., MAKOVÁ, J., PETROVÁ, J., HLEBA, L., KOŠŤÁLOVÁ, D., CINKOCKI, R. 2019. Soil amended with organic fertilizers as a source of actinomycetes with high potential as biocontrol agents. *Journal of Microbiology, Biotechnology and Food Sciences*, 8(6), 1352–1359. http://dx.doi.org/10.15414/jmbfs.2019.8.6.1352-1359.

KARNWAL, A. 2017. Isolation and identification of plant growth promoting rhizobacteria from maize (*Zea mays* L.) rhizosphere and their plant growth promoting effect on rice (*Oryza sativa* L.). *Journal of Plant Protection Research*, 57(2), 144–151. https://doi.org/10.1515/jppr-2017-0020.

KAUR, A., DEVI, S.R., VYAS, P. 2018. Stress-tolerant antagonistic plant growth-promoting rhizobacteria from *Zea mays*. *Journal of Plant Protection Research*, 58(2), 115–123. https://doi.org/10.24425/119127.

KEJELA T., THAKKAR V.R., THAKOR P. 2016. *Bacillus* species (BT42) isolated from *Coff ea arabica* L. rhizosphere antagonizes *Colletotrichum gloeosporioides* and *Fusarium oxysporum* and also exhibits multiple plant growth promoting activity. *BMC Microbiology*, 16 (277). http://dx.doi.org/10.1186/s12866-016-0897-y.

KHAN, A., SINGH, P., SRIVASTAVA, A. 2018. Synthesis, nature and utility of universal iron chelator–Siderophore: A review. *Microbiological research*, 212-213, 103-111. http://dx.doi.org/10.1016/j.micres.2017.10.012.

KUMAR, V., NARULA, N. 1999. Solubilization of inorganic phosphates and growth emergence of wheat as affected by *Azotobacter chroococcum* mutants. *Biology and Fertility of Soils*, 28(3), 301-305. http://dx.doi.org/10.1007/s003740050497.

LANE, D.J. 1991. 16S/23S rRNA sequencing. In: STACKEBRANDT, E.; GOODFELLOW, M., (Eds.). Nucleic acid techniques in bacterial systematics. New York: John Wiley and Sons, 115-175.

LEE, S., KA, J.O., SONG, H.G. 2012. Growth promotion of *Xanthium italicum* by application of rhizobacterial isolates of Bacillus aryabhattai in microcosm soil. *The Journal of Microbiology*, 50(1), 45–49. http://dx.doi.org/10.1007/s12275-012-1415-z.

LI, B., WANG,B., PAN P, LI, P., QI, Z., ZHANG, Q., SHI, C., HAO, W., ZHOU, B., LIN, R. 2019. *Bacillus altitudinis* strain AMCC 101304: a novel potential biocontrol agent for potato common scab. *Biocontrol science and technology*, 29(10), 1009-1022. http://dx.doi.org/10.1080/09583157.2019.1641791.

LU, X., ZHOU, D., CHEN, X., ZHANG, J., HUANG, H., WEI, L. 2017. Isolation and characterization of *Bacillus altitudinis* JSCX-1 as a new potential biocontrol agent against *Phytophthora sojae* in soybean [*Glycine* max (L.) Merr.]. *Plant and Soil*, 2017, 416(1-2), 53-66. http://dx.doi.org/10.1007/s11104-017-3195-z.

MARRA, L. M., OLIVEIRA, S. M. DE, SOARES, C. R. F. S., MOREIRA, F. M. de S. 2011. Solubilisation of inorganic phosphates by inoculant strains from tropical legumes. *Scientia Agricola*, 68(5), 603-609. http://dx.doi.org/10.1590/s0103-90162011000500015.

MASALHA, J., KOSEGARTEN, H., ELMACI, Ö., MENGEL, K. 2000. The central role of microbial activity for iron acquisition in maize and sunflower. *Biology and Fertility of Soils*., 30(5-6), 433-439. http://dx.doi.org/10.1007/s003740050021.

MELNICHUK, T. EGOVVTSEVA, A., ABDURASHYTOV, S., KAMENEVA, I., YAKUBOVSKAJA, A., RADCHENKO, A., GANOTSKAYA, T., RADCHENKO, L. 2019. Associative to triticum aestivum L. bacteria as a source of strains for biotechnology of the rhizosphere. *Journal of Microbiology, Biotechnology, and Food Sciences*, 8(5) 1194-1197. https://dx.doi.org./10.15414/jmbfs.2019.8.5.1194-1197.

MENDES, R., GARBEVA, P., RAAIJMAKERS, J.M. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS microbiology reviews*, 37(5), 634-663. http://dx.doi.org/10.1111/1574-6976.12028.

MRKOVACKI, N., DJALOVIC, I., JOSIC, D., BJELIC, D., BRDAR-JOKANOVIC, M. 2016. The effect of PGPR strains on microbial abundance in maize rhizosphere in field conditions. *Ratarstvo i Povrtarstvo*, 53(1), 15–19. http://dx.doi.org/10.5937/ratpov53-8224.

MUKHTAR, S., SHAHID, I., MEHNAZ, S., MALIK, K.A. 2017. Assessment of two carrier materials for phosphate solubilizing biofertilizers and their effect on growth of wheat (*Triticum aestivum* L.). *Microbiological Research*, 205, 107–117. http://dx.doi.org/10.1016/j.micres.2017.08.011.

NEILANDS, J.B.; LEONG, S.A. 1986. Siderophores in relation to plant growth and disease. *Annual Review of Plant Physiology*, 37(1), 187-208. http://dx.doi.org/10.1146/annurev.pp.37.060186.001155.

PANDA, B., RAHMAN, H., PANDA, J. 2016. Phosphate solubilizing bacteria from the acidic soils of Eastern Himalayan region and their antagonistic effect on fungal pathogens. *Rhizosphere*, 2, 62–71. http://dx.doi.org/10.1016/j.rhisph.2016.08.001.

PARK, Y.G., MUN, B.G., KANG, S.M., HUSSAIN, A., SHAHZAD, R., SEO, CH.W., KIM, AH.Y., LEE S.U., OH, K.Y., Lee D.Y., LEE, I.J., YUN, B.W. 2017. *Bacillus aryabhattai* SRB02 tolerates oxidative and nitrosative stress and promotes the growth of soybean by modulating the production of phytohormones. *Plos One*.12(3), e0173203. http://dx.doi.org/10.1371/journal.pone.0173203.

PAILAN, S., GUPTA, D., APTE, S., KRISHNAMURTHI, S., SAHA, P. 2015. Degradation of organophosphate insecticide by a novel Bacillus aryabhattai strain SanPS1, isolated from soil of agricultural field in Burdwan, West Bengal, India. *International Biodeterioration & Biodegradation*, 10(3), 191–195. http://dx.doi.org/10.1016/j.ibiod.2015.05.006.

PODILE, A.R., KISHORE, G.K.2006. Plant growth-promoting rhizobacteria. Plant-Associated Bacteria. Kluwer Academic Publishers, 195–230. http://dx.doi.org/10.1007/1-4020-4538-7_6.

PRAVEEN KUMAR, G., MIR HASSAN AHMED, S.K., DESAI, S., LEO, DANIEL AMALRAJ, E., RASUL, A. 2014. *In vitro* screening for abiotic stress tolerance in potent biocontrol and plant growth promoting strains of *Pseudomonas* and *Bacillus* spp. *International Journal of Bacteriology*, 6, 1-6. http://dx.doi.org/10.1155/2014/195946.

PUENTE, M. E., LI, C. Y., BASHAN, Y. 2004. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings. *Plant Biology*, 6(5), 643-650. http://dx.doi.org/10.1055/s-2004-821101.

RAAIJMAKERS, J.M., PAULITZ, T.C., STEINBERG, C., ALABOUVETTE, C., MOËNNE-LOCCOZ Y.. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and soil*, 2009, 321 (1-2), 341-361. http://dx.doi.org/10.1007/s11104-008-9568-6.

RAMESH, A., SHARMA, S.K., SHARMA, M.P., YADAV, N., JOSHI, O.P. 2014. Inoculation of zinc solubilizing *Bacillus aryabhattai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of central India. *Applied Soil Ecology*, 2014 (73), 87–96. http://dx.doi.org/10.1016/j.apsoil.2013.08.009.

RIBEIRO, C.M., CARDOSO, E.J.B.N. 2012. Isolation, selection and characterization of root-associated growth promoting bacteria in brazil pine (*Araucaria angustifolia*). *Microbiological Research*. 167(2), 69–78. http://dx.doi.org/10.1016/j.micres.2011.03.003.

SAITOU, N., NEI, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4(4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454.
SANTOS, S., NETO, I.F.F., MACHADO, M.D., SOARES, H.M.V.M., SOARES, EV. 2014. Siderophore production by *Bacillus megaterium*: effect of growth phase and cultural conditions. *Applied Biochemistry and Biotechnology*, 172(1), 549-560. http://dx.doi.org/10.1007/s12010-013-0562-y.
SAXENA, A.K., KUMAR, M., CHAKDAR, H., ANUROOPA, N., BAGYARAJ., D.J. 2019. *Bacillus* species in soil as a natural resource for plant health and nutrition. *Journal of Applied Microbiology*, 128(6), 1583–1594. http://dx.doi.org/10.1111/jam.14506.
SGROY, V., CASSÁN, F. MASCIARELLI, O., DEL PAPA, M.F., LAGARES, A., LUNAET, V. 2009. Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte *Prosopis strombulifera*. *Applied microbiology and Biotechnology*, 2009, 85(2), 371-381. https://doi.org/10.1007/s00253-009-2116-3.
SHAHID, M., HAMEED, S., IMRAN, A., ALI, S., VAN ELSAS, J.D.2012. Root colonization and growth promotion of sunflower (*Helianthus annuus* L.) by phosphate solubilizing Enterobacter sp. Fs-11. *World Journal of Microbiology and Biotechnology*, 28(8), 2749-2758. http://dx.doi.org/10.1007/s11274-012-1086-2.
SHIVAJI, S., CHATURVEDI, P., SURESH, K., REDDY, S., DUTT, B., WAINWRIGHT, M., NARLIKAR, V., BHARGAVA, M. 2006. *Bacillus aerius* sp. nov., *Bacillus aerophilus* sp. nov., *Bacillus stratosphericus* sp. nov. and *Bacillus altitudinis* sp. nov., isolated from cryogenic tubes used for collecting air samples from high altitudes. *Journal of Systematic and Evolutionary Microbiology*, 56(7), 1465–1473. http://dx.doi.org/10.1099/ijs.0.64029-0.
SHIVAJI S, CHATURVEDI P, BEGUM Z, PINDI PK, MANORAMA R, PADMANABAN DA, SHOUCHE, Y. S., PAWAR, S., VAISHAMPAYAN, P., DUTT, C. B. S., DATTA, G. N., MANCHANDA, R. K., RAO, U. R., BHARGAVA, P. M., NARLIKAR, J. V. 2009. *Janibacter hoylei* sp. nov., *Bacillus isronensis* sp. nov. and *Bacillus aryabhattai* sp. nov., isolated from cryotubes used for collecting air from the upper atmosphere. *International Journal of Systematic and Evolutionary Microbiology*, 59(12), 2977–2986. https://doi.org/10.1099/ijs.0.002527-0.
SCHWYN, B., NEILANDS, J. B. 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, 160(1), 47-56. http://dx.doi.org/10.1016/0003-2697(87)90612-9.
SILINI-CHERIF, H., SILINI, A., GHOUL, M., YADAV, S. 2012 Isolation and characterization of plant growth promoting traits of a rhizobacteria: Pantoea agglomerans lma2. *Pakistan Journal of Biological Sciences*, 15(6), 267–276. http://dx.doi.org/10.3923/pjbs.2012.267.276.
SUN, Z., LIU, K., ZHANG, J., ZHANG, Y., XU, K., YU, D., WANG, J., HU, L., CHEN, L., LI, CH. 2017. AA producing Bacillus altitudinis alleviates iron stress in Triticum aestivum L. seedling by both bioleaching of iron and up-regulation of genes encoding ferritins. *Plant and Soil*, 419(1-2), 1-11. http://dx.doi.org/10.1007/s11104-017-3218-9.
TAMURA, K., PETERSON, D., PETERSON, N., STECHER, G., NEI, M., KUMAR, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28(10), 2731–2739. http://dx.doi.org/10.1093/molbev/msr121.
TIAN, F., DING, Y., ZHU, H., YAO, L., DU, B. 2009. Genetic diversity of siderophore-producing bacteria of tobacco rhizosphere. *Brazilian Journal of Microbiology*, 40(2), 276–284. http://dx.doi.org/10.1590/s1517-83822009000200013.
TAO, G-C., TIAN, S-J., CAI, M-Y., XIE, G-H.2008. Phosphate-solubilizing and -mineralizing abilities of bacteria isolated from soils. *Pedosphere*, 18(4), 515–23. http://dx.doi.org/10.1016/s1002-0160(08)60042-9.
VACHERON, J., DESBROSSES, G., BOUFFAUD, M-L., TOURAINE, B., MOËNNE-LOCCOZ, Y., MULLER, D., LEGENDRE, L., WISNIEWSKI-DYÉ, F., PRIGENT-COMBARET, C. 2013. Plant growth-promoting rhizobacteria and root system functioning. *Frontiers in Plant Science*, 4(356). http://dx.doi.org/10.3389/fpls.2013.00356.
VERMA, P., YADAV, A.N., KHANNAM, K.S., PANJIAR, N., KUMAR, S., SAXENA, A.K., SUMAN, A. 2015. Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with wheat (Triticum aestivum) from the northern hills zone of India. *Annals of Microbiology*, 65(4), 1885–1899. http://dx.doi.org/10.1007/s13213-014-1027-4.
VURUKONDA, S.S.K.P., VARDHARAJULA, S., SHRIVASTAVA, M., SKZ, A. 2016. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological research*, 184, 13-24. http://dx.doi.org/10.1016/j.micres.2015.12.003.
YADAV, A.N., VERMA, P., SINGH, B., CHAUHAN, V.S., SUMAN, A., SAXENA, A.K. 2017. Plant growth promoting bacteria: biodiversity and multifunctional attributes for sustainable agriculture. *Advances in Biotechnology and Microbiology*, 5(5), 1-16. http://dx.doi.org/10.19080/aibm.2017.05.5556671.
YOUSUF, J., THAJUDEEN, J., RAHIMAN, M., KRISHNANKUTTY, S.P., ALIKUNJ, A. A., ABDULLA, M.H. 2017. Nitrogen fixing potential of various heterotrophic *Bacillus strains* from a tropical estuary and adjacent coastal regions. *Journal of Basic Microbiology*, 57(11), 922–932. http://dx.doi.org/10.1002/jobm.201700072.
ZHEN, S., KAIQI, L., CHANGXU, L., JIAN, Y., RUICHENG, J., XUNLI, L. 2011. Isolation and characterization of a potential biocontrol *Brevibacillus laterosporus*. *African Journal of Microbiology Research*, 5(18), 2675-2681. http://dx.doi.org/10.5897/ajmr11.335.
ZHANG, W., HE, L., WANG, Q., SHENG, X. 2016. Inoculation with endophytic *Bacillus megaterium* 1Y31 increases Mn accumulation and induces the growth and energy metabolism-related differentially-expressed proteome in Mn hyperaccumulator hybrid pennisetum. *Journal of Hazardous Materials*, 300, 513-521. http://dx.doi.org/10.1016/j.jhazmat.2015.07.049.