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 (soil texture), 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 suppression 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/H₂O (7.32) soil reaction. Genotype KESSOS is a two-liner hybrid of maize, designed for grain, very well resistant to dry stress, easily 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 plates 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 H₂O, 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 Pikovskaya’s (PKV) 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 + halo zone / 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 under uninoculated control. The experiment was three replicates and mean was calculated.

**Production of siderophores**

Siderophore production was detected using Chrome Azurul 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 and cause considerable losses under field conditions. *Fusarium* species and the soil type, the determined numbers of rhizobacteria were of the numerous abundance of microorganisms in agricultural soils increases the proportion of bacteria that have a significant 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’-AGAGTTTGATCTGGCTCAG-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 MgCl₂, 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).

**RESULTS AND DISCUSSION**

**Quantitative analysis of rhizobacteria**

Quantitative representation of bacteria in the rhizosphere of maize was 7.4 × 10⁶ 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 10⁹ 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 (Mrokváčová 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 (Charoussová 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] | Siderophores | Antifungal activity [%] |
|--------------|--------|--------------|--------------|------------------------|
| KMiJP17R085 | 1.9    | 3.65         | NP           | 0 (+)                  |
| KMiJP17R086 | 0      | 2.83         | NP           | 71.43 (+++)            |
| KMiJP17R087 | 1.82   | 1.39 PP      | PP           | 35.71 (+)              |
| KMiJP17R088 | 1.74   | 2.71 PP      | PP           | 14.43 (+)              |
| KMiJP17R089 | 1.29   | 15.74 PP     | PP           | 14.28 (+)              |
| KMiJP17R090 | 1.65   | 10.56 PP     | PP           | 27.14 (+)              |
| KMiJP17R091 | 2.96   | 3.13 PP      | PP           | 20.72 (+)              |
| KMiJP17R092 | 0.16   | 1.65 PP      | PP           | 47.86 (+)              |
| KMiJP17R093 | 1.8    | 3.43 PP      | PP           | 41.43 (+)              |
| KMiJP17R094 | 1      | 2.58 PP      | PP           | 24.29 (+)              |
| KMiJP17R095 | 0      | 4.08 PP      | PP           | 64.29 (+)              |

- 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 μg.ml⁻¹ in Pikovskaya’s broth with L-tryptophan as a precursor for 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 μg.ml⁻¹) reported by several authors (De La Torre-Ruiz et al., 2016; Karnwal, 2017) in the same tested dose of tryptophan 100 μg.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 μg.ml⁻¹) and KmiJP17B090 (10.56 μg.ml⁻¹) produced relatively high levels of IAA. According to Barazani and Friedmann (1999) value IAA above 13.5 μg.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 utilisable form that represents a possible mechanism of plant growth promotion (Asnafuzzaman 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 KmiJP1B091 (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 be to 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 (Neiandls 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 Phialospora piriocola. 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 phytopathogens, 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 to 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 was strongly 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).
Identification and characterization of the bacterial isolates

Based on the results (Table 1) from the PGPR traits isolates KniJP17B089, KniJP17B090 and KniJP17B091 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 tests, 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 Serratia (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 Pseudomonas. 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).

The strains screened 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). KniJP17B089 was most closely related to Bacillus altitudinis (similarity index 100 %), KniJP17B090 to Bacillus aryabhattai (similarity index 99 %) and KniJP17B091 to Bacillus megaterium with similarity index 99 %. The presence of the species B. megaterium was not surprising, but the other two identified species (B. altitudinoides and B. aryabhattai) are among the less frequently occurring or occurring in specific conditions. 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 AM1013 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 that can induce differential solubilization and the production 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 phosphate 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. amyloliquefaciens, 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 CrI and CrX 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 neighbour joining 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 KniJP17B089, B. aryabhattai strain KniJP17B090 and B. megaterium strain KniJP17B091 showed the best properties of PGPR 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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