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Phosphate solubilizing and PGR activities of ericaceous shrubs microorganisms isolated from Mediterranean forest soil

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ABSTRACT

Many soil microorganisms are able to transform insoluble forms of phosphorus into an accessible soluble form, thus contributing to plant nutrition through exhibiting other beneficial traits such as production of organic acids, siderophore indole acetic acid (IAA) and production of hydrogen cyanide (HCN). Achieving this purpose, ericoid fungi and actinobacteria were isolated from roots and rhizospheric soil of *Calluna vulgaris* L., belonging to the ericaceous family. All isolates were shown to be able to solubilize insoluble phosphate in liquid cultures and to produce transparent halos of solubilization on PVK solid medium. The actinobacteria isolate AH6 was the most efficient compared to others, producing 145.5 mg/L of phosphate and 141 µg/L of IAA. However, fungi isolate S2 and S3 had high solubilization capacity and produced a high concentration of IAA in comparison with S1, which was a good siderophore producer.

We applied a sequencing approach by amplifying the ITS region for fungi and 16S for actinobacteria. Most of the actinobacteria isolates belong to the *Streptomyces* genus while fungi were identified as related to ericoid mycorrhizal fungi. To evaluate the effectiveness of selected rhizobacteria and symbiotic fungi isolates and to confirm their role as biofertilizers, inoculation experimentations on plants are required.

1. Introduction

Phosphorus (P) is an essential macronutrient, the more requested by plant. It plays an important role in many physiological activities such as cell division, photosynthesis, and development of good root system and utilization of carbohydrate (Sharma et al., 2011), despite phosphorus is widely and abundantly distributed in soil under both its inorganic and organic forms, many soils throughout the world are deficient in P due to the fact that is not easily accessible for both plant growth and metal immobilization (Park et al., 2011). In soils, insoluble P compounds can be solubilized by phosphatase enzymes, organic acids and complexing agents produced by plants and microorganisms (Drigo and Donn, 2017). Among the wide diversity of symbiotic and phosphate-solubilizing soil microorganisms (PSM) involved in P-solubilization processus, ericaceous family shrubs can establish root-fungus associations with several fungal belonging to different taxa (Gorzelak et al., 2012). Such multiple symbiotic interactions occur mainly with ericoid mycorrhizal (ErM) fungi and *Phialocephala fortinii* belonging to dark septate endophytes (DSE) (Bruzone et al., 2014). Many studies have shown that ErM enhances plant performance through plant growth promotion activities. For example, previous studies showed that ErM play an important role in nutrient cycles, in heath land and in decomposing organic matter (Lin et al., 2011). They enhance the solubilization of insoluble P compounds and they provide a direct conduit for the transfer of nutrients to the host plant (Vohník and Albrechtová, 2011; Ramasamy et al., 2016; Hamim et al., 2017). Besides, actinomycetes constitute another important group of soil borne microorganisms. They play important ecological roles in soil nutrient cycling (Franco-Correa et al., 2010). These bacteria are known for their economic importance as producers of biologically active substances, such as antibiotics,

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vitamins, enzymes and diverse antimicrobial metabolites that could be a benefit for plant growth (Hamdali et al., 2008a; 2008b; Rey and Dumas, 2017) and for protecting the roots from pathogenic fungi (Belyagoubi, 2014).

The aim of this study was to isolate from a peculiar biotope of *Calluna vulgaris* L., a shrub belonging to the Ericaceous family plant, symbiotic fungi and actinomycetes able to release soluble phosphate and exhibiting different plant growth promoting (PGP) activities. The solubilization mechanism used by these selected strains was investigated and molecular characterization of the most efficient solubilizing and PGPR isolates was achieved.

2. Materials and methods

2.1. Root and rhizospheric soil sampling

Calluna vulgaris L. was collected in a forest area at the region of Malloussa, Tangier in the northwest of Morocco (Table 1). Its botanical classification was carried out at National Herbarium in Rabat. Roots from four plants were sampled and soil samples were randomly collected and analyzed for pH, total nitrogen (N) (Kjeldahl, 1883) and total phosphorus (P) (Olsen and Sommers, 1982).

2.2. Isolation of endophytic fungi

The roots from *Calluna vulgaris* L. were cleaned of debris, rinsed with deionized water and were then sequentially surface-sterilized in ethanol (70%) (30s), sodium hypochlorite (1.65%) (15s), and rinsed in sterile deionized water (5 min).

Three to five surface-sterilized root pieces (length 0.5–1 cm) were then placed onto modified Melin Norkrans Agar media (MMN) (Marx and Bryan, 1975) in a 9 cm diameter petri-dish and incubated at 25 °C in the dark. Mycelia growing out the root were transferred. Cultures were checked for sporulation and slow growing.

2.3. Isolation of total bacteria and actinomycetes

Sample of rhizospheric soil was first mixed, suspended in sterile distilled water (1 g in 10 mL) homogenized and treated for 10–15 min by sonication. Sample was serially diluted up to 10^{-6} and actinobacteria was enumerated by plating and spreading 0.1 mL of 10^{-4}, 10^{-5} and 10^{-6} over the surface of the Soil Extracts Agar (SEA) prepared as follow: 1 L of distilled water and 35 g of Co-composting Time Extracts Agar (CTEA) were mixed overnight at 25 °C. After filtration and sterilization at 120 °C for 15 min, agar (15 g) was added to the collected filtrate ().

The pH was adjusted to 7. The SEA was supplemented with 40μg/mL actidione to inhibit the development of fungi and 10 μg/mL nalidixic acid to inhibit the Gram-negative bacteria capable of swarming without affecting the growth of actinomycetes (Bizuye et al., 2013). The number of colonies was determined after 7 days and 9 days at 28 °C. Actinobacteria were recognized on the basis of morphological features following recommendations given by International *Streptomyces* Project (ISP) (Hamdali et al., 2008a; Rashad et al., 2015).

Properties of *Calluna vulgaris* sampled soil in Melloussa area in Morocco.

Location	35°45′56.66″ N 5°35′39.79″ W
Elevation (m ASL)[1]	377
Vegetation	Ericaceous shrubs
Ericaceous Species	*E.arborea* *E.australis* *E.multiflora* *E.umbellata* *C. vulgaris*

[1] Above Sea Level.

2.4. Screening for fungi and actinomycetes able to use tricalcic-phosphate (TCP) as sole phosphate source

The isolates were grown on Pikovskayas (PVK) (1948) agar plates containing 0.5% $Ca_3(PO_4)_2$ as P source. To measure the solubilization halos, the inoculated PVK plates were incubated at 28 °C for 7 days. The diameter of the halo of solubilization was calculated by subtracting the colony diameter from the total diameter. Only isolates surrounded by clear halos were considered as P solubilizers.

2.5. Estimation of the ability of the selected isolates to release soluble phosphate from TCP

A selection of actinomycetes and endophytic fungi able to use tricalcic-phosphate (TCP) as sole phosphate source was carried out by plating colonies on PVK liquid containing: 10 g/L glucose 0.5 g/L $(NH_4)_2$ SO_4; 0.2 g/L NaCl; 0.2 g/L KCl, 0.1 g/L $MgSO_4,7H_2O$; 0.002 g/L $MnSO_4$ H_2O; 0.5 g/L yeast extract and 5 g/L $Ca_3(PO_4)_2$ as sole phosphate source.

All flasks were incubated at 28 ± 2 °C with shaking for seven days. The cultures were centrifuged at 13000 rpm for 20 min and the P of supernatant was determined by the colorimetric method as described by Ames (1966). The amount of soluble P was detected from the standard curve of KH_2PO_4. Dissolved P concentration was determined by subtracting the P concentration of control from the final concentration of soluble P obtained in the inoculated broths. The pH was determined using a pH meter.

2.6. Phosphatase activity

The acid phosphatase activity in the culture medium used for P solubilization experiment was measured by the method based on the hydrolysis of p-nitrophenyl phosphate as described by Behera et al. (2017). Culture filtrate was incubated with p-nitrophenyl phosphate and modified universal buffer. After 1 h the hydrolysis reaction of p-nitrophenyl phosphate by phosphatase was terminated by adding 0.5 M $CaCl_2$and 0.5 M NaOH solution.

2.7. Indole acetic acid (IAA) production

Selected isolates based on their ability to solubilize P were analyzed for IAA production (Sipahutar and Vangnai, 2017). The selective bacterial strains were grown in a minimal medium (50 mM KH_2PO_4, 50 mM K_2HPO_4, 5 mM $MgSO_4$, 25 mM $(NH_4)_2SO_4$, 1% glucose) amended with 0.05% of L-tryptophan at 25 °C for 14 days in a shaking incubator at 180 rpm.

A separate broth medium inoculated with sterile Milli-Q water served as control treatment.

A syringe filter collected the culture filtrates. One mL of aliquot of the supernatant was mixed vigorously with 2 mL of Salkowski's reagent (Park et al., 2011). The mixture was incubated for 30 min at 25 °C in the dark.

2.8. Hydrocyanic acid (HCN) production

Production of hydrocyanic acid (HCN) was measured according to Bakker Albert and Schippers (1987). The test reveals color changes from orange to dark red indicating HCN production by the isolates.

2.9. Test of siderophore excretion

Chrome Azurol S medium (CAS) agar plates are used for siderophores production by the method of Schwyn and Neilands (1987). After incubation for 7 days at 28 °C, the disks were surrounded by a

zone of color change (blue to yellow-orange) of the CAS that is due to iron chelation. Fungi and bacteria that produce siderophores form an orange or bluish color, which contrasts with the blue-green coloring of the culture medium around the siderophore producing strain. The size of the zones and the intensity of the color change were estimated and compared to the controls.

The PGP activities were carried out in cultures of the control ericoid mycorrhizal strains MVA1 (Vohník et al., 2012), RER4 and RER6 (Vohnik et al., 2013).

2.10. Amplification of the 16S rDNA of the selected actinomycetes isolates

One mL overnight culture was used for DNA extraction as described by Bio Basic Inc product information. The 16S rDNA was amplified using the PCR method with Taq DNA polymerase and universal primers PA (50-AGAGTTTGATCCTGGCTCAG-30) and PH (50 AAGGAGGTGAT CCAGCCGCA-30). Amplification was carried out in 18-mL reaction mixture containing 0.2 U of Taq polymerase (Applied Biosystems), 5-µl Gold reaction buffer (Applied Biosystems), 0.4 mM dNTP, 1 mM of each primer and 100 ng of genomic DNA. Reaction conditions were: PCR cycling parameters used were initial denaturation step for 3 min at 97 °C, then 35 cycles with denaturation at 94 °C for 30s, annealing at 55 °C for 30s and extension at 72°Cfor 45s, with a final extension at 72 °C for 10 min. PCR products were checked for length, quality and quantity by gel electrophoresis (1.5% agarose in 0.5% TAE).

2.11. 11Molecular determination of endophytic fungi (DNA extraction and ITS amplification)

Fungal DNA was extracted from 50 to 150 mg fresh mycelia using wizard Genomic DNA Purification Kit® (Promega). Amplifications of the ITS rDNA regions were performed using the primer pairs ITS1and ITS4 (White et al., 1990) and the GoTaq® DNA Polymerase kit (Promega) following manufacturer's instructions. The PCR cycling parameters used were initial denaturation step for 3 min at 94 °C, then 35 cycles with denaturation at 94 °C for 30s, annealing at 55 °C for 30s and extension at 72 °C for 45s, with a final extension at 72 °C for 10 min.

2.12. Sequencing of isolated strains

PCR products of fungi and actinomycetes were checked for length, quality and quantity by gel electrophoresis (1.5% agarose in 0.5% TAE) and double direction sequenced by Eurofins MWG GmbH (Ebersberg, Germany), using the same primers pair. The sequences were corrected and assembled using Chromaslite v2.1.1 (Technelysium Pty). Multiple alignments were first performed with MUSCLE on Phylogeny.fr (Dereeper et al., 2008) before using MEGA 4 (Tamura et al., 2007).

The sequences obtained were compared for similarity with sequences present in the genomic database banks, using the 'NCBI Blast' program available at: ncbi-nlm-nih.gov website. The selected fungi sequences have been deposited in the GenBank database under accessions No. KU986751 − KU986834.

2.13. Statistical analysis

The data are reported as means ± SD (standard error) for 3 replications. The results were subjected to analysis of variance (ANOVA) at the significance level of 0.05 followed by the Newman-Keuls *t*-test using the SPSS statistical software.

3. Results and discussion

3.1. Soil characteristics

Soil samples were collected from the rhizosphere of an ericaceous soil in the area of Tangier in the north of Morocco, located at 377 m above the sea level and characterized by sub-humid climate with rainfall ranging from 300 to 1000 mm/year. The soil has sandy texture (57%), acid pH (5.5) with total N (0.05%), organic matter (2%), carbon (1.1%). C/N (25) and P (4.3 mg/kg). The sampling site characteristics are given in Table 1. This indicates that humification is slow with the presence of different stages of humus status. In general the soil has a normal organic matter content and is slightly poor in nitrogen and phosphorus. The particularity of this environment is a soil with low levels of mineral supplements, acidic pH, poor or free drainage (Pikovskaya, 1948). These harsh edaphic conditions are generally considered as the best natural environment for ericaceous shrubs (Ames, 1966). Most ericaceous species characteristically grow on nutrient-poor, acidic soil, which causes the complexation of mineral elements by the cations and makes them unavailable to the plant. The capacity of mycorrhizal fungi to access mineral nutrients may determine the survival of ericaceous plants, and help in the uptake of both N and P. Their community structure is important because their presence influences ecosystem function and the competitive relationships of their host plant (Bruzone et al., 2015; Hamim et al., 2017).

3.2. Isolation of total bacteria, actinomycetes and fungi

Roots of ericaceous plants are colonized by a scope of fungi, some of which shape ericoid mycorrhizae, basically characterized by the development of hyphal coils in the cortical cells of the hair roots of ericaceous plants (Bruzone et al., 2015). Functionally, ericoid mycorrhizal fungi are highly efficient in taking up organically bound nutrient elements, which benefit their host plants growing in nutrient-poor and acidic soils conditions (Hazard et al., 2014). The formation of mycorrhizal symbiosis is widely viewed as critical to the survival of ericaceous plants under such nutrient-stressed conditions (Walker et al., 2011). Ericoid mycorrhizal fungi have also been shown to degrade organic molecules such as protein, chitin, cellulose, hemicellulose, starch and more recalcitrant compounds such as polyphenols (Zhang et al., 2016). Additionally, ericaceous roots also harbour other root-associated microorganisms, with unknown ecological functions (Debabrata et al., 2017), such as the actinomycete, Gram-positive saprophytic microscopic organisms, which are generally dispersed in soil and other earth bound environments. They contribute together to the turnover of complex biopolymers of natural matter, for example, chitin and lignocellulose in biological systems (Franco-Correa et al., 2010). They are also well known as producers of many secondary metabolites, with application in agriculture, such as phosphate solubilizing. The cooperation happens among plants and microorganisms, including actinomycetes and fungi in the fascinating zone of the rhizosphere (Franco-Correa et al., 2010).

To the best of our knowledge, this is the first report on the presence of ericoid fungi and actinobacteria able to solubilize the insoluble TCP phosphate from rhizospheric soil under ericaceous shrubs in the northwest of Morocco, which exhibit several plant-beneficial activities. The distribution of total flora of the *Calluna vulgaris* soil extracts showed that the total microbial flora was more abundant on nutrient agar (GN) (95.10^5 ufc/g) and synthetic minimum medium (SMM) (39.10^5 ufc/g). However, on Nautiyal medium synthetic (NCN) we have noticed a significantly lower number of 12.10^5 CFU/g. This result is normal because the nutrient agar is a rich medium that allows the growth of total cultivable microbial flora, but SMM and NCN medium have TCP as sole phosphorus form to be used to evaluate the phosphate-solubilizing flora. The total fungal flora was determined on PDA medium, which accounted for 26.10^4 ufc. However, the total actinomycetes were about 17.10^4 ufc. It's noticed that the amount of total fungi (60%) is higher than the actinomycetes amount (40%) at the sampled soil of *Calluna vulgaris*. Our results have shown the presence of ericoid fungi related to mycorrhizal fungi sp. The three selected fungi strains belong to the Helotiales order, they are able to solubilize insoluble phosphate (TCP).

Table 2
Identification of endophytes fungi isolated from roots of *Calluna vulgaris* L. of Ericaceae and known taxa from the Genbank and EMBL nucleotide databases.

Strain	Best Blast match	Similarity (%)	Accession	Lineage
S1	*Ericoid mycorrhizal* sp.	93	AF072301.1	Helotiales
S2	*Ericoid mycorrhizal* sp.	93	AF072296.1	Helotiales
S3	*Ericoid endophyte* sp.	98	AF252845.1	Helotiales

3.3. Characterization of the selected isolates

Fungal isolates were classified into non-sporulating strain. They were predominantly dark-colored, ranging from grey to black olive and from light to dark brown with hyphae showing simple septa, and sterile mycelia. This group was slow growing generally producing less than 0.5 mm/day. The three selected fungi were identified as ericoid mycorrhizal fungi (93% similarity) (Table 2). This study has shown also the presence of actinomycetes, and different rhizobacteria. However, the proportion of actinomycetes able to grow on insoluble phosphate was low. The non-abundance of these strains could simply be due to a lower content of organic matter, considering that organic matter probably promotes a better development of actinomycetes. Also this soil is nitrogen-poor and there is a few actinomycetes able to fix atmospheric nitrogen (Hamdali et al., 2008a). Also under acidic soils conditions actinomycetes have to adapt other mechanism than organic acids production like chelator secretion or phosphatase enzymes to mobilize organic phosphate (Hamdali et al., 2008b).

3.4. Characterization of actinomycetes and other rhizobacteria

Seven of the selected isolates were shown to belong to the genus of *Streptomyces*. The sequencing of the 16S RNA in these strains (Table 3) confirmed this classification. AH7, AH6 and AH5 isolates exhibited 99 and 100% sequence identity to *Streptomyces* sp. AH4 isolates exhibited 99% sequence identity to *Streptomyces thermocarboxydus*. AH2 exhibited 99% identity to *Streptomyces fimbriatus*. AH1 isolate exhibited 99% identity to *Streptomyces werraensis* and AH3 isolate exhibited 100% sequence identity to *Streptomyces labedae*.

Table 3
Percentage of sequence identity to the 16s RNA sequence of other actinomycetes strains.

Code	Description	Accession	Query cover (%)	Ident (%)
AH7	*Streptomyces* sp.	KT021816.1	99	99
AH6	*Streptomyces* sp.	KX279638.1	100	100
AH5	*Streptomyces* sp.	KT021816	100	100
AH4	*Streptomyces thermocarboxydus*	HQ238376.1	100	99
AH3	*Streptomyces labedae*	EU294135.1	100	100
AH2	*Streptomyces fimbriatus*	EU841630.1	100	99
AH1	*Streptomyces werraensis*	LC128333	100	99
BH1	*Rhizobium* sp.	KJ831224	100	100
BH2	*Burkholderia ambifaria*	NZCP009798	99	99
BH3	*Paenibacillus* sp.	AM900509	99	100

The analysis showed the presence of rhizobacteria belonging to *Burkholderia* sp., *Rhizobium* sp., *Paenibacillus* sp. To achieve our purpose, we have chosen three strains of fungi (S1, S2, S3) and three of actinomycetes (AH4, AH5, AH6) that showed a good phosphorus solubilizing ability. The selected strains will be evaluated *in vitro* for multiple (PGP) activities.

3.5. Abilities of the selected isolates to release soluble phosphate

Phosphorus is one of the major elements in plant mineral nutrition. It is required in large amounts for adequate growth and development of plants (Balemi and Negisho, 2012). It plays an important role in all major metabolic processes occurring in plant including photosynthesis, energy transfer, macromolecular biosynthesis and respiration (Rashad et al., 2015). However it is restricted for crops nutrition because it is present in the insoluble, immobilized, and precipitated form (Debabrata et al., 2017).

The selected strains S1, S2, S3, AH4, AH5, AH6 showed different abilities to release soluble phosphate from TCP (Fig. 1). Phosphate release ranged from 18.1 to 145.5 mg/L, a significant difference was found between strains (Table 4). AH6 was the most efficient actinomycetes strains releasing 145.5 mg/L soluble P in the growth medium. S2 was the most efficient ericoid fungi strains releasing 61.2 mg/L. The control strains RER4, RER6 and MVA1 were the most efficient fungi releasing 123.2 mg/L, 103.5 mg/L, and 103 mg/L, respectively. We have noticed acidification of the growth medium for all the strains; even a slight acidification was noticeable toward the end of growth for AH4, recalling that the initial pH was 6.8 (Fig. 2). This suggests that the process of solubilization involves the excretion of organic acids. All the selected strains were able to solubilize TCP on the basis of halos solubilization on the classical Pikovskaya (Table 4). All isolates were able to produce phosphatase with different levels. Phosphatase does not act directly on inorganic P solubilization, but phosphatase activity may participate in lowering the pH of the culture medium by the dephosphorylating action and the production of acids (Balemi and Negisho, 2012).

Our study has demonstrated that the ability to solubilize TCP obviously varies from one strain to another, some being much more efficient than others. Among all strains growing on liquid PVK, isolates AH6 and S2 were found to be the most powerful phosphate solubilizers in PVK. This actinobacterial strain improved in SMM medium with TCP to have a solubilizing activity much higher than that reported for actinomycetes from Moroccan mines in SMM containing RP^K as sole phosphate source (Hamdali et al., 2008a).

The ability of mycorrhizal, symbiotic fungi and actinomycetes to

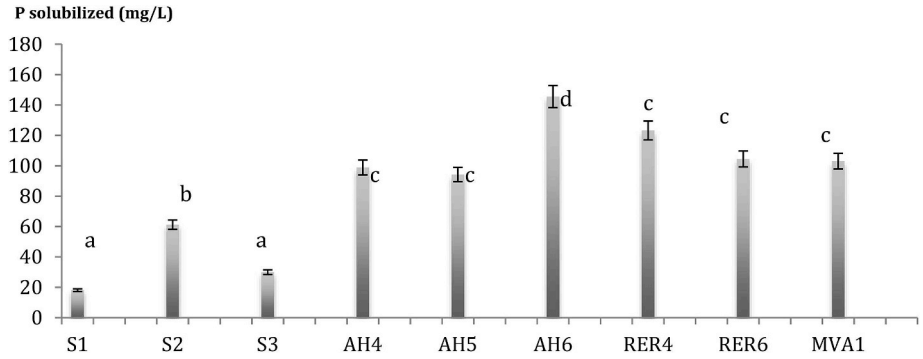

Fig. 1. Concentration of soluble phosphate released in the supernatant of cultures of nine-selected ericoid fungi and actinomycetes strains. Different letters indicate significant differences at $p < 0.05$. Newman-Keuls *t*-test was used to compare means.

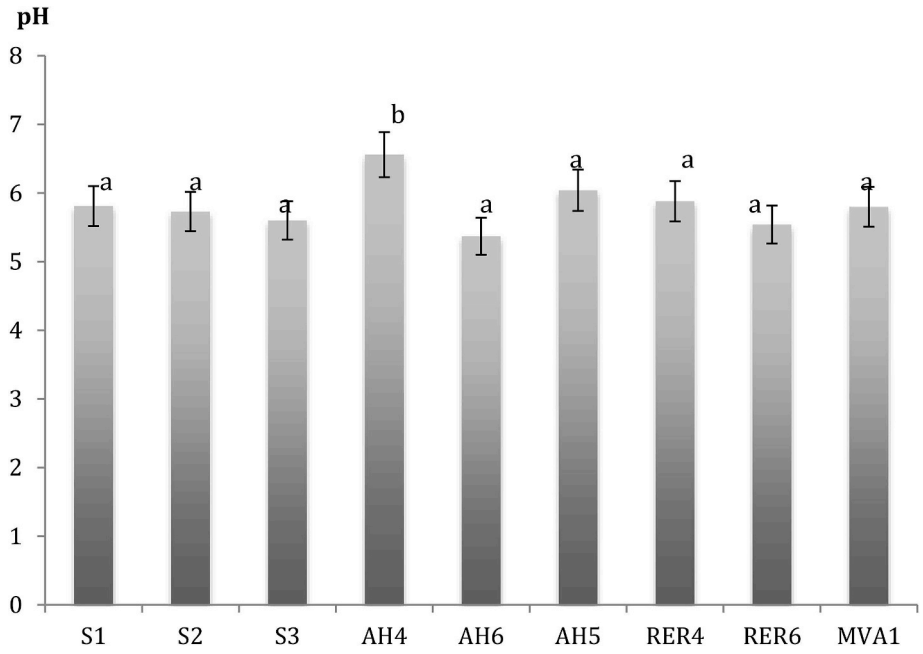

Fig. 2. Final pH of the culture supernatant of the nine selected solubilizing ericoid fungi and actinomycetes strains.
Different letters indicate significant differences at $p < 0.05$. Newman-Keuls *t*-test was used to compare means.

release phosphate is well documented (Bagyaraj, 2014; Debabrata et al., 2017). These authors have reported similar results affirming the phosphate solubilizing ability of a number of ericoid mycorrhizal fungi. Wurzburger et al. (2011) have reported that a number of isolates, mainly *Oidiodendron maius* Barron ericoid mycorrhizal fungi, were capable of solubilizing sparingly soluble zinc phosphate in agar media besides. Gibson and Mitchell (2004) have demonstrated the ability of four *Hymenoscyphus ericae* type isolates to solubilize phosphate under varying nutrient conditions such as in the presence of different carbon concentrations and nitrogen forms to confirm the result of this study.

Plant growth promoting rhizobacteria present in the soil employ different strategies to make use of unavailable forms of phosphorus and in turn also help in making phosphorus available for absorption by plants (Gibson and Mitchell, 2004). The reports in the literature suggest that microbial solubilization of mineral phosphate might be either due to the excretion of organic acids causing acidification of the external medium or to the excretion of chelating substances such as siderophores (Gibson and Mitchell, 2004; Hamdali et al., 2008a). These authors have also indicated that actinomycetes and fungi can solubilize insoluble phosphate in soil by the production of organic acids and acidic protons (H^+ ions), hydroxyl ions. Produced phosphatase enzymes also mineralize organic phosphate (P) by increasing the concentration of soluble phosphate in soil that can improve plant phosphate nutrition and growth (Hamdali et al., 2008b). In our study, all symbiotic fungi and actinomycetes have shown high phosphatase activity to mobilize inorganic phosphate.

3.6. In vitro screening of isolates for multiples (PGP) activities

3.6.1. Production of indole acetic acid (IAA)

Screening results of PGP activities of selected ericoid fungi and actinomycetes are shown in Table 4. IAA production was detected in all isolates with significantly different amounts, ranging from 10.8 to 141 µg/L. The highest concentration (141 µg/L) was produced by the isolate AH6 while the lowest values 10.8 and 11.3 µg/L were obtained by S1 and RER4, respectively (Fig. 3; Table 4). The IAA is able to solubilize P also and promote growth (Ahemad and Kibret, 2014). Park et al. (2011) have obtained 12–34 mg/L IAA production from PSM isolated from soil, indicating that the isolated have plant growth promoting effect. Plant hormones are regulators that may influence plant development and growth, and IAA is one of the most physiologically active auxins and it has positive effect on root growth (Ahemad and Kibret, 2014).

Interestingly the majority of the selected strains of ericoid fungi and actinomycetes were able to produce IAA from tryptophan added to the culture medium, which is in agreement with numerous studies that demonstrated that IAA is the common product of tryptophan metabolism of several rhizobacteria (Ahemad and Kibret, 2014; Rodríguez-Caballero et al., 2017). The production of IAA by our strains was high in comparison with the control strains, especially for AH6 and S3 and

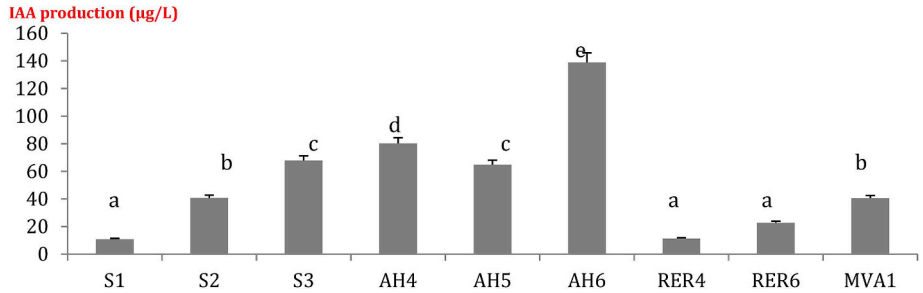

Fig. 3. IAA production by different selected strains.
Different letters indicate significant differences at $p < 0.05$. Newman-Keuls *t*-test was used to compare means.

therefore, the ability of those strains to produce IAA is important in the application of the isolated to phyto-stabilization. Up to 80% of rhizobacteria can synthesize IAA, it is proposed to act in conjunction with endogenous IAA in plant to stimulate cell proliferation and enhance the host's uptake of minerals and nutrients from the soil (Balemi and Negisho, 2012). High IAA production resulted in high plant growth (Park et al., 2011). This phytohormone is involved in physiological processes; it affects plant cell division, extension, and differentiation (Balemi and Negisho, 2012; Garbaye, 2013). It plays a very important role in the stimulation of elongation and proliferation of root hairs and lateral roots (Khan et al., 2016; Shilev, 2013; Napier and Napier, 2017). The IAA has been associated with the plant growth promoting effect of several rhizospheric microorganisms that allows the elongation of the root system to better absorb mineral elements (Martinez-Viveros et al., 2010; Nadeem et al., 2013).

3.6.2. Production of hydrogen cyanide (HCN)

HCN is a co-product of ethylene biosynthesis, a key phytohormone that can affect plant growth and development in a large number of different ways including promoting root initiation, inhibiting root elongation and activating the synthesis of other plant hormones (Silveira et al., 2016). The HCN test revealed that ericoid fungi isolate S1, S2, S3 and AH4 produced high quantity of HCN, while AH5 and AH6 produced less. We have noticed that reference strains did not show ability to synthetize hydrogen cyanide (Table 4). Besides, the selected fungi and actinomycetes were assessed for HCN production. This volatile acid is one of the effective antagonistic compounds specifically against fungi (Silveira et al., 2016). All selected strains were HCN positive at the same level as other isolates such as *Pseudomonas* sp., identified as the most efficient bacteria by the production of hydrogen cyanide (HCN) (Ahmed and Holmström, 2014; Yien Ting et al., 2014; Thampi and Bhai, 2017). In the present investigation, the isolate was potentially able to produce HCN in plate agar. This allelochemical is a secondary metabolite commonly produced by other bacteria such as *P. fluorescens, P. aeruginosa*, and *Chromobacterium violaceum* (Thampi and Bhai, 2017) and appeared to be active against *Sclerotium rolfsii* and *R. solani* (Silveira et al., 2016).

3.6.3. Siderophore production

Many problems of nutrient accumulation in soil, especially acidic soil, were concerned, that iron deficiency limited plant growth in that type of soil. In recent study, siderophores production by actinomycetes focused on its ability to indirectly promote plant growth by capturing ferric iron in soil and provides nutrients by the specific uptake system to stimulate plant growth factors like (Tamreihao et al., 2016). Many researchers have reported that siderophore production by actinomycetes is also one of the microbial mechanisms to improve phosphorus availability to plants, thus enhancing the growth and yield (Ramyasmruthi et al., 2012; Tamreihao et al., 2016). Microorganisms have evolved specialized mechanisms for the assimilation of iron, including the production of low molecular weight iron-chelating compounds known as siderophores, which transport this element into their cells (Ahmed and Holmström, 2014). The production of siderophores is a PGP feature that may play an important role in biocontrol and to mobilize phosphorus by mineral chelation (Hamdali et al., 2008a).

Siderophores solubilize and sequester iron from the soil, but they may also play an indirect role in plant growth promotion (Park et al., 2011). The CAS test has shown that all the strains produced the siderophores. The most efficient producers of siderophores as judged by the size of the zone and the intensity of the color change of the Cas-Agar were: S1, AH4, AH5 (Fig. 4; Table 4), whereas the strains S2, S3 secreted little followed by AH6. However the reference strains RER4 and MVA1 produced little siderophores. These molecules have high affinity for iron ion Yien Ting et al. (2014). Our study demonstrated that all selected fungi and actinomycetes strains excrete chelator as revealed by the blue CAS-agar test. Our finding is in agreement with Haselwandter et al. (1992); Sujatha and Ammani (2013); Gaonkar and Bhosle (2013); Ahemad and Kibret (2014), who confirmed that most bacteria and mycorrhizal fungi are capable of producing siderophores containing a variety of functional groups under pure culture conditions. Subsequently this has a benefit effect on ericaceous plant growth.

The literature describes the role of siderophores that can solubilize the sequestered iron from the soil (Hamdali et al., 2008a). They also play a role in mobilizing heavy metals because they show a great affinity for heavy metal ions, which increases phytoextraction capacity (Sujatha and Ammani, 2013; Gaonkar and Bhosle, 2013). The production of siderophores by microorganisms can be beneficial to plants in two ways. First, siderophore formation can solubilize iron previously unavailable to the plant (Ramyasmruthi et al., 2012; Gull and Hafeez, 2012; Schindler et al., 2017). Secondly, siderophore production by nonpathogenic microorganisms can also suppress growth of pathogenic microorganisms by depriving the pathogens of iron (Prabhu et al., 1996; Ramyasmruthi et al., 2012). Besides, siderophores form stable complexes with phosphorus adsorbents (aluminium, iron and calcium) and thus increase phosphate solubilization.

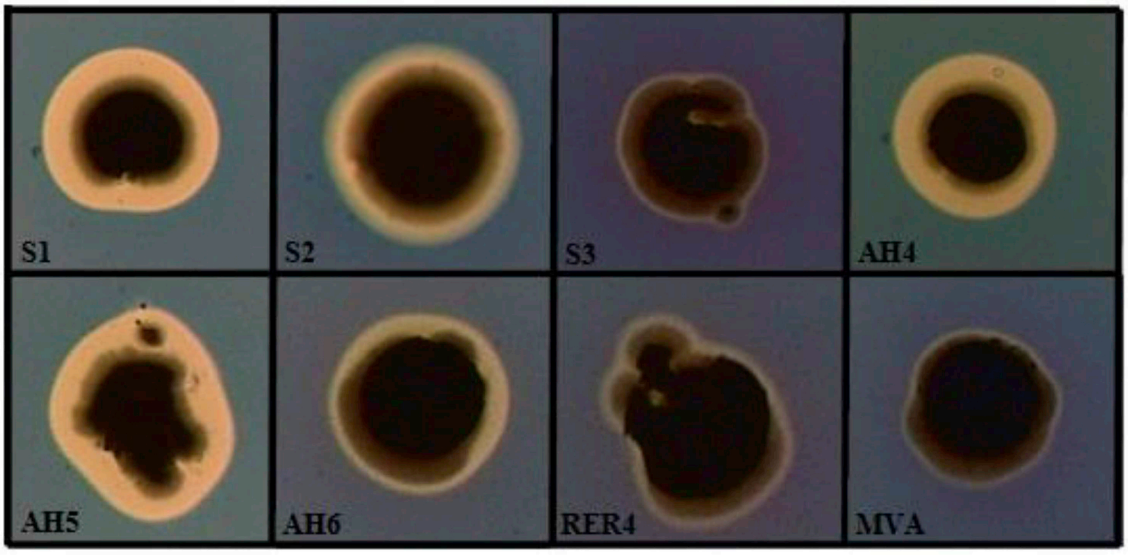

Fig. 4. Siderophore revelation on CAS-blue agar plate for selected strains. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Biochemical tests of selected isolates.

Isolates	Halos (cm)	HCN	Siderophores	phosphatase	IAA (µg/l)	P (mg/l)	pH
S1	+ 0 .1 (± 0.0)	+ + +	+ + +	+ +	10.8a (± 1.6)	18.1a (± 0.1)	5.8a (± 0.1)
S2	+ + 0.8 (± 0.4)	+ + + +	+ +	+ + +	40.7b (± 0.9)	62.2b (± 0.0)	5.7a (± 0.1)
S3	+ 0.3 (± 0.0)	+ + +	+ +	+ +	67.8c (± 1.2)	30 a (± 0.1)	5.6a (± 0.1)
RER4	+ 0.7 (± 0.2)	-	+	+ + +	11.3a (± 1.9)	123.2 c (± 0.1)	5.9a (± 0.0)
RER6	+ 0.7 (± 0.1)	-	+	+ +	20.7a (± 3.5)	104.5c (± 0.1)	5.5a (± 0.0)
MVA1	+ 0.1 (± 0.7)	-	+	+ +	40.5b (± 1.50)	103 c (± 0.5)	5.8 a (± 0.0)
AH4	+ + + 1.6 (± 0.1)	+ + +	+ + +	+ + +	80.3d (± 1.9)	98.8c (± 0.1)	6.5b (± 0.0)
AH5	+ 0.1 (± 0.0)	+ +	+	+ + +	64.8c (± 1.6)	94.2 c (± 0.1)	6 a (± 0.0)

Values are means of triplicates. Means in the same column followed by the same letter are not significantly different at $p > 0.05$ Newman-Keuls test; ± values indicate standard errors of the means.

Mycorrhizal plants harbour higher population of microorganisms in the rhizosphere, thus making it difficult for the pathogen to compete and gain access to the root (Bagyaraj, 2014). Microorganisms producing siderophores, chelating agents that have high affinity for ferric iron and thus fungistatic to many pathogens, were observed in higher numbers in the rhizosphere of mycorrhizal plants (Bagyaraj, 2014). In our case, it would be beneficial to inoculate siderophore producer strains to *Vaccinium corymbosum,* a plant belonging to the ericaceous family, to form an ericoid mycorrhizal symbiosis which is sensitive to iron chlorosis, growth in acid soil and prevent from pathogens (Liu et al., 2017).

Many authors have confirmed the ability of actinobacteria to enhance plant growth directly or indirectly by their multiple plant growth promoting properties (Hamdali et al., 2008b; Nadeem et al., 2013). The genus *Streptomyces* is known as one of the major sources of bioactive natural products (Calvaruso et al., 2013; Devadass et al., 2016). In addition, about 75% of natural antibiotics that are isolated from actinomycetes were produced by streptomyces genus (Rajivgandhi et al., 2016).

Besides, the mycorrhizal fungi (ErM) constitute another key group of soil-borne microorganisms known to play a critical role on agricultural sustainability especially after forming a symbiosis with roots of ericaceous species. The involvement of mycorrhizal fungi in plant nutrition has been the most studied function of mycorrhizae. ErM fungi positively influence growth, competitiveness of their host species by enhancing nutrient uptake. In particular, a great number of reports dealed with mineral nutrient uptake, Devin and Leopol (2016); Wahbi et al. (2016). In general, mycorrhizal fungi influence and are influenced by the activities of other microorganisms in soil (Thakur et al., 2007; Legay et al., 2016; Bizabani et al., 2016; Drigo and Donn, 2017). Many studies have reported that mycorrhizal plants harboring actinomycetes. Therefore, the actinomycetes and mycorrhiza consortium might produce an efficient biofertilizer specific to develop Ericaceous shrubs adapted to Mediterranean forest soil.

4. Conclusions

The present work contributes to evaluate the ericoid fungi and actinobacteria from roots and rhizospheric soil of *Calluna vulgaris* L. The results make several isolates attractive as phosphate solubilizers and throw light on the phosphate solubilizing genes that could be further studied to find out the mechanism of this process. Our results emphasize the importance of some selected strains characterized by several phytobenefic activities *in vitro* and provide a promoting plant growth potential through the release of indole acetic acid (IAA), siderophores and the production of hydrogen cyanide (HCN) such as for the S2, AH6 and AH4 isolates, and the possible exploitation of the results for biotechnological applications. However, further studies are required to assess their effectiveness and confirm their role as biofertilizers.

Conflicts of interest

State any potential conflicts of interest here or “The authors declare no conflict of interest".

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References

Ahemad, M., Kibret, M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J. King Saud Univ. Sci. 26, 1.
Ahmed, E., Holmström, S.J.M., 2014. Siderophores in environmental research: roles and applications. Microb. Biotech. 7, 196.
Ames, B.N., 1966. Assay of inorganic phosphate, total phosphate and phosphatases. Methods Enzymol. 8, 115.
Bagyaraj, D.J., 2014. Mycorrhizal fungi. Proc Indian Natn Sci Acad Spl. Sec. 80, 415.
Bakker Albert, W., Schippers, B., 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp-mediated plant growth-stimulation. Soil Biol. Biochem. 4, 19–451.
Balemi, T., Negisho, K., 2012. Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production. J. Soil Sci. Plant Nutr. 12, 547.
Behera, B.C., Himendra, Y.H., Singh, S.K., Sethi, B.K., Mishra, R.R., Kumari, S., Thatoi, H., 2017. Alkaline phosphatase activity of a phosphate solubilizing *Alcaligenes faecalis*, isolated from Mangrove soil. Biotechnology 1 (1), 101–111. https://doi.org/10.1016/j.biori.2017.01.003.
Belyagoubi, L., 2014. Antibiotiques produits par des bactéries (actinomycètes et bactéries lactiques) issus de différents écosystèmes naturels Algériens. PhD thesis. University of Aboubakr Belkaïd-Tlemcen, Alger, Algeria.
Bizabani, C., Fontenla, S., Dames, J.F., 2016. Ericoid fungal inoculation of blueberry under commercial production in South Africa. Sci. Hortic. (Canterb.) 209, 173–177.
Bizuye, A., Moges, F., Andualem, B., 2013. Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town, North West Ethiopia. Asian Pac. J. Trop. Dis. 3, 375.
Bruzone, S., Fontenla, B., Vohník, M., 2014. Is the prominent ericoid mycorrhizal fungus *Rhizoscyphus ericae* absent in the Southern Hemisphere's Ericaceae? A case study on the diversity of root mycobionts in Gaultheria spp. from northwest Patagonia, Argentina. Mycorrhiza 25, 25.
Bruzone, S., Fontenla, B., Vohník, M., 2015. Is the prominent ericoid mycorrhizal fungus

Rhizoscyphus ericae absent in the Southern Hemisphere's Ericaceae A case study on the diversity of root mycobionts in *Gaultheria* spp. from northwest Patagonia, Argentina. Mycorrhiza. https://doi.org/10.1007/s00572-014-0586-3.

Calvaruso, C., Turpault, M.P., Frey-Klett, P., Uroz, S., Pierret, M.C., Tosheva, Z., Kies, A., 2013. Increase of apatite dissolution rate by Scots pine roots associated or not with *Burkholderia glathei* PML1 (12) Rp in open-system flow microcosms. Geochem. Cosmochim. Acta 106, 287.

Debabrata, N., Bihari, R.M., Singh, V.M., 2017. Documentation of five potassium- and phosphorus-solubilizing bacteria for their K and P-solubilization ability from various minerals. Biocatal. Agric. Biotechnol. 10, 174.

Dereeper, A., Guignon, V., Blanc, G., 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36, 465.

Devadass, B.J., Paulraj, M.G., Ignacimuthu, S., Theoder, P.A.S., Dhabi, N.A.A., 2016. Antimicrobial activity of soil actinomycetes isolated from western ghats in Tamil nadu, India. J. Bacteriol. Mycol, Open Access 3 (2), 00059. https://doi.org/10.15406/jbmoa.2016.03.00059.

Devin, R., Leopol, D., 2016. Ericoid fungal diversity: challenges and opportunities for mycorrhizal research. Fungal Ecol. 24, 114.

Drigo, B., Donn, S., 2017. Trading carbon between arbuscular mycorrhizal fungi and their hyphae-associated microbes. In: Mycorrhizal Mediation of Soil, vol 22. pp. 395.

Franco-Correa, M., Quintana, A., Duque, C., Rodríguez, C.S.M.X., Barea, J.M., 2010. Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. Appl. Soil Ecol. 45, 209.

Gaonkar, T., Bhosle, S., 2013. Effect of metals on a siderophore producing bacterial isolate and its implications on microbial assisted bioremediation of metal contaminated soils. Chemosphere 93, 9.

Garbaye, J., 2013. La symbiose mycorhizienne: Une association entre les plantes et les champignons. Éditions QUAE, Versailles.

Gibson, B.R., Mitchell, D.T., 2004. Nutritional influences on the solubilization of metal phosphate by ericoid mycorrhizal fungi. Mycol. Res. 108, 947.

Gorzelak, M.A., Hambleton, S., Massicotte, H.B., 2012. Community structure of ericoid mycorrhizas and root-associated fungi of *Vaccinium membranaceum* across an elevation gradient in the Canadian Rocky Mountains. Fungal Ecol. 5, 36.

Gull, M., Hafeez, F.Y., 2012. Characterization of siderophore producing bacterial strain *Pseudomonas fluorescens* Mst 8.2 as plant growth promoting and biocontrol agent in wheat. Afr. J. Microbiol. Res. 6, 6308.

Hamdali, H., Bouizgane, B., Hafidi, M., Lebrihi, A., Virolle, M.J., Ouhdouch, Y., 2008a. Screening for rock phosphate solubilizing actinomyceles from Moroccan phosphate mines. Appl. Soil Ecol. 38, 12.

Hamdali, H., Hafidi, M., Virolle, Mj, Ouhdouch, Y., 2008b. Rock phosphate solubilizing Actinomycetes: screening for plant growth promoting activities. World J. Microbiol. Biotechnol. https://doi.org/10.1007/s11274-008-9817-0.

Hamim, A., Miché, L., Douaik, A., Mrabet, R., Ouhammou, A., Duponnois, R., Hafidi, M., 2017. Diversity of fungal assemblages in roots of Ericaceae in two Mediterranean contrasting ecosystems. C. R. Biol. 340, 226–237. doi.org/10.1016/j.crvi.2017.02.003.

Haselwandter, K., Dobemigg, B., Beck, W., Jung, G., Cansiert, A., Winkelmannt, G., 1992. Isolation and identification of hydroxamate siderophores of ericoid mycorrhizal fungi. Biometals 5, 51.

Hazard, C., Gosling, P., Mitchell, D.T., Doohan, F.M., Bending, G.D., 2014. Diversity of fungi associated with hair roots of ericaceous plants is affected by land use. FEMS Microbiol. Ecol. 87, 586–600 PMID: 24741702.

Khan, A., Halo, B.A., Elyassi, A., Ali, S., Al-Hosni, K., Hussain, J., Al-Harrasi, A., Lee, I., 2016. Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*, Electron. J. Biotechnol. 21, 58.

Kjeldahl, J., 1883. Neue Methode zur Bestimmung des Stickstoffs in organisch en Körpern. Z. für Anal. Chem. 22, 36.

Legay, N., Grassein, F., Binet, M.N., Arnoldi, C., Personeni, E., Perigon, S., Poly, F., Pommier, T., Puissant, J., Clément, Jc, Lavorel, S., Mouhamadou, B., 2016. Plant species identities and fertilization influence on arbuscular mycorrhizal fungal colonisation and soil bacterial activities. Appl. Soil Ecol. 98, 132.

Lin, L.C., Lee, M.J., Chen, J.L., 2011. Decomposition of organic matter by the ericoid mycorrhizal endophytes of Formosan rhododendron (*Rhododendron formosanum* Hemsl.). Mycorrhiza 21, 331.

Liu, X., Fu, J.W., Da Silva, E., Shi, X.X., Cao, Y., Rathinasabapathi, B., Chen, Y., Ma, L.Q., 2017. Microbial siderophores and root exudates enhanced goethite dissolution and Fe/As uptake by As-*hyperaccumulator Pteris vittata*. Environ. Pollut. 223, 230.

Marx, D.H., Bryan, W.C., 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infected with the fungal symbiont *Pisolithus tinctorius*. For. Sci. 21, 245.

Matínez-Viveros, O., Jorquera, M.A., Crowley, D.E., Gajardo, G., Mora, M.L., 2010. Mechanisms and practical considerations involved in plant growth promotion by *rhizobacteria*. J. Soil Sci. Plant Nutr. 10, 293.

Nadeem, S.M., Naveed, M., Zahir, Z.A., Asghar, H.N., 2013. Plant-microbe interactions for sustainable agriculture: fundamentals and recent advances. In: Arora, N.K. (Ed.), Plant Microbe Symbiosis: Fundamentals and Advances. Springer, India, pp. 51.

Napier, R.M., 2017. Auxins. In: Thomas, Brian, Murray, Brian G., Murphy, Denis J. (Eds.), Encyclopedia of Applied Plant Sciences, second ed. Academic Press, Oxford, pp. 367.

Olsen, S.R., Sommers, L.E., 1982. Phosphorus. In: Page, A.L., Miller, R.H., Keeny, D.R. (Eds.), Methods of Soil Analysis, Part **2**. Madison. Am Soc Agr, pp. 403.

Park, J.H., Bolana, N.B., Megharaja, M., Naidua, R., 2011. Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. J. Hazard Mater. 185, 829.

Pikovskaya, R.I., 1948. Mobilization of phosphorous in soil in connection with vital activity of some microbial species. Microbiologiya 17, 362.

Prabhu, V., Biolchini, P.F., Boyer, G.L., 1996. Detection and identification of ferricrocin produced by ectendomycorrhizal fungi inthe genus *Wilcoxina*. Biometals 9, 229.

Rajivgandhi, G., Vijayan, R., Kannan, M., Santhanakrishnan, M., Manoharan, N., 2016. Molecular characterization and antibacterial effect of endophytic actinomycetes *Nocardiopsis sp*. GRG1 (KT235640) from brown algae against MDR strains of *uropathogens*. Bioact. Mater. 1, 140.

Ramasamy, K., Kiyoon, K., Parthiban, S., Murugaiyan, S., Rangasamy, A., Tongmin, S., 2016. Arbuscular mycorrhizal fungi and associated bacteria isolated from salt-affected soil enhances the tolerance of maize to salinity in coastal reclamation soil. Agric. Ecosyst. Environ. 231–233.

Ramyasmruthi, S., Pallavi, O., Pallavi, S., Tilak, K., Srividya, S., 2012. Chitinolytic and secondary metabolite producing *Pseudomonas fluorescens* isolated from *Solanaceae* rhizosphere effective against broad spectrum fungal phytopathogens. Asian J. Plant Sci. Res. 2, 16.

Rashad, F.M., Fathy, H.M., El-Zayat, A.S., Elghonaimy, A.H., 2015. Isolation and characterization of multifunctional *Streptomyces* species with antimicrobial, nematicidal and phytohormone activities from marine environments in Egypt. Microbiol. Res. 175, 34.

Rey, T., Dumas, B., 2017. Plenty is No plague: streptomyces symbiosis with crops. Trends Plant Sci. 22, 30.

Rodríguez-Caballero, G., Caravaca, F., Fernández-González, A.J., Alguacil, M.M., Fernández-López, M., Roldán, A., 2017. Arbuscular mycorrhizal fungi inoculation mediated changes in rhizosphere bacterial community structure while promoting revegetation in a semiarid ecosystem. Sci. Total Environ. 838, 584–585.

Schindler, F., Merbold, L., Karlsson, S., Sprocati, A.R., Kothe, E., 2017. Seasonal change of microbial activity in microbially aided bioremediation. J. Geochem. Explor. 174, 4.

Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160, 47.

Sharma, S., Kumar, V., Tripathi, R.B., 2011. Isolation of phosphate solubilizing microorganism (PSMs) from soil. J. Microbiol. Biotechnol. Res. 1, 90.

Shilev, S., 2013. Soil rhizobacteria regulating the uptake of nutrients and undesirable elements by plants. In: In: Arora, N.K. (Ed.), Plant Microbe Symbiosis: Fundamentals and Advances, vol 147. Springer, India, pp. 50.

Silveira, D., Parada, A., Sala, R., Marino, V., Cardoso, B.N., Jurandy, E., Godoy, E.L., Cipriano, P., Aparecido, M., 2016. Nitrogen metabolism and growth of wheat plant under diazotrophic endophytic bacteria inoculation. Appl. Soil Ecol. 107, 313.

Sipahutar, M.K., Vangnai, A.S., 2017. Role of plant growth-promoting *Ochrobactrum* sp. MC22 on triclocarban degradation and toxicity mitigation to legume plants. J. Hazard Mater. 329, 38.

Sujatha, N., Ammani, K., 2013. Siderophore production by the isolates offluorescent *Pseudomonads*. Int. J. Cur. Res. Rev. 5, 1.

Tamreihao, K., Ningthoujam, D.S., Nimaichand, S., Singh, E.S., Reena, P., Singh, S.H., Nongthomba, U., 2016. Biocontrol and plant growth promoting activities of a *Streptomyces corchorusii strain UCR3-16* and preparation of powder formulation for application as biofertilizer agents for rice plant. Microbiol. Res. 192, 260.

Tamura, K., Dudley, J., Nei, M., kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596.

Thakur, D., Yadav, A., Gogoi, B.K., Bora, T.C., 2007. Isolation and screening of *Streptomyces* in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. J. Mycol. Med. 17, 242–249.

Thampi, A., Bhai, R.S., 2017. Rhizosphere actinobacteria for combating Phytophthora capsici and Sclerotium rolfsii, the major soil borne pathogens of black pepper (*Piper nigrum L.*). Biol. Control 109, 1.

Vohník, M., Albrechtová, J., 2011. The co-occurrence and morphological continuum between ericoid mycorrhiza and dark septate endophytes in roots of six European *Rhododendron species*. Folia Geobot. 46, 373.

Vohník, M., Sadowsky, J.J., Kohout, P., Lhotáková, Z., Nestby, R., Kolařík, M., 2012. Novel root-fungus symbiosis in Ericaceae: sheathed ericoid mycorrhiza formed by a hitherto undescribed basidiomycete with affinities to trechisporales. PLoS One 7 (6), e39524. https://doi.org/10.1371/journal.pone. 0039524.

Vohnik, M., Mrnka, L., Lukesova, T., Bruzone, M.C., Kouhout, P., Fehrer, J., 2013. The cultivable endophytic community of Norway spurce ectomycorrhizass from microhabitas lacking ericaceous hosts is dominated by ericoid mycorrhizal *Meliniomyces variabilis*. Fungal Ecol. 6, 281.

Wahbi, S., Maghraoui, S., Hafidi, M., Sanguin, H., Oufdou, K., Prin, Y., Duponnois, R., Galiana, A., 2016. Enhanced transfer of biologically fixed N from faba bean to intercropped wheat through mycorrhizal symbiosis. Appl. Soil Ecol. 107, 91.

Walker, J.F., Aldrich-Wolfe, L., Riffel, A., Barbare, H., Simpson, N.B., Trowbridge, J., Jumpponen, A., 2011. Diverse Helotiales associated with the roots of three species of Arctic *Ericaceae* provide no evidence for host specificity. New Phytol. 191, 515.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfland, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: a Guide to Methods and Applications. Academic Press, San Diego, CA, USA, pp. 315.

Wurzburger, N., Higgins, B.P., Hendrick, R.L., 2011. Ericoid mycorrhizal root fungi and their multicopper oxidases from a temperate forest shrub. Ecol. Evol. https://doi.org/10.1002/ece3.67.

Yien Ting, A.S., Hermanto, A., Peh, K.L., 2014. Indigenous actinomycetes from empty fruit bunch compost of oil palm: evaluation on enzymatic and antagonistic properties, Biocatal. Agric. Biotechnol. 3, 310.

Zhang, Y., Ni, J., Tang, F., Pei, K., Luo, Y., Jiang, L., Lifu Sun, L., Liang, Y., 2016. Root-associated fungi of *Vaccinium carlesii* in subtropical forests of China: intra- and inter-annual variability and impacts of human disturbances. Sci. Rep. 6, 22399. https://doi.org/10.1038/srep22399.