*Research Article*

# Plant Growth Promotion by Endophytic Bacteria Isolated from Rice (*Oryza sativa*)

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## Abstract

Endophytic bacteria are widely studied because of their plant-growth-promoting benefits. One hundred and twenty-six endophytic bacteria were isolated from rice roots and stems in this study. Based on their partial 16S rRNA gene sequences, they were characterized as members of phyla *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*. Among these, isolates 1017, 1048 and 3037 likely represented novel bacterial species of genera *Pedobacter*, *Sphingomonas* and *Paenibacillus*, respectively, based on their relatively low sequence similarities (<98.5%) with recognized bacterial species. All isolates were tested for their growth promotion in rice seedlings *in vitro*. The increases of fresh weight ranging from 2.30 to 3.18 fold were observed in rice seedlings that were inoculated with twelve bacterial isolates when compared to the water-treated control group. These isolates were members of genera *Bacillus* (ten isolates), *Micrococcus* (one isolate) and *Acinetobacter* (one isolate). The presence of *nifH*, siderophore production, indole-3-acetic acid (IAA) synthesis and ACC-deaminase activity were determined in these twelve isolates. The most common characteristic was the *nifH* gene that was detected in five isolates. The result obtained in our study demonstrated the diversity of endophytic bacteria in rice and their potential application as biofertilizers.

**Keywords**: ACC deaminase; endophytic bacteria; IAA, *nifH*, *Oryza sativa*; plant growth promotion; siderophores

## 1. Introduction

Plant-microbe interactions have been a major area of study in both microbiology and plant biology. While phytopathogenic bacteria detrimentally affect the plant host by causing diseases, a larger number of bacteria have positive effects on plant growth. The latter group is termed plant-growth-promoting bacteria (PGPB). PGPB that colonize intercellular spaces in internal tissues of the plant host are also recognized as endophytic bacteria [1-3].

Increasing the nutrient availability for the plant uptake is one of the direct mechanisms through which endophytic bacteria exert their positive effects on the plant host. Nitrogen is one of the major nutrients and an important limiting factor for plant growth and development [4]. Bacteria produce the nitrogenase enzyme to convert atmospheric nitrogen into nitrogenous compounds that are readily available for the plant uptake. Nitrogenase is composed of the dinitrogenase reductase subunit encoded by *nifH* and the heterotetrameric component

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encoded by *nifD* and *nifK* [5]. Because *nifH* is conserved among various groups of bacteria, the presence of the gene is also used to indirectly investigate the nitrogen-fixing activity [4]. Previous studies demonstrated the increase of growth and the nitrogen content of rice plants when the plants were co-cultivated with nitrogen-fixing bacteria [6-7]. Siderophore biosynthesis is another mechanism which bacteria employ to increase the iron uptake by plants because iron is mostly found in the insoluble ferric ($Fe^{3+}$) form [8-9]. In a previous study, inoculation of a siderophore-producing bacterium reduced the symptoms of iron deficiency in mung bean plants that were grown in iron-deficient soil [10].

Another type of plant-growth-promoting mechanism by endophytic bacteria is the modulation of the phytohormones auxin and ethylene. IAA enhanced plant growth and is also produced by bacteria in several genera [8]. Previous studies showed that inoculation of IAA-producing bacteria was able to induce expression of auxin-responsive genes as well as promote plant growth compared to the non-inoculated control group [13-15]. Additionally, strains of endophytic bacteria are capable of producing ACC deaminase that converts 1-aminocyclopropane-1-carboxylic (ACC) acid, the precursor of ethylene biosynthesis, into ammonia and α-ketobutyrate. This decreases the ethylene level and prevents overproduction of ethylene that leads to growth reduction in plants [11-12]. A report showed that enhancement of salt tolerance in maize was achieved by inoculating the plants with ACC-deaminase-producing endophytic bacteria [16].

We reported here molecular characterization of endophytic bacteria isolated from roots and stems of rice plants. Plant growth promotion was determined in rice seedlings *in vitro*. The presence of the *nifH* gene as well as other plant-growth-promoting activities including production of siderophore, IAA and ACC deaminase were also examined.

## 2. Materials and methods

### 2.1 Bacterial isolation

Samples of rice plants were collected from Bangkok, Chonburi and Supanburi provinces, Thailand. Roots and stems were cleaned with running tap water and cut into small pieces. Surface sterilization was performed using 10% (v/v) NaHClO that was added with a few drops of Tween-20. Samples were rinsed with sterilized distilled water five times and ground using a mortar and a pestle. Ground tissues were placed in glass bottles containing sterilized distilled water and shaken on a rotary shaker to obtain bacterial suspension. Serial dilutions of the suspension was prepared up to the $10^{-3}$ concentration and plated on nutrient agar (NA; HiMedia) and tryptone soya agar (TSA; HiMedia) plates. Bacterial isolates were obtained after incubation at 30°C for 7 days. All isolates were purified and further grown on NA plates. Control plates were obtained by plating the water that was used for the final rinse on NA and TSA plates.

### 2.2 DNA extraction and amplification of the 16S rRNA gene

Bacterial cells were grown on NA plates and scraped off for DNA extraction using a GF-1 Bacterial DNA Extraction Kit (Vivantis, Malaysia) according to the manufacturer's protocol. Bacterial DNA was used for amplification of the nearly complete 16S rRNA gene with the universal primers 41F and 1492R [17].

### 2.3 DNA sequencing and pairwise alignment analysis

16S rRNA gene PCR products were purified using a Gel/PCR Purification Kit (Favorgen Biotech Corp, Taiwan) according to the protocol provided by the manufacturer. DNA fragments were partially sequenced with the primer 1492R. The pairwise alignment analysis of partial 16S rRNA gene sequences of all isolates was performed on the EzTaxon database [18].

**2.4 Phylogenetic analysis**

Almost-complete 16S rRNA gene fragments of isolates 1017, 1048 and 3037 were sequenced using universal primers 41F, 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 800R (5′-TACCAGGGTATCTAATCC-3′). 16S rRNA gene sequences of known bacterial species were obtained from the GenBank database. These sequences were subjected to the multiple alignment analysis using Clustal W. Gaps and ambiguous bases were manually removed and adjusted. The neighbour-joining method [19] was used to reconstruct the phylogenetic tree, and the Kimura 2-parameter model [20] was used to calculate evolutionary distances. The confidence level of each clade was determined using the bootstrap analysis based on 1,000 re-samplings [21].

**2.5 Plant growth promotion**

Endophytic bacteria were grown on NA plates at 30ºC for two days. Bacterial cells were scraped off the medium and re-suspended in sterilized distilled water. The cell concentration was adjusted to McFarland standard No. 0.5. Rice seeds were washed thoroughly with soap and rinsed under tap water. Seeds were surface-sterilized with 95% ethanol for 10 minutes and dried on sterilized filter paper. Subsequently, they were soaked in 50 mL of sterilized distilled water supplemented with one mL of 10% providone-iodine for two hours and rinsed with sterilized distilled water again. Ten seeds were inoculated with each bacterial suspension for two hours and then transferred to a glass bottle containing 0.6% agar. Seeds were kept in the dark for three days and subsequently grown in the growth chamber with the growth conditions of 25$\pm$2ºC and the 16hr/8hr (light/dark) cycle for an additional 16 days. Seedlings were removed from the medium and measured for fresh weight. The control group was prepared by submerging rice seeds in sterilized distilled water. Growth promotion by bacteria was determined as the ratio of the fresh weights of the bacterium-inoculated group to the control group. All experiments were performed in triplicates. Statistically significant differences between means were determined using Duncan's Multiple Range Test at the significant level of $P$=0.05.

**2.6 Amplification of partial *nifH***

Amplification of the partial *nifH* gene fragment was carried out with bacterial genomic DNA using the 19F and 407R primers as described by Ji *et al.* [4]. The temperature conditions were as follow: 94ºC, 3 min; 30 cycles of 1 min at 94ºC, 1 min at 50ºC, and 50 sec at 72ºC; 72ºC for 5 min. PCR products were examined using electrophoresis in 1.5% agarose gel.

**2.7 Production of siderophores**

Endophytic bacteria were grown on chrome azurol S (CAS) agar plates [22] and incubated at 30ºC for seven days. The presence of yellow or orange halo around bacteria indicated the positive result.

**2.8 IAA production**

Nutrient broth (NB; HiMedia, India) containing 5mM L-tryptophan was inoculated with bacteria and incubated at 30ºC for 48 hours on the rotary shaker. Two hundred µL of the culture supernatant was obtained by centrifugation and tested for IAA production using the Salkowski's reagent [23].

**2.9 ACC deaminase production**

Bacterial isolates were grown on NA plates at 30ºC for 48 hours. Subsequently, cells were scraped off the medium and washed with one mL of Dworkin and Foster (DF) salt minimal medium [24] by resuspension and centrifugation. Bacterial cells were collected and used to prepare bacterial suspension in one mL of DF salt minimal medium. Two µL of the suspension were inoculated on DF salt minimal agar containing 2mM 1-aminocyclopropane-1-carboxylic acid as the sole nitrogen source. Plates were incubated at 30ºC for four days. Positive bacterial isolates were determined based on their ability to utilize ACC as the nitrogen source. Negative and positive control groups were obtained by inoculating the suspension on DF salt minimal

agar and DF salt minimal agar supplemented with 2mM $(NH_4)_2SO_4$, respectively.

## 3. Results and Discussions

### 3.1 Bacterial isolation and molecular characterization

A total number of 126 culturable bacterial isolates were obtained from the samples in this study. Seventy-two and 54 isolates were obtained from roots and stems, respectively (Table 1). They were initially characterized based on the pairwise-alignment analysis of the partial 16S rRNA gene sequences using the EzTaxon database. The results showed that they were classified into four phyla and 31 genera. In roots, the majority (66.7%) of the isolates were members in the phylum *Firmicutes*. It consisted of five different genera, and the predominant group (36 isolates) belonged to the genus *Bacillus*. The second largest phylum (19.44%) was *Proteobacteria*. Isolates in this phylum belonged to ten different genera with *Acinetobacter* as the major genus (four isolates). 12.50% and 1.39% of the isolates were members in phyla *Bacteroidetes* (four genera) and *Actinobacteria* (one genus), respectively. In contrast, *Proteobacteria* (eleven genera) and *Firmicutes* (five genera) constituted the largest and the second-largest groups in stems. *Burkholderia* (six isolates), *Acinetobacter* (five isolates), *Pseudomonas* (five isolates) and *Pantoea* (four isolates) were found as the predominant genera of the phylum *Proteobacteria* while *Bacillus* (eleven isolates) was the major genus of the phylum *Firmicutes.* The remaining isolates were composed of bacteria that belonged to phyla *Actinobacteria* (5.56%; two genera) and *Bacteroidetes* (3.70%; two genera).

**Table1.** Affiliation of endophytic bacteria isolated from rice stems and roots. Numbers in parentheses represent the numbers of isolates that were classified as members in each genus.

| Phylum | Genus | |
|---|---|---|
| | **Roots** | **Stems** |
| *Actinobacteria* | *Micrococcus* (1) | *Curtobacterium* (1), *Microbacterium* (2), |
| *Bacteroidetes* | *Chryseobacterium* (6), *Flavobacterium* (1), *Myroides* (1), *Pedobacter* (1) | *Chryseobacterium* (1), *Mucilaginibacter* (1), |
| *Firmicutes* | *Bacillus* (36), *Fictibacillus* (3), *Halobacillus* (1), *Paenibacillus* (2), *Staphylococcus* (6) | *Bacillus* (11), *Fictibacillus* (3), *Lactococcus* (2), *Lysinibacillus* (1), *Staphylococcus* (3) |
| *Proteobacteria* | *Acinetobacter* (4), *Citrobacter* (1), *Cronobacter* (1), *Dickeya* (1), *Enhydrobacter* (2), *Enterobacter* (1), *Escherichia* (1), *Novosphingobium* (1), *Pseudomonas* (1), *Sphingomonas* (1) | *Acinetobacter* (5), *Aeromonas* (2), *Burkholderia* (6), *Enterobacter* (1), *Klebsiella* (1), *Novosphingobium* (1), *Ochrobactrum* (2), *Pantoea* (4), *Pseudacidovorax* (1), *Pseudomonas* (5), *Sphingomonas* (1) |

Differences in the diversity of endophytic bacteria between previous studies have been observed. Using the culture-independent method, Sun *et al*. reported the composition of endophytic bacteria from rice roots that were collected in China [25]. They found that the majority (59.26%) of the bacteria belonged to the genera *Gallionella* and *Burkholderia* of the phylum *Proteobacteria.* Other phyla including *Firmicutes*, *Bacteroidetes*, *Deinococcus-Thermus* and *Acidobacteria* were found as minor groups. Although a metagenomic study indicated that the predominant root colonizers of rice plants collected in Brazil were also members of the phylum *Proteobacteria*, the predominant genera in this study were *Enterobacter* and *Rhizobium* [26]. In our

study, 66.67% (48 isolates) of endophytic bacteria from roots were affiliated with the phylum *Firmicutes* while the predominant group in stems was related to the phylum *Proteobacteria*. Previous studies have demonstrated the effects of various factors on the number and the diversity of endophytic bacteria in plants. These included environmental conditions, plant species, plant cultivars and phytopathogens [27-31]. We surmised that the discrepancy between endophytic bacterial compositions found in the previous studies and the present study was partly due to the differences in environmental conditions because rice samples were grown in geographically different countries.

Almost all isolates showed the similarity values higher than 98.5% with recognized bacterial species. However, relatively low sequence similarities were observed between isolates 1017, 1048 and 3037 and *Pedobacter tournerimensis* TF5-37.2-LB10$^T$ (96.6% sequence similarity), *Sphingomonas changbaiensis* NBRC 104936$^T$ (97.4%) and *Paenibacillus vulneris* CCUG 53270$^T$ (98.2%), respectively. Stackebrandt and Ebers reported that 16S rRNA gene sequence similarities below 98.7% were highly correlated with the DNA-DNA relatedness lower than 70% [32] that has been established as the threshold to assign bacterial strains to different species [33]. The low levels of sequence similarities observed in isolates 1017, 1048 and 3037 suggested that they may represent novel bacterial species in their corresponding genera. In order to understand their phylogenetic relationship with other recognized bacterial species, multiple-alignment and phylogenetic analyses using nearly-complete 16S rRNA gene sequences were carried out. The result obtained from reconstruction of the phylogenetic tree is shown in Fig.1. Isolates 1017 formed a distinct clade with *P. tournerimensis* TF5-37.2-LB10$^T$ and *Pedobacter xinjiangensis* 12157$^T$ with the bootstrap value of 67%. Isolate 1048 was placed in the same cluster with other *Sphingomonas* species. It was phylogenetically related to *S. changbaiensis* NBRC 104936$^T$, and this was significantly supported by the high bootstrap value of 87%. Isolate 3037 was found in the cluster consisting of *Paenibacillus* species. Its closest relatives were *P. vulneris* CCUG 53270$^T$ and *Paenibacillus rigui* WPCB173$^T$ at the 96% bootstrap value. Our result was consistent with several previous studies that demonstrated isolation of a number of novel bacterial species as endophytes of rice and other plants [34-37]. This suggested that plant internal tissues are an important isolation source for the study of microbial taxonomy.

In a previous study, *Pedobacter trunci*, *Pedobacter daejeonensis* and *Pedobacter silvilitoris* have been recovered from woody plants [38]. *Sphingomonas roseiflava*, *Sphingomonas rosa*, *Sphingomonas gei* are examples of *Sphingomonas* species that were described as endophytic bacteria [39-41]. However, up to the date of the preparation of this manuscript, there were no reports of novel bacterial species in genera *Pedobacter* and *Sphingomonas* that were initially characterized as endophytic bacteria of rice. In contrast, *Paenibacillus hunanensis* was the only species of the genus *Paenibacillus* that was isolated from internal tissues of rice seeds [42]. Thus, isolates 1017, 1048 and 3037 that were obtained in our study may represent additional novel bacterial species of genera *Pedobacter*, *Sphingomonas* and *Paenibacillus* that were able to colonize rice plants.

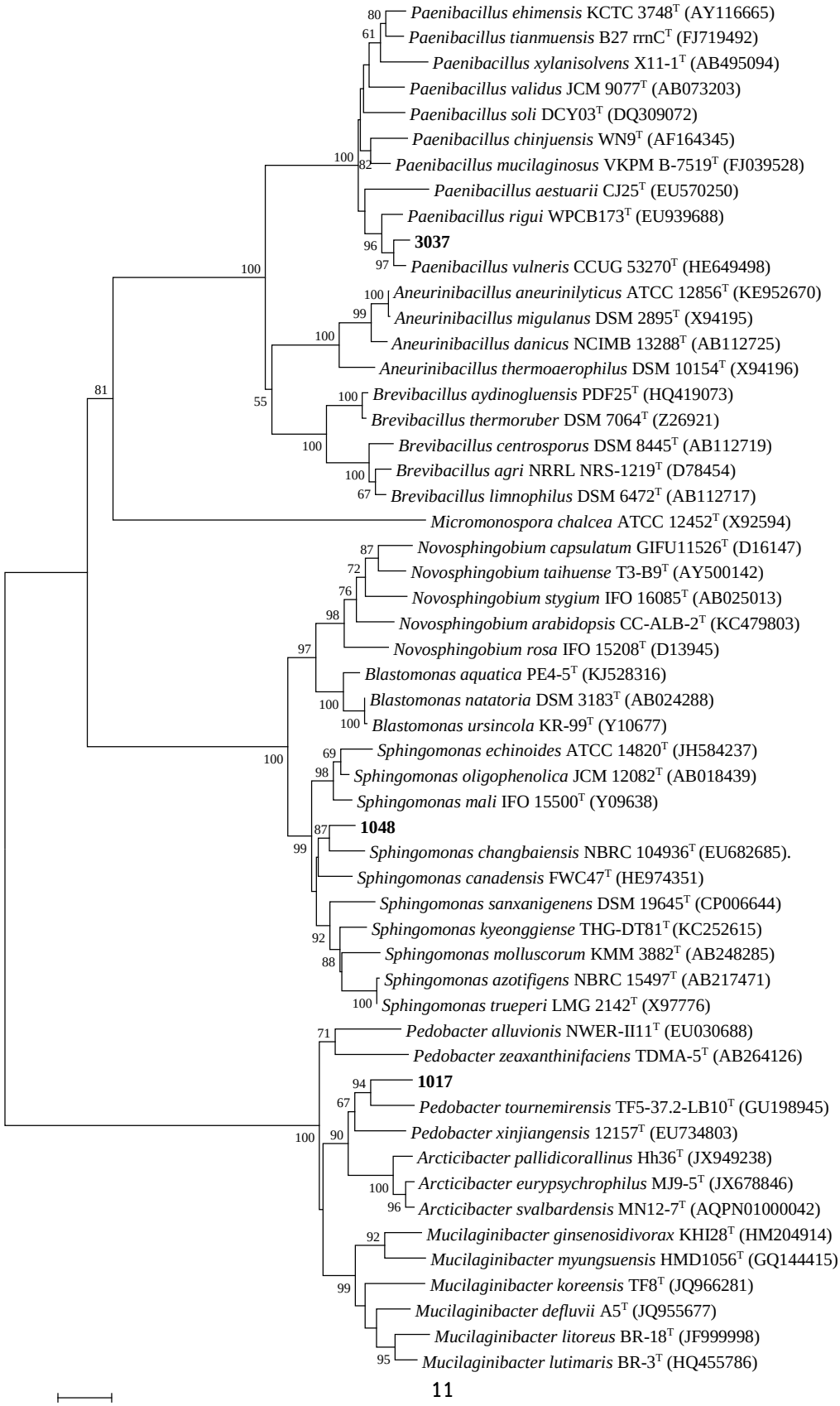
*Paenibacillus ehimensis* KCTC 3748$^T$ (AY116665)
*Paenibacillus tianmuensis* B27 rrnC$^T$ (FJ719492)
*Paenibacillus xylanisolvens* X11-1$^T$ (AB495094)
*Paenibacillus validus* JCM 9077$^T$ (AB073203)
*Paenibacillus soli* DCY03$^T$ (DQ309072)
*Paenibacillus chinjuensis* WN9$^T$ (AF164345)
*Paenibacillus mucilaginosus* VKPM B-7519$^T$ (FJ039528)
*Paenibacillus aestuarii* CJ25$^T$ (EU570250)
*Paenibacillus rigui* WPCB173$^T$ (EU939688)
**3037**
*Paenibacillus vulneris* CCUG 53270$^T$ (HE649498)
*Aneurinibacillus aneurinilyticus* ATCC 12856$^T$ (KE952670)
*Aneurinibacillus migulanus* DSM 2895$^T$ (X94195)
*Aneurinibacillus danicus* NCIMB 13288$^T$ (AB112725)
*Aneurinibacillus thermoaerophilus* DSM 10154$^T$ (X94196)
*Brevibacillus aydinogluensis* PDF25$^T$ (HQ419073)
*Brevibacillus thermoruber* DSM 7064$^T$ (Z26921)
*Brevibacillus centrosporus* DSM 8445$^T$ (AB112719)
*Brevibacillus agri* NRRL NRS-1219$^T$ (D78454)
*Brevibacillus limnophilus* DSM 6472$^T$ (AB112717)
*Micromonospora chalcea* ATCC 12452$^T$ (X92594)
*Novosphingobium capsulatum* GIFU11526$^T$ (D16147)
*Novosphingobium taihuense* T3-B9$^T$ (AY500142)
*Novosphingobium stygium* IFO 16085$^T$ (AB025013)
*Novosphingobium arabidopsis* CC-ALB-2$^T$ (KC479803)
*Novosphingobium rosa* IFO 15208$^T$ (D13945)
*Blastomonas aquatica* PE4-5$^T$ (KJ528316)
*Blastomonas natatoria* DSM 3183$^T$ (AB024288)
*Blastomonas ursincola* KR-99$^T$ (Y10677)
*Sphingomonas echinoides* ATCC 14820$^T$ (JH584237)
*Sphingomonas oligophenolica* JCM 12082$^T$ (AB018439)
*Sphingomonas mali* IFO 15500$^T$ (Y09638)
**1048**
*Sphingomonas changbaiensis* NBRC 104936$^T$ (EU682685).
*Sphingomonas canadensis* FWC47$^T$ (HE974351)
*Sphingomonas sanxanigenens* DSM 19645$^T$ (CP006644)
*Sphingomonas kyeonggiense* THG-DT81$^T$ (KC252615)
*Sphingomonas molluscorum* KMM 3882$^T$ (AB248285)
*Sphingomonas azotifigens* NBRC 15497$^T$ (AB217471)
*Sphingomonas trueperi* LMG 2142$^T$ (X97776)
*Pedobacter alluvionis* NWER-II11$^T$ (EU030688)
*Pedobacter zeaxanthinifaciens* TDMA-5$^T$ (AB264126)
**1017**
*Pedobacter tournemirensis* TF5-37.2-LB10$^T$ (GU198945)
*Pedobacter xinjiangensis* 12157$^T$ (EU734803)
*Arcticibacter pallidicorallinus* Hh36$^T$ (JX949238)
*Arcticibacter eurypsychrophilus* MJ9-5$^T$ (JX678846)
*Arcticibacter svalbardensis* MN12-7$^T$ (AQPN01000042)
*Mucilaginibacter ginsenosidivorax* KHI28$^T$ (HM204914)
*Mucilaginibacter myungsuensis* HMD1056$^T$ (GQ144415)
*Mucilaginibacter koreensis* TF8$^T$ (JQ966281)
*Mucilaginibacter defluvii* A5$^T$ (JQ955677)
*Mucilaginibacter litoreus* BR-18$^T$ (JF999998)
*Mucilaginibacter lutimaris* BR-3$^T$ (HQ455786)
0.02

**Fig.1.** Phylogenetic relationships based on nearly-complete 16S rRNA gene sequences between isolates 1017, 1048 and 3037 and other recognized bacterial species using the neighbour-joining method. The confidence level of each clade was determined by the bootstrap analysis based on 1,000 replicates. Only values higher than 60% are shown. Bar indicates 0.02 nucleotide substitutions.

### 3.2 Plant growth promotion by endophytic bacteria

Plant growth promotion was determined based on the ratio between the fresh weight of rice seedlings that were inoculated with endophytic bacteria and that of the control group. After 16 days of co-cultivation with endophytic bacteria, the fresh-weight ratios higher than two were observed in seedlings that were inoculated with twelve bacterial isolates (Table 2). Ten of the twelve isolates were members of the genus *Bacillus*, while isolates 3010 and 3014 were characterized as *Micrococcus* sp. and *Acinetobacter* sp., respectively. The highest ratio ($3.18\pm0.30$) was observed with isolate 3010.

Several bacterial strains belonging to genera *Bacillus*, *Micrococcus* and *Acinetobacter* have been demonstrated as plant-growth-promoting bacteria. For example, inoculation of *Bacillus oryzicola* YC7007$^{T}$, isolated from rice roots, increased the germination rates and the number of tillers of rice plants [35]. A bacterial strain that was characterized as *Micrococcus* sp. was found promoting the vegetative growth of cow pea roots, shoots and leaves [43]. *Acinetobacter johnsonii* 3-1 was isolated from sugar beet roots, and inoculation of the bacterium enhanced the growth of sugar beet plants [44]. Additionally, inoculation of several strains of endophytic bacteria isolated from rice landraces was able to significantly increase the growth rate of root length of the commercial rice cultivar by approximately three fold [45]. Taken together, our results and these examples emphasized the benefits of endophytic bacteria for plant growth.

### 3.3 Plant-growth-promoting activities

The twelve isolates were examined for their plant-growth-promoting traits including the presence of the *nifH* gene, siderophore synthesis, IAA production and ACC-deaminase activity (Table 2). Seven isolates displayed at least one characteristic. The presence of the *nifH* gene was the most common characteristic and detected in isolates 3003, 3008, 3010, 3012 and 3016. Isolate 3014 was able to produce siderophores and IAA. The only trait found in isolate 3013 was the ability to produce ACC-deaminase. Additionally, isolate 3010 that yielded the highest fresh-weight ratio of rice seedlings exhibited all of the tested plant-growth-promoting characteristics. This was consistent with previous studies. Ji *et al.* isolated *nifH*-containing endophytic bacteria from Korean rice cultivars and demonstrated their ability to promote growth in rice plants [4]. These bacteria were also able to produce IAA and siderophores as well as solubilize phosphate. Other previous studies also showed that inoculation of ACC-deaminase-producing bacteria could promote growth, stress tolerance and nutrient uptake in plants [16, 46-47]. However, we found that isolates 3004, 3005, 3006, 3015 and 3022 were tested negative for all the examined traits. This result suggests the involvement of other mechanisms that have also been proposed as plant-growth-promoting activities including phosphate solubilization as well as cytokinin and gibberellin production [4].

**Table 2.** Growth promotion in rice seedlings by endophytic bacteria and bacterial plant-growth-promoting activities. Different letters indicate significance differences between means (n=3). (+ : positive, − : negative).

| Isolates | Affiliation | Fresh-weight Ratio (Mean±S.D.) | Plant-growth-promoting characteristics | | | |
|---|---|---|---|---|---|---|
| | | | *nifH* | Siderophores | ACC-deaminase | IAA |
| 3003 | *Bacillus* | $2.30\pm0.23^{a,b}$ | + | − | − | − |
| 3004 | *Bacillus* | $2.34\pm0.18^{a,b,c}$ | − | − | − | − |
| 3005 | *Bacillus* | $2.70\pm0.62^{a,b,c}$ | − | − | − | − |
| 3006 | *Bacillus* | $3.05\pm0.42^{b,c}$ | − | − | − | − |
| 3008 | *Bacillus* | $2.64\pm0.19^{a,b,c}$ | + | − | − | − |
| 3010 | *Micrococcus* | $3.18\pm0.30^{c}$ | + | + | + | + |
| 3012 | *Bacillus* | $3.14\pm0.22^{b,c}$ | + | − | − | − |
| 3013 | *Bacillus* | $2.94\pm0.59^{a,b,c}$ | − | − | + | − |
| 3014 | *Acinetobacter* | $2.14\pm0.42^{a}$ | − | + | − | + |
| 3015 | *Bacillus* | $2.56\pm0.35^{a,b,c}$ | − | − | − | − |
| 3016 | *Bacillus* | $2.58\pm0.70^{a,b,c}$ | + | − | − | − |
| 3022 | *Bacillus* | $2.97\pm0.63^{a,b,c}$ | − | − | − | − |

## 4. Conclusion

In the present study, we found that endophytic bacteria isolated from rice roots and stems composed of members in phyla *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria.* Based on the phylogenetic analysis of nearly-complete 16S rRNA gene sequences, isolates 1017, 1048 and 3037 may represent novel species of genera *Pedobacter*, *Sphingomonas* and *Paenibacillus*, respectively. Phenotypic and chemotaxonomic characterizations are required in order to confirm their taxonomic classification. Significant growth promotion in rice seedlings was observed when they were treated with twelve bacterial isolates. Seven of these isolates displayed some plant-growth-promoting traits. The most common characteristic was the presence of the *nifH* gene. Five other isolates yielded negative results in all tested traits. According to the data obtained in our study, these isolates are potential candidates for future application as biofertilizers.

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