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Plant growth enhancing effects by a siderophore-producing endophytic streptomycete isolated from a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105)

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Abstract An endophytic *Streptomyces* sp. GMKU 3100 isolated from roots of a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105) showed the highest siderophore production on CAS agar while phosphate solubilization and IAA production were not detected. A mutant of *Streptomyces* sp. GMKU 3100 deficient in just one of the plant growth promoting traits, siderophore production, was generated by inactivation of a *desD*-like gene encoding a key enzyme controlling the final step of siderophore biosynthesis. Pot culture experiments revealed that rice and mungbean plants inoculated with the wild type gave the best enhancement of plant growth and significantly increased root and shoot biomass and lengths compared with untreated controls and siderophore-deficient mutant treatments. Application of the wild type in the presence or absence of ferric citrate significantly promoted plant growth of both plants. The siderophore-deficient mutant clearly showed the effect of this important trait involved in plant–microbe interaction in enhancement of growth in rice and mungbean plants supplied with sequestered iron. Our results highlight the value of a substantial understanding of the relationship of the plant growth promoting properties of endophytic actinomycetes to the plants. Endophytic actinomycetes, therefore, can be applied as potentially safe and environmentally friendly biofertilizers in agriculture.

Keywords Endophyte · *Streptomyces* · Siderophore · Plant growth promotion · Rice (*Oryza sativa* L.) · Mungbean (*Vigna radiata* (L.) Wilczek)

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Introduction

Endophytic actinomycetes can be defined as those Gram-positive filamentous bacteria that generally inhabit soil and rhizosphere and can colonize the internal tissues of plants without causing any evident damage or morphological changes in the plants (Kunoh 2002; Hasegawa et al. 2006). They interact with plants

as free-living non-symbiotic bacteria similar to *Pseudomonas* spp. (Mercado-Blanco and Bakker 2007) and could be considered as being involved in a neutral interaction. Reflecting the enormous plant diversity in different niches and ecosystems, many reports indicate that plants are rich reservoirs for diversity and discovery of novel taxa of actinomycetes (Inbar et al. 2005; Hasegawa et al. 2006; Zin et al. 2007; Bascom-Slack et al. 2009; Qin et al. 2011). Endophytic actinomycetes were isolated from live tissues of various type of plants such as wheat (Coombs et al. 2004; Sadeghi et al. 2012), rice (Tian et al. 2004), tomato (Tan et al. 2006; Fialho de Oliveira et al. 2010), banana (Cao et al. 2005), cowpea (Dimkpa et al. 2008), cucumber (El-Tarabily et al. 2009), medicinal plants (Qin et al. 2009), eaglewood (Nimnoi et al. 2010), blue lupin (Trujillo et al. 2010), chickpea (Misk and Franco 2011) and neem tree (Verma et al. 2011).

Recently, there has been an increasing number of reports that these endophytic actinomycetes are clearly beneficial to plants. They protect plants against phytopathogens by the production of antibiotics or chitinolytic enzymes to inhibit fungal pathogens, thus being considered as potential biocontrol agents of plant diseases (Coombs et al. 2004; Tian et al. 2004; Cao et al. 2005; Zin et al. 2007; Quecine et al. 2008; El-Tarabily et al. 2009; Misk and Franco 2011). They also directly promote plant growth such as by the production of phytohormones (auxins, cytokinins and gibberellins), production of siderophores to scavenge ferric iron from the environment, solubilization of inorganic phosphate, fixing nitrogen and suppression of stress ethylene in plant by production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Cao et al. 2005; Tan et al. 2006; Dimkpa et al. 2008; El-Tarabily et al. 2009; Trujillo et al. 2010; Fialho de Oliveira et al. 2010; Misk and Franco 2011; Verma et al. 2011; Sadeghi et al. 2012). However, whether these plant growth enhancers form an active interaction between plant and bacterium is poorly studied. In this work, we investigated plant growth promoting properties by endophytic actinomycetes isolated from Thai rice plant cultivars. We firstly demonstrated a clear link between a genotype and a phenotype of a siderophore producing endophyte that delineated this single trait towards plant growth promotion. We also showed that an endophytic streptomycete isolated from a rice plant could increase growth parameters in different host plants.

Materials and methods

Isolation and identification of endophytic actinomycetes

Endophytic actinomycetes were isolated from healthy Thai rice cultivars, Thai glutinous rice plants (*Oryza sativa* L. cv. RD6) and Thai jasmine rice plants (*O. sativa* L. cv. KDML105) collected from Pathum Thani Rice Research Center, Pathum Thani province, Thailand. The excised plant materials (leaves, stems and roots) were surface-sterilized and endophytic actinomycetes were isolated according to the protocol previously described by Indananda et al. (2010). The plant samples were initially washed in running tap water and sterilized by sequential immersion in 95 % (v/v) ethanol for 10 min and 1 % sodium hypochlorite for 15 min and then the surface-sterilized plant materials were washed in sterile water three times. The leaves, stems and roots were then soaked in 10 % (w/v) $NaHCO_3$ solution for 10 min to retard the growth of endophytic fungi. The plant materials were crushed and the resulting solution was spread onto starch-casein agar (SCA; Küster and Williams 1964) supplemented with 50 mg/ml nalidixic acid, 2.5 U/ml penicillin G, 50 mg/ml nystatin and 50 mg/ml cycloheximide. The crushed leaf, stem and root debris was also placed on SCA. Colonies of endophytic actinomycetes appeared on the agar after incubation at 28 °C for 4–5 weeks. The pure isolates were identified by 16S rRNA gene amplification using the primers and conditions described by Indananda et al. (2010). The 16S rRNA gene sequences of the isolates were determined by direct sequencing of the PCR products. The sequences were compared with those of 16S rRNA genes in the GenBank and EzTaxon-e (Kim et al. 2012) databases to indicate close relationships with valid species.

Determination of phosphate solubilization, IAA and siderophore production

Phosphate solubilization potential of endophytic actinomycetes was determined by growing actinomycetes in tryptic soy broth (TSB) in a rotary shaker at 200 rpm, 28 °C for 5 days. 100 µl of cell culture was dropped onto Pikovskaya agar (Pikovskaya 1948) containing tricalcium phosphate and incubated at 28 °C for 5 days. The presence of clear zones around

the microbial colonies was used as indicator for positive strains.

Indole-3-acetic acid (IAA) was assayed based on the colorimetric method described by Glickmann and Dessaux (1995) with some modifications. Endophytic actinomycetes were inoculated into 5 ml TSB supplemented with tryptophan (500 μg/ml) in a rotary shaker at 200 rpm, 28 °C in the dark for 7 days. The cultures were centrifuged at 12,000 rpm for 5 min and 1 ml supernatant was mixed with 2 ml Salkowski's reagent (Gordon and Weber 1951) and incubated for 30 min at room temperature. Development of a pink color indicated IAA production.

Siderophore assay was done using the chrome azurol S (CAS) assay (Schwyn and Neilands 1987). An agar plug of 5-day growth of endophytic actinomycetes on YM agar was placed onto a CAS agar plate and incubated at 28 °C for 2 days. A positive strain was indicated by an orange halo around the agar plug.

Construction of siderophore-deficient mutant

A partial *desD*-like gene was amplified from genomic DNA of *Streptomyces* sp. GMKU 3100 using specific primers, ATT083F (5′-TGCTTCGTCGCCAACAAC GG-3′) and ATT083R (5′-CTGGAGCCGGTTGAGG CAGGA-3′). The PCR reaction was carried out in a final volume of 20 μl containing 0.2 mM of each dNTP, 5 % DMSO, 10 pmol of each primer, 2 μl of 10× *Taq* DNA polymerase buffer and 0.5 units of *Taq* DNA polymerase (Fermentas, USA). The PCR program was as follows: 3 min at 95 °C for 1 cycle; 30 s at 95 °C, 30 s at 68 °C, 1 min and 30 s at 72 °C for 30 cycles and 4 min at 72 °C for 1 cycle. The PCR fragment was confirmed by DNA sequencing and then cloned into an integrating vector, pIJ8671 (Sun et al. 1999) to obtain pIJ8671/*desD* prior to transformation of *E. coli* ET12567(pUZ8002) (MacNeil et al. 1992). Intergeneric conjugation was performed according to Phornphisutthimas et al. (2010). The mycelium of a 24-h culture of *Streptomyces* sp. GMKU 3100 was used as recipient to conjugate with donor *E. coli* ET12567(pUZ8002)/pIJ8671/*desD* on mannitol soya agar (MS; Hobbs et al. 1989) containing 10 mmol/l $MgCl_2$. The mutants were selected by thiostrepton resistance. The mutant was tested for siderophore production using the CAS assay described above (Schwyn and Neilands 1987). The mutant was verified to result from integration of pIJ8671/*desD* into the chromosome by amplification of the thiostrepton resistance gene using specific primers, ATT012 and ATT013, and PCR condition as described by Phornphisutthimas et al. (2010). The absence of a 5.4-kb long PCR product was also determined comparing by comparison of the recombinant plasmid pIJ8671/*desD* using specific primers, ATT012 and Apr N-2 (5′-CCC CGGCGGTGTGCT-3′; Choi et al. 2004). The PCR reaction was carried out using 0.1 unit of Phusion High-Fidelity DNA polymerase (Finnzymes, Finland) with 10 % DMSO as follows: 3 min at 98 °C for 1 cycle; 30 s at 98 °C, 30 s at 69.5 °C, 3 min at 72 °C for 30 cycles and 5 min at 72 °C for 1 cycle.

Pot culture growth conditions

Healthy rice seeds (*Oryza sativa* L. cv. KDML105) were immersed in water for 24 h before surface-sterilization. Rice and mungbean seeds (*Vigna radiata* (L.) Wilczek cv. CN72) were then surface-disinfected with 70 % ethyl alcohol for 1 min followed by a 1 % solution of sodium hypochlorite for 5 min. The seeds were washed six times for 1 min each with sterile distilled water. The seeds were then transferred onto sterilized moistened tissue paper in a tissue culture flask and incubated at room temperature in the dark for 2 days for seed germination. The pruned-root dip method (Musson et al. 1995) was used to inoculate the seedlings. The roots were trimmed using a sterilized scalpel and the seedlings were then immediately placed in sterile glass beakers containing a 10^8 CFU/ml spore suspension of *Streptomyces* sp. GMKU 3100 and its mutant and soaked for 4 h. Ten seedlings were used for each experiment and replicated twice. The seedlings were then transferred to a pot containing autoclaved vermiculite overnight at room temperature. Hoagland's solution (Hoagland and Arnon 1950) supplemented with and without a less complex iron source, 10 μM ferric citrate, was added to the pot in order to assess the role of bacterial siderophores (Sharma et al. 2003). The pots were placed in the greenhouse and supplied with water once a day for 2–4 weeks.

Evaluation of shoot and root length and biomass

After 14- and 28-day growth of rice and mungbean plants, respectively, in the greenhouse, they were surface-sterilized and examined for endophytes

according to the protocol previously described (Indananda et al. 2010). Root and shoot lengths, root and shoot fresh weights, and root and shoot dry weights were observed. All measurements of root and shoot parameters were conducted on ten seedlings for each condition. The one-way ANOVA and Tukeys multiple range tests (TMRT) were calculated using SPSS (version 11.5) at $p = 0.05$ to determine the efficacy of wild type, mutant and untreated control in promotion of root and shoot development with and without 10 μM ferric citrate.

Results

Identification of endophytic actinomycetes isolated from rice plants

Nine unique endophytic actinomycetes were isolated from the interior tissues of roots of Thai glutinous rice plants (*Oryza sativa* L. cv. RD6; two isolates, namely GMKU 366 and GMKU 367) and Thai jasmine rice plant (*O. sativa* L. cv. KDML 105; seven isolates, namely GMKU 368, GMKU 369, GMKU 370, GMKU 372, GMKU 3100, GMKU 3101 and GMKU 3102). No actinomycete was isolated from leaves or stems of rice plants. The 16S rRNA gene sequences compared to the Genbank database suggested that the endophytes belonged to the genera *Streptomyces* (GMKU 3100, GMKU 3101 and GMKU 3102) and *Microbispora* (GMKU 366, GMKU 368, GMKU 369 and GMKU 372). A novel described genus, *Actinophytocola oryzae* BCC 31372^T (GMKU 367; Indananda et al. 2010), and a novel described species, *Actinoallomurus oryzae* BCC 31373^T (GMKU 370; Indananda et al. 2011), were also discovered and validated in this collection. In addition, we found that *Microbispora* spp. appeared on SCA in larger numbers compared to other actinomycetes.

Plant growth promotion determination

The endophytic actinomycetes were determined for their plant growth promotion abilities on phosphate solubilization, and production of IAA and siderophores. None of them could solubilize phosphate or produce IAA. Five strains could produce only siderophores (GMKU 366, GMKU 367, GMKU 370, GMKU 3100 and GMKU 3102). Among these strains, *Streptomyces* sp. GMKU 3100 showed the widest orange halo of siderophore production on CAS agar (Fig. 1a). The complete 16S rDNA sequence of strain GMKU 3100 revealed 99.9 % identity (1,506 nucleotides; GenBank accession number JQ922248) with the *Streptomyces somaliensis* NBRC 12916^T (accession number AB184243) and *Streptomyces albidoflavus* DSM 40455^T (accession number Z76676) type strains. This strain was selected for further investigation of siderophore production related to its ability to facilitate plant growth.

Siderophore-deficient mutant

To investigate the effect of bacterial siderophores on plant growth, a siderophore-deficient mutant was constructed in order to compare its ability to that of the *Streptomyces* sp. GMKU 3100 wild type. The *desD* gene was targeted for gene disruption since it codes for a siderophore synthetase which catalyzes the key step in desferrioxamine biosynthesis in *S. coelicolor* (Barona-Gómez et al. 2004). Based on the published sequences of the *desD*-like genes available in databases, specific primers for *desD* were successfully designed. A partial *desD*-like gene was amplified from the genome of *Streptomyces* sp. GMKU 3100 to give a fragment of 1.2 kb (Fig. 1c). Conceptual translation of the partial gene (GenBank accession number JX204383) revealed 99 % identity to DesD of *S. albus* (accession number ZP_06590848). Insertional inactivation of *desD* in the siderophore biosynthetic gene cluster of *Streptomyces* sp. GMKU 3100 was performed by introduction of pIJ8671/*desD* into the strain by intergeneric conjugation. pIJ8671/*desD* was therefore integrated at the homologous position between the partial *desD*-like gene in the plasmid and the intact *desD* in the chromosome by single crossover recombination (Fig. 1b). Siderophore-deficient mutants were screened and characterized by (i) resistance to thiostrepton; (ii) absence of siderophore production on CAS agar (Fig. 1a); (iii) presence of a 0.8-kb amplicon of the thiostrepton resistance gene (Fig. 1d); and (iv) absence of a 5.4-kb long amplicon present in pIJ8671/*desD* (Fig. 1e). In addition, the mutants showed a morphology on MS agar in growth of mycelium and spore formation similar to that of wild type. However, slightly slower growth of the mutants was observed compared to the wild type (i.e. late spore formation).

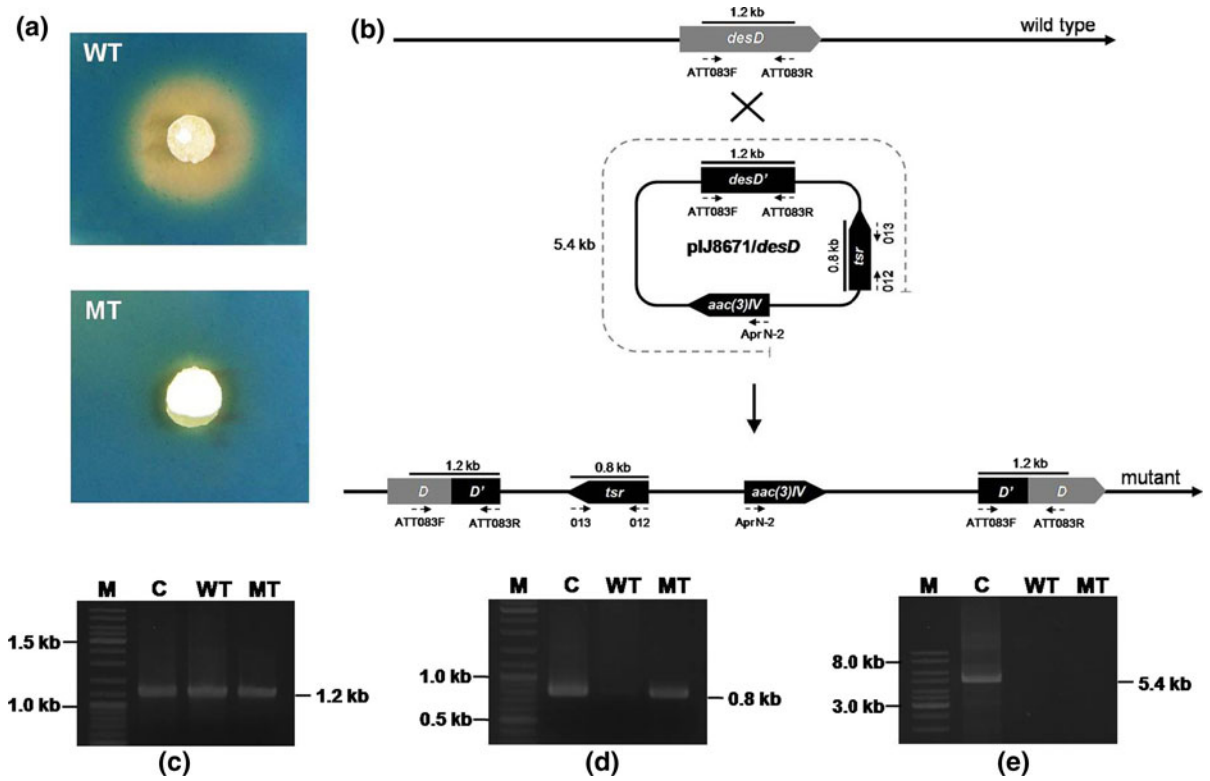

Fig. 1 Characterization of *Streptomyces* sp. GMKU 3100 and the siderophore-deficient mutant. **a** *Orange* halo of siderophore production on CAS agar of the wild type (*above*) and the mutant (*below*). **b** Illustration of the insertion inactivation of the *desD* gene by single cross-over recombination. *Small arrows* indicate primers used for amplification tests. **c** PCR amplification tests for the 1.2-kb *desD* gene sequence using primers, ATT083F and ATT083R. **d** PCR amplification tests for the 0.8-kb thiostrepton resistance gene sequence using primers, ATT012 and ATT013. **e** PCR amplification tests for the 5.4-kb long amplicon in pIJ8671/*desD* using primers ATT012 and Apr N-2. *M* 1-kb ladder, *C* pIJ8671/*desD*, *WT Streptomyces* sp. GMKU 3100, *MT* siderophore-deficient mutant

Effect on plant growth promotion

Streptomyces sp. GMKU 3100 and its siderophore-deficient mutant were investigated for their ability for plant growth enhancement by re-inoculation of both strains into seedlings of rice and mungbean with and without 10 μM ferric citrate (Fe-citrate). In addition, the wild type and the mutant could be re-isolated from the inoculated 14-day rice plants and 28-day mungbean plants in every condition. Growth parameters (root and shoot lengths, root and shoot fresh weights, and root and shoot dry weights) of rice and mungbean plants inoculated with the wild type and the mutant strains were recorded after 14 and 28 days, respectively. Plants inoculated with *Streptomyces* sp. GMKU 3100 appeared statistically significantly ($p < 0.05$) greater in plant growth parameters compared with uninoculated plants and plants inoculated with the mutant, both with and without Fe-citrate (Figs. 2, 3). In the presence of Fe-citrate, the root and shoot lengths, and root and shoot fresh/dry weights of rice and mungbean plants increased with GMKU 3100 inoculation even more remarkably than without Fe-citrate (Figs. 2, 3). Compared with the uninoculated plants and siderophore mutant treated plants, GMKU 3100 obviously built up the number of roots, increasing the root fresh weight in both rice and mungbean plants (Figs. 2a, 3a). However, the untreated plants and siderophore-deficient mutant inoculated plants showed equal plant growth parameters with no statistically significant difference (Figs. 2, 3).

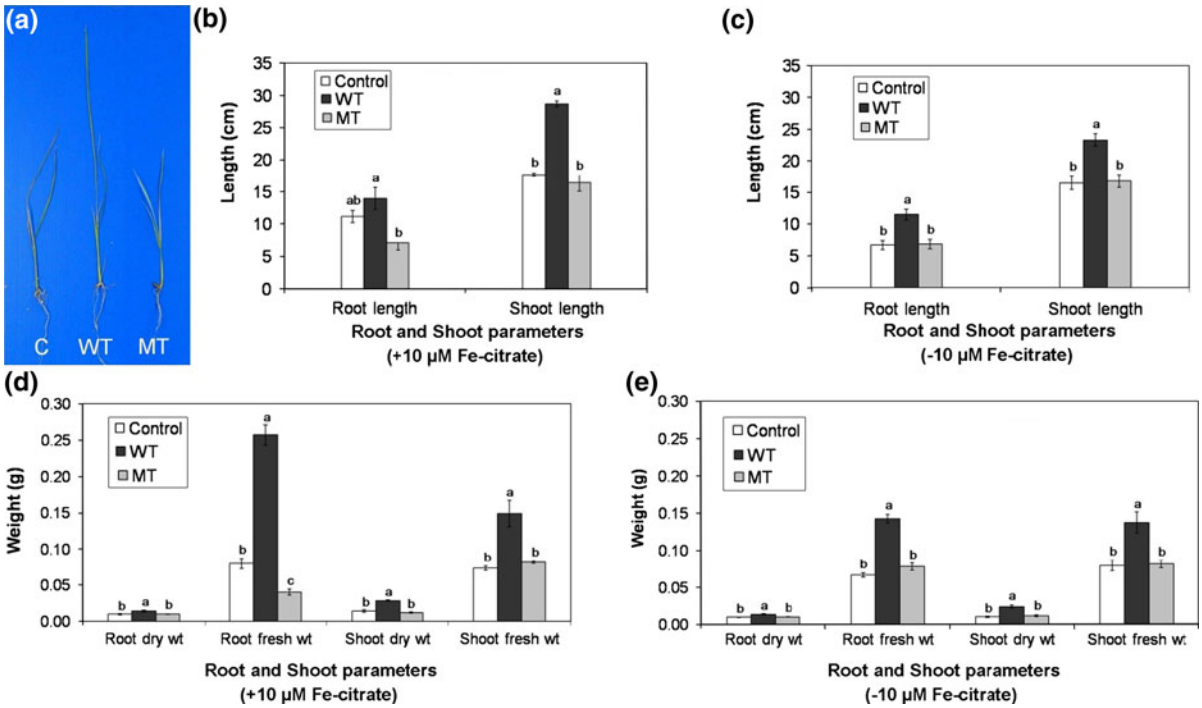

Fig. 2 Plant growth parameters of rice plants (*Oryza sativa* L. cv. KDML105) inoculated with *Streptomyces* sp. GMKU 3100 and the siderophore-deficient mutant after 14 days. **a** 14-day rice plants; **b** root and shoot lengths with 10 µM Fe-citrate; **c** root and shoot lengths without 10 µM Fe-citrate; **d** root dry/fresh weights and shoot dry/fresh weights with 10 µM Fe-citrate; **e** root dry/fresh weights and shoot dry/fresh weights without 10 µM Fe-citrate; *C* uninoculated plant (control), *WT Streptomyces* sp. GMKU 3100, *MT* siderophore-deficient mutant. Data are the mean of 10 replicates. Means designated with *different letters* are significantly different ($p = 0.05$). *Error bars* show standard deviation ($n = 10$)

With Fe-citrate, the percentage increase in root and shoot lengths for GMKU 3100-inoculated rice plants was 125 and 160 % greater, respectively, compared with the untreated controls (Fig. 2b). The percent increase in root dry and fresh weights for GMKU 3100-inoculated rice plants were 140 and 320 % greater, respectively, compared with the untreated plants (Fig. 2d). The percentage increase in shoot dry and fresh weights for GMKU 3100-inoculated rice plants were about 200 % greater than that of the controls (Fig. 2d). Compared with the uninoculated controls, GMKU 3100 enhanced the lengths 115 and 120 % higher in roots and shoots of mungbean plants, respectively in the presence of Fe-citrate (Fig. 3b). GMKU 3100-inoculated mungbean plants had root dry and fresh weights about 200 % higher than the untreated controls (Fig. 3d). In the same condition, shoot dry and fresh weights of GMKU 3100-inoculated mungbean plants increased 190 and 170 %, respectively, compared with uninoculated plants (Fig. 3d).

Discussion

Endophytic bacteria could be found within different parts such as leaves, stems, roots and seeds inside rice plants (Mano and Morisaki 2008). However, more diverse actinobacteria were detected from roots than other parts of rice plants (Tian et al. 2007). In our work, endophytic actinomycetes could only be isolated from the roots. It was suggested that roots are the most abundant sources of actinomycete endophytes (Sardi et al. 1992; Taechowisan et al. 2003). This may be due to the fact that endophytic populations of the root originate from the rhizosphere (Germida et al. 1998). The presence of endophytic actinomycetes inside the root tissues has an important role with regard to plant development and may also protect against soil-borne pathogens (Sardi et al. 1992). Several genera of actinomycetes were isolated from rice plants such as *Actinoplanes*, *Amycolatopsis*, *Dactylosporangium*, *Frankia*, *Micromonospora*, *Rhodococcus* and *Streptomyces* (Tian et al. 2007; Naik

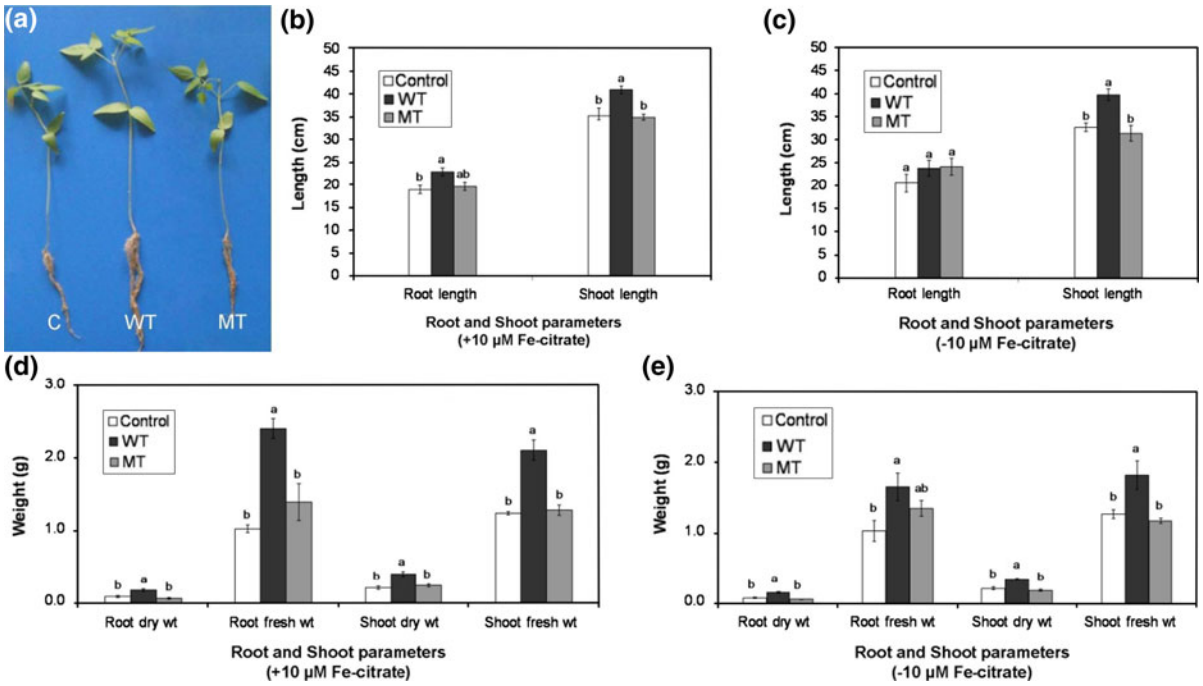

Fig. 3 Plant growth parameters of mungbean plants (*Vigna radiata* (L.) Wilczek cv. CN72) inoculated with *Streptomyces* sp. GMKU 3100 and the siderophore-deficient mutant after 28 days. **a** 28-day mungbean plants; **b** root and shoot lengths with 10 µM Fe-citrate; **c** root and shoot lengths without 10 µM Fe-citrate; **d** root dry/fresh weights and shoot dry/fresh weights with 10 µM Fe-citrate; **e** root dry/fresh weights and shoot dry/fresh weights without 10 µM Fe-citrate; *C* uninoculated plant (control), *WT* *Streptomyces* sp. GMKU 3100, *MT* siderophore-deficient mutant. Data are the mean of 10 replicates. Means designated with different letters are significantly different ($p = 0.05$). *Error bars* show standard deviation ($n = 10$)

et al. 2009). However, we found different genera of non-streptomycetes (*Actinoallomurus*, *Actinophytocola* and *Microbispora*) isolated from rice plants compared with previous reports. Besides, we observed a large number of colonies of *Microbispora* spp. from our isolation. It seems that *Microbispora* spp. predominantly occupy root tissues of rice plants. Although rather few strains were isolated from roots of Thai rice plants, it is noteworthy that one new genus and one new species were proposed (Indananda et al. 2010; 2011) out of nine strains, which indicated that Thai rice plants are rich bio-resources for discovery of new taxa of endophytic actinomycetes. These results are in agreement with the finding that rice represents a rich reservoir for a wide diversity of actinobacteria (Tian et al. 2007).

It was confirmed that *Streptomyces* sp. GMKU 3100 was truly endophytic because it could be isolated from surface-sterilized rice and mungbean plants up to 14 and 28 days, respectively, after inoculation. This indicated that the endophytic streptomycete tested was able to localize and multiply within plant tissues without causing any obviously deleterious effects to both host plants, but influencing plant development. Furthermore, the endophytic streptomycete isolated from cereal plants could effectively colonize leguminous plants such as mungbean and could remarkably enhance growth of mungbean plants in the similar way as in the plant from which it was originally isolated. It was previously concluded that actinobacteria are not host-specific colonizers, but can adapt themselves to live as free-living endophytes (Misk and Franco 2011). We suggest that endophytic actinomycetes may migrate back and forth between the rhizosphere and the inside of the plants and can neutrally colonize and help plant growth in a wide host range of plants.

Recently, endophytic actinomycetes have been recognized as new members of the plant growth promoting bacterial community (PGPB) due to their properties of plant growth enhancement and protection of plants from infectious diseases (El-Tarabily et al. 2009; Misk and Franco 2011; Verma et al. 2011).

However, phosphate solubilization and IAA production were not detected in our endophytic actinomycetes isolated from rice. Only siderophore production could be detected from five strains (56 %), which could exhibit extensive rice growth-promotion benefits. Siderophores are relatively low molecular weight ferric iron specific chelating agents produced by bacteria and fungi under iron-limiting conditions (Neilands 1995). Soil *Streptomyces* spp. have been reported to produce certain kinds of siderophores (Imbert et al. 1995; Barona-Gómez et al. 2006). Microbial siderophores have a positive correlation with plant growth promotion; therefore, production of siderophores is one of the key factors that should be considered for primary screening (Crowley et al. 1991; Glick et al. 1999). There were reports on screening for siderophore production from endophytic actinomycetes (Nimnoi et al. 2010; Fialho de Oliveira et al. 2010; Ruanpanun et al. 2010) and on the relationship between plant growth enhancing effects and biocontrol of plant pathogens (Cao et al. 2005; Tan et al. 2006; Misk and Franco 2011; Verma et al. 2011; Sadeghi et al. 2012) but little work has been done on the effect of the single trait of siderophore production on plant growth parameters (Dimkpa et al. 2008).

In the work firstly reported here, a clear link between a genotype and a phenotype of a siderophore producing endophytic streptomycete related to plant growth enhancement. A mutant of *Streptomyces* sp. GMKU 3100 deficient in siderophore production was constructed. The key gene, *desD*, coding for a siderophore synthetase catalyzing the final step of desferrioxamine biosynthesis in *S. coelicolor* (Barona-Gómez et al. 2004) was targeted for gene disruption. The insertional inactivation of this gene, therefore, yielded a mutant in which production of siderophores was completely abolished. The previous disruption of *desD* in *S. coelicolor* resulted in a mutant that did not produce desferrioxamines because the final oligomerization step in the biosynthetic pathway was disrupted, causing the abrogation of the production of desferrioxamines (Barona-Gómez et al. 2004). The feasibility of *desD* gene disruption in endophytic *Streptomyces* sp. GMKU 3100 gives new possibility to study the biological role of the single trait of bacterial siderophore production with the host plants.

In this study, pot culture experiments were carried out to evaluate the effect of the siderophore-producing endophytic *Streptomyces* sp. GMKU 3100 on plant growth promotion in rice and mungbean plants in comparison with the siderophre-deficient mutant. When the wild type was applied, it gave the best enhancement of plant growth and significantly increased root and shoot biomass and lengths compared with the untreated control and the siderophore-deficient mutant. Furthermore, application of the wild type in the presence or absence of Fe-citrate significantly promoted plant growth of both rice and mungbean plants. The results suggested that the enhancement of rice and mungbean plant growth was solely due to siderophore production from *Streptomyces* sp. GMKU 3100 since the treatments which received the inoculated siderophore-deficient mutant were even less effective as the uninoculated control in reduction of biomass and elongation of roots and shoots. The production of siderophores is one of the important mechanisms used by plant growth-promoting bacteria to promote plant growth (Crowley et al. 1991; Glick et al. 1999). The increase in plant growth of rice and mungbean plants by the endophytic *Streptomyces* sp. GMKU 3100 in our study is supported by other observations where siderophore-producing endophytic actinomycetes were also shown to enhance plant growth, as in tomato (Tan et al. 2006), banana (Cao et al. 2005), cowpea (Dimkpa et al. 2008); chickpea (Misk and Franco 2011), neem tree (Verma et al. 2011) and wheat (Sadeghi et al. 2012). However, the effects of plant growth from previous reports are not derived from siderophores alone but combined with other plant growth promoting properties such as IAA production and phosphate solubilization. Furthermore, their data emphasize the role of plant growth promoting actinomycetes on the antagonistic effect to phytopathogens of plants. In our study, we generated a mutant of the endophytic *Streptomyces* sp. GMKU 3100 deficient in the single trait of plant growth promotion. The siderophore-deficient mutant could be used to clearly evaluate the effect of this important trait involved in plant–microbe interaction in enhancement of growth in rice and mungbean plants. It is evident from our results that siderophore-producing endophytic *Streptomyces* sp. GMKU 3100 increased root and shoot biomass and lengths of rice and mungbean plants by supplying the plants with sequestered iron.

Production of siderophores is also important for antagonism to phytopathogens and improving growth of the plant (Tan et al. 2006). It was suggested that siderophore-producing endophytic actinomycetes also

have very significant antagonistic activity against plant pathogens (Cao et al. 2005; Tan et al. 2006; Dimkpa et al. 2008; Misk and Franco 2011; Verma et al. 2011; Sadeghi et al. 2012). Therefore, it will be useful to investigate further the antagonistic effect of this wild type strain and the mutant against rice diseases. More understanding of plant growth-promoting properties of endophytic actinomycetes suggest that these bacteria merit further investigation for potentially safe and environmentally friendly biofertilizers which can help us limit the use of chemical fertilizers in agriculture.

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References

Barona-Gómez F, Wong U, Giannakopulos AE, Derrick PJ, Challis GL (2004) Identification of a cluster of genes that directs desferrioxamine biosynthesis in *Streptomyces coelicolor* M145. J Am Chem Soc 126:16282–16283

Barona-Gómez F, Lautru S, Francou F-X, Leblond P, Pernodet J-L, Challis GL (2006) Multiple biosynthetic and uptake systems mediate siderophore-dependent iron acquisition in *Streptomyces coelicolor* A3(2) and *Streptomyces ambofaciens* ATCC 23877. Microbiology 152:3355–3366

Bascom-Slack CA, Ma C, Moore E, Babbs B, Fenn K, Greene JS, Hann BD, Keehner J, Kelley-Swift EG, Kembaiyan V, Lee S-J, Li P, Light DY, Lin EH, Schorn MA, Vekhter D, Boulanger L-A, Hess WM, Vargas PN, Strobel GA, Strobel SA (2009) Multiple, novel biologically active endophytic actinomycetes isolated from upper amazonian rainforests. Microb Ecol 58:374–383

Cao L, Qiu Z, You J, Tan H, Zhou S (2005) Isolation and characterization of endophytic streptomycete antagonists of *Fusarium* wilt pathogen from surface-sterilized banana roots. FEMS Microbiol Lett 247:147–152

Choi SU, Lee CK, Hwang YI, Kinoshita H, Nihira T (2004) Cloning and functional analysis by gene disruption of a gene encoding a c-butyrolactone autoregulator receptor from *Kitasatospora setae*. J Bacteriol 186:3423–3430

Coombs JT, Michelsen PP, Franco CMM (2004) Evaluation of endophytic actinobacteria as antagonists of *Gaeumannomyces graminis* var. tritici in wheat. Biol Control 29:359–366

Crowley DE, Wang YC, Reid CPP, Szaniszlo PJ (1991) Mechanisms of iron acquisition from siderophores by microorganisms and plants. Plant Soil 130:179–198

Dimkpa C, Svatoš A, Merten D, Büchel G, Kothe E (2008) Hydroxamate siderophores produced by *Streptomyces acidiscabies* E13 bind nickel and promote growth in cowpea (*Vigna unguiculata* L.) under nickel stress. Can J Microbiol 54:163–172

El-Tarabily KA, Nassar AH, Hardy GESJ, Sivasithamparam K (2009) Plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes. J Appl Microbiol 106:13–26

Fialho de Oliveira M, Germano da Silva M, Van Der Sand ST (2010) Anti-phytopathogen potential of endophytic actinobacteria isolated from tomato plants (*Lycopersicon esculentum*) in southern Brazil, and characterization of *Streptomyces* sp. R18(6), a potential biocontrol agent. Res Microbiol 161:565–572

Germida JJ, Siciliano SD, Freitas R, Seib AM (1998) Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). FEMS Microbiol Ecol 26:43–50

Glick BR, Patten CL, Holguin G, Penrose DM (1999) Biochemical and genetic mechanisms used by plant growth promoting bacteria. World Scientific, River Edge

Glickmann E, Dessaux Y (1995) A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61:793–796

Gordon SA, Weber RP (1951) Colorimetric estimation of indole acetic acid. Plant Physiol 26:192–195

Hasegawa S, Meguro A, Shimizu M, Nishimura T, Kunoh H (2006) Endophytic actinomycetes and their interactions with host plants. Actinomycetologica 20:72–81

Hoagland DR, Arnon DI (1950) The water-culture method of growing plants without soil. California Agricultural Experiment Station, Circular 347

Hobbs G, Frazer CM, Gardner DCJ, Cullum JA, Oliver SG (1989) Dispersed growth of *Streptomyces* in liquid culture. Appl Microbiol Biotechnol 31:272–277

Imbert M, Be′chet M, Blondeau R (1995) Comparison of the main siderophores produced by some species of *Streptomyces*. Curr Microbiol 31:129–133

Inbar E, Green SJ, Hadar Y, Minz D (2005) Competing factors of compost concentration and proximity to root affect the distribution of streptomycetes. Microb Ecol 50:73–81

Indananda C, Matsumoto A, Inahashi Y, Takahashi Y, Duangmal K, Thamchaipenet A (2010) *Actinophytocola oryzae* gen. nov., sp. nov., isolated from root of Thai glutinous rice plants, a new member of the family *Pseudonocardiaceae*. Int J Syst Evol Microbiol 60:1141–1146

Indananda C, Thamchaipenet A, Matsumoto A, Inahashi Y, Duangmal K, Takahashi Y (2011) *Actinoallomurus oryzae* sp. nov., an endophytic actinomycete isolated from root of Thai jasmine rice plant. Int J Syst Evol Microbiol 61:737–741

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kunoh H (2002) Endophytic actinomycetes: attractive biocontrol agents. J Gen Plant Pathol 68:249–252

Küster E, Williams ST (1964) Selection of media for isolation of streptomycetes. Nature 202:928–929

MacNeil DJ, Gewain KM, Ruby CL, Dezeny G, Gibbons PH, MacNeil T (1992) Analysis of *Streptomyces avermitilis*