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Indole-3-Acetic Acid Production by Endophytic *Streptomyces* sp. En-1 Isolated from Medicinal Plants

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Abstract Plant-associated actinobacteria are rich sources of bioactive compounds including indole-derived molecules such as phytohormone indole-3-acetic acid (IAA). In view of few investigations concerning the biosynthesis of IAA by endophytic actinobacteria, this study evaluated the potential of IAA production in endophytic streptomycete isolates sourced from medicinal plant species *Taxus chinensis* and *Artemisia annua*. By HPLC analysis of IAA combined with molecular screening approach of *iaaM*, a genetic determinant of streptomycete IAA synthesis via indole-3-acetamide (IAM), our data showed the putative operation of IAM-mediated IAA biosynthesis in *Streptomyces* sp. En-1 endophytic to *Taxus chinensis*. Furthermore, using the co-cultivation system of model plant *Arabidopsis thaliana* and streptomycete, En-1 was found to be colonized intercellularly in the tissues of *Arabidopsis*, an alternative host, and the effects of endophytic En-1 inoculation on the model plant were also assayed. The phytostimulatory effects of En-1 inoculation suggest that IAA-producing *Streptomyces* sp. En-1 of endophytic origin could be a promising candidate for utilization in growth improvement of plants of economic and agricultural value.

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Introduction

As a predominant form of phytohormone auxin, indole-3-acetic acid (IAA) is ubiquitously present in higher plants, algae, mosses, and lichens. In addition to plants, a wide range of microbes can produce IAA, particularly those in association with plant rhizosphere [1] and those living within plant internal tissues, the so-called endophytes [2–4]. Auxin-producing microbes are documented to involve many species of bacteria, fungi, and yeasts [5], as exemplified by *Agrobacterium* spp., *Pseudomonas savastanoi* pv. *savastanoi*, *Pseudomonas putida* [1], *Pseudomonas chlororaphis* [6], *Bradyrhizobium* spp. [7], *Azospirillum* spp. [8], *Rhizoctonia* [9], *Colletotrichum* [10], *Saccharomyces cerevisiae* [11], *Streptomyces* spp. [4, 12–14]. Recent findings about IAA serving as a signaling molecule in certain plant-associated microbes [15, 16] suggested that it might exert an indispensable impact in microbe-plant interactions [17, 18].

Endophytes, referring to a community of harmless microorganisms colonizing inside plant tissues, are ubiquitous in terrestrial plants [19–21]. Besides synthesizing a variety of bioactive secondary metabolites, endophytes have been demonstrated to improve plant health in response to environmental stresses. The beneficial effects of endophytes may be through producing plant growth promoters such as phytohormone auxins [22], secreting siderophores for Fe acquisition [23], antagonizing plant pathogens [24, 25] and activating plant defense pathways [26]. Therefore studies on endophytes stand out as a hot topic in the field of microbe-plant interactions as well as production of bioactive compounds of microbial origin [27, 28].

It is widely accepted that plant-associated microbes possess multiple routes for IAA biosynthesis, four major tryptophan (Trp)-dependent pathways and one Trp-independent pathway, the latter of which remains debatable.

The indole-3-acetamide (IAM) pathway has been found in both phytopathogenic and symbiotic bacteria [18, 29], which involves two enzymatic steps. The first step is the conversion of the precursor Trp to an intermediate IAM by Trp-2-monooxygenase (encoded by *iaaM*), followed by the second step in which IAM is hydrolyzed into IAA by the enzyme IAM hydrolase (encoded by *iaaH*). In addition, production of IAA has been reported in various streptomycete species, including scab-causing pathogens such as *S. scabies* and non-pathogenic species like *S. coelicolor*, *S. lividans*, and *S. albidoflavus* [12–14]. Notwithstanding the above advancements, there is a paucity of understanding about the IAA production of endophytic origin. In view of that actinomycetes including *Streptomyces* spp. are prolific producers of bioactive compounds, an investigation is needed to reveal the producing capacity of endophytic streptomycetes to medicinal plants.

This study aims to address such questions as whether the IAM pathway is implicated in the IAA biosynthesis of endophytic streptomycetes using En-1, IFB-A02, and IFB-03 isolated in our lab and how the microbial IAA affects plant development by using the co-cultivation system consisting of endophytic streptomycete isolate En-1 and model plant *Arabidopsis thaliana*. To the best of our knowledge, we are first to report on investigation of Trp-dependent IAA biosynthesis via IAM by an endophytic streptomycete strain isolated from medicinal plant *Taxus* sp. Moreover, plant-growth-stimulating endophytes of *Streptomyces* genus like En-1 reported herein would offer additional advantage to the bio-control formulation problem owing to the spore-forming ability.

Materials and Methods

Isolation of Endophytic Actinomycetes

The healthy fresh twigs and leaves of *Taxus chinensis* were collected from Zhongshan botanical garden, Nanjing, Jiangsu Province, P. R. China (March 2011). The collected plant materials were immediately placed into the sterile bags and brought back to the laboratory, followed by the isolation protocol as described in Lin et al. [26]. In brief, the thoroughly-cleaned, surface-sterilized plant tissues were ground in 5 ml of sterile physiological saline (pH 7.0), followed by the serial dilution plating onto the modified Gause No.1 synthetic media supplemented with 25 μg ml^{-1} of kanamycin and 25 μg ml^{-1} of nystatin (for isolating actinomycetes), and the beef extract peptone media supplemented with 25 μg ml^{-1} of nystatin (for isolating bacteria). Among the isolates, most of which were bacteria, one strain morphologically resembling streptomycetes (i.e., colony texture, spore-chain arrangement, and the geosmin production) was of primary interest because the aim of this study is to search for bioactive metabolites of endophytic origin and streptomycetes are well-known prolific producers of secondary metabolites with pharmaceutical and agronomic activities. After incubation at 27–28 °C for up to 4 weeks, putative actinomycete colonies were microscopically examined, isolated, and purified. The spore-containing agar plugs of pure culture of interest, designated as En-1, were stored at −80 °C in 15–20 % glycerol.

Phylogenetic Analyses

Genomic DNA of *Actinomyces* sp. En-1 was extracted from the 48 h-cultured mycelia using the protocol in Lin et al. [26]. The 16S rDNA of En-1 was PCR-amplified, sequenced [26], and has been deposited in Genbank database (accession number: JX985444). Phylogenetic analyses based on 16S rDNA sequences were done using BLASTN and then confirmed by performing the multiple sequence alignment. Phylogenetic tree was constructed with the neighbor-joining (NJ) method, in which the reference *Streptomyces* spp. such as *S. coelicolor*, *S. griseus*, *S. scabies*, and as well as the newly documented endophytic streptomycete isolates IFB-A02 and -A03 (Genbank accession number: HQ317204—317205) were included for comparing their hierarchy relationship. The stability of tree topology was evaluated using BOOTSTRAP based on 1,000 pseudoreplications.

Microbial Growth Conditions

The strains used herein were endophytic streptomycetes En-1 (isolated from *Taxus chinensis*), IFB-A02, and IFB-A03 (sourced from *Artemisia annua*, [26]), the latter two of which were the endophytic isolates maintained in our laboratory. These strains were grown in tryptic soy medium and routinely maintained in *ISP2* (International Streptomyces Project 2) medium. The liquid cultures were performed in a rotary shaker at 200 rpm, 28 °C.

Determination of Microbial IAA

For determination of IAA, three endophytic streptomycete strains En-1, IFB-A02, and -A03 were cultured for 48 h in tryptic soy broth (TSB). The 10 % of microbial inoculum was transferred to 25 ml of the amended TSB supplemented with 5 mM Trp (Sigma, USA). The cultures were incubated in the dark at 200 rpm, 28 °C with agitation in a rotary shaker. The cultures were sampled at different time intervals after addition of Trp. Initial screening of

microbial IAA was performed with microplate method [30] using Salkowski's reagent. Quantitative assay of IAA was performed by high performance liquid chromatography (HPLC). The cultures were harvested until the maximum level of IAA was reached (usually falling into the stationary phase of streptomycete), as indicated by the microplate method based on Salkowski's reaction. Cultures were centrifuged at 10,000×*g* for 15 min at 4 °C and the supernatants were obtained. The extraction of IAA was performed from the supernatants according to Manulis et al. [13] with slight modifications as follows: experiments were carried out in the dark to avoid IAA degradation and the supernatant pH was acidified to pH 2.0 with 5.0 N HCl to insure efficient extraction of IAA with ethyl acetate. The culture supernatants were extracted with ethyl acetate twice, followed by evaporation at 40 °C under vacuum. The resulting residues were dissolved in 2 ml of methanol, and analyzed for the presence of IAA using an Agilent 1200 HPLC on a C-18 reversed-phase column eluting with methanol–1 % acetic acid in water (40:60 v/v) as the mobile phase at a flow rate of 1 ml min^{-1}. The pure authentic IAA and IAM compounds were spiked into the column as standards, respectively. A 10 µl aliquot was analyzed and the presence of IAA and IAM in the specimens was determined at the identical retention time to that of the respective standard.

To elucidate the role of IAM as an intermediate in Trp-dependent IAA synthesis, 5 mM IAM was supplied to the microbial cultures in place of Trp. Cultures were incubated at the similar conditions with Trp-supplemented experiments, and subjected to microplate assay (Salkowski's) and HPLC as afore-described.

Detection of *iaaM*, the Key IAA Biosynthetic Gene via IAM Pathway

Genomic DNAs of streptomycete isolates were extracted as previously described [26]. The key gene *iaaM* associated with IAM-mediated IAA biosynthesis was examined by PCR amplification with the respective genomic DNA of streptomycete isolate serving as a template. The specific primers for *iaaM*, designed from known sequences of IaaM of model microorganism *Streptomyces coelicolor* A3(2) (Genbank accession No.: NP_625735) were used in PCR amplification as follows:

*iaaM*_F(5′-ATGACGTCCACCGTGCCCAACGCG-3′) and *iaaM*_R(5′-CTAGTCCTCGGGGAGTTCCACGGG-3′). The thermal profile of PCR was 94 °C for 5 min, followed by 27 cycles of 95 °C for 40 s, 59 °C for 30 s, and 72 °C for 2 min, with a final extension at 72 °C for 5 min. The corresponding PCR products were equally loaded on gel electrophoresis with image recorded.

Transcriptional Analysis of *iaaH*, the IAM Hydrolase Gene in Cultures of *Streptomyces* sp. En-1

Cultures of *Streptomyces* sp. En-1 supplemented with Trp were harvested at 72 h, followed by centrifugation (4,500×*g*, 5 min) to remove supernatants. Total RNA extraction was carried out from the cells with Trizol reagent (Takara, Dalian, China) according to the manufacturer's instructions and DNA was digested by DNase. cDNA was generated from 4 µg of DNA-free RNA using the First-Strand cDNA Synthesis kit (Invitrogen Co, Shanghai, China). The gene-specific primers for PCR amplification of *iaaH*, generated from the conserved regions of *S. coelicolor* A3(2) (accession No.: NP_625736.1) and *S. scabies* 87-22 (accession No.: YP_003493062.1) were *iaaH*_F(5′-GAGTTCCCGGAGAACGTCCGCGCC-3′) and *iaaH*_R(5′-CGCTGCATGTAGACCAAGAGCTT-3′). To normalize the RNA levels, *rrn* gene (encoding 16S rRNA) was used as internal control [31]. PCR thermal profile was 94 °C for 5 min, followed by 35 cycles at 95 °C for 15 s, and 60 °C for 45 s. The samples derived from the similarly cultured cells of *S. coelicolor* A3(2) and *Streptomyces* sp. IFB-A02 were used as positive and negative reference strains, respectively. Water control served as an experimental negative control with which PCR reactions were performed as the template in place of cDNA.

The Plant Assay of En-1

Surface-sterilized seeds of *Arabidopsis thaliana* Col-0 were germinated in sterile jars (Shanghai Jiafeng Horticultural Appliance Co., China) containing half-strength MS medium (sugar-free, 0.8 % plant agar, pH 5.8) and allowed to germinate under a 14 h photoperiod at 65 µmol $m^{-2}s^{-1}$ at 22–23 °C in the growth chamber. A model test system was established with *Arabidopsis* seedlings grown in the 24-well tissue culture plate. Each well contained 1.7 ml of 1/2 MS liquid medium. Standardized *Arabidopsis* seedlings bearing 4–6 leaves were transferred aseptically into every well of the tissue culture plate. En-1 culture suspension (2×10^7 CFU ml^{-1}) was supplied in 0.3 ml to the well where a standardized *Arabidopsis* seedling was grown in 1/2 MS nutrients, while identical volume of sterile saline water was applied to the control. Before transplanting, twenty-four 5-mm (diameter) holes were melted into the lids of plates, individually corresponding to the middle position of each well. The pre-made holes were sealed with Parafilm to maintain sterile conditions (also keep moist) during the initial transplanting period and the Parafilms were removed while the seedlings grew proximity to the top of wells. The plates with lids were kept at 22–23 °C under same conditions as the above-mentioned. Fresh weight of the plants was measured at 20 days after transplanting. The experiments in triplicate were performed by three repetitions.

Transmission Electron Microscopic (TEM) Examination of Endophytic Colonization In-planta

The stems and leaves of 6-week-old endophyte-preinoculated *Arabidopsis* plantlets were subjected to sample preparation as previously described [26]. Ultra-thin sections of 60–70 nm were examined under an H-7560 transmission electron microscope (TEM, Hitachi, Japan) operating at 80 kV.

Statistical Analysis

As for in-planta bioassay of endophytic En-1, the values represent mean ± SD (standard deviation). *T* test was used to analyze whether the significant difference exists between the treated samples and control. The accepted level of statistical significance was $P < 0.05$.

Results

Streptomycetes are abundant producers of many bioactive metabolites, among which are tryptophan (Trp)-derived compounds including IAA, and other indolic derivatives [16, 32]. The phytohormone IAA has been reported to be detectable in 80 % of bacteria living in the rhizosphere (rhizobacteria) [33]. But few data are available for the production of IAA by spore-bearing actinobacteria endophytic to plants. Given recent findings concerning the involvement of IAA in several types of microbe-plant interactions [16], we adopt a combination of chemical analysis, genetic approach, and microscopic examination to evaluate the producing potential of IAA and the putative operation of IAM pathway in endophytic streptomycete species En-1, and to investigate the effects of endophytic inoculation on plant.

Sequence Alignment and Phylogenetic Analysis

The isolate En-1 resembled *Streptomyces* species in morphologic aspects including the spore-chain features, production of volatile geosmin, and texture of the colony surface. Analysis of the 16S rDNA gene sequence by BLASTN further substantiated that En-1 could be a member under the genus *Streptomyces* with 98–99 % identity. As shown in Fig. 1, the strain En-1, in spite of belonging to the genus *Streptomyces*, was not a close relative to either of the newly documented endophytic *Streptomyces* spp. IFB-A02 and IFB-A03 (Genbank accession No.: HQ317204—317205), which were isolated from another medicinal plant species *Artemisia annua*. In addition, En-1 was phylogenetically distant from phytopathogenic species *S. scabies*, *S. acidiscabies*, and *S. turgidiscabies*.

The Potential of IAA Production in En-1 and Evidence for IAM Pathway

The biosynthesis of IAA was investigated in several phytopathogenic and soil-dwelled non-pathogenic streptomycetes [13], suggesting that the enzyme Trp-2-monooxygenase is responsible for the first step of IAM-mediated IAA synthetic pathway, which is critical for the production of IAA [13]. Recent studies unraveled that the genetic determinant of Trp-2-monooxygenase is the *iaaM* gene [16, 34]. Compelling evidence has supported the proposition that the genome of model microorganism *Streptomyces coelicolor* A3(2) harbors the key gene *iaaM*, which shows 95 % identity with *Streptomyces scabies* 87–22 [12]. These bioinformatics studies facilitate the genetic screening of *iaaM* gene in streptomycete's genome. The IAA biosynthetic pathway via IAM may also be elucidated by analyzing the transcription of *iaaH* (coding for IAM hydrolase) via RT-PCR. More recent data revealed that *S. coelicolor* A3(2) chromosome contains *iaaH* (accession No.: NP_625736.1), another gene for IAM-mediated IAA synthesis, sharing 90.5 % identity with that of *S. scabies* 87–22 (accession No.: YP_003493062.1).

From our preliminary assay of IAA with microplate method [30] based on Salkowski's reaction, pink-red color formation was recorded for endophytic strain En-1 but not for other two endophytes IFB-A02 and -A03, indicating that IAA production is likely to occur in En-1. Further HPLC analysis (Fig. 2) revealed the presence of IAA in the in vitro culture metabolites of endophytic En-1 when supplemented with Trp. Moreover, the detectable level of IAM (Fig. 2b), a pivotal intermediate of microbial IAA biosynthesis, was displayed when 5 mM Trp was added into the cultures of En-1, suggesting that IAM pathway is likely to be functional in En-1. In order to corroborate this, the intermediate IAM at 5 mM was fed into the cultures of En-1 (IAA producer) in comparison with those of non-IAA-producing strain IFB-A02 equally supplied by the same amounts of IAM. For the En-1 cultures, the distinct peak of IAA was shown at expense of IAM (Fig. 2c vs. d), whereas no detectable IAA was displayed for the IFB-A02 cultures (Fig. 2d), which was a non-IAA-producer as indicated by our initial microplate assay. Collectively, chemical data herein pointed toward the operation of IAM pathway during IAA synthesis in the endophytic strain En-1.

For substantiating the occurrence of IAM-mediated IAA synthesis pathway, the first IAM pathway gene *iaaM* was PCR-amplified using streptomycete gene-specific primers. As shown in Fig. 3a, endophytic strain En-1 displayed the genomic presence of *iaaM* as the positive control *S. coelicolor* A3(2) did. However, no band corresponding to the size of *iaaM* (1,698 bp) was shown for either of endophytic IFB-A02 and -A03, suggesting that IFB-A02 and -A03

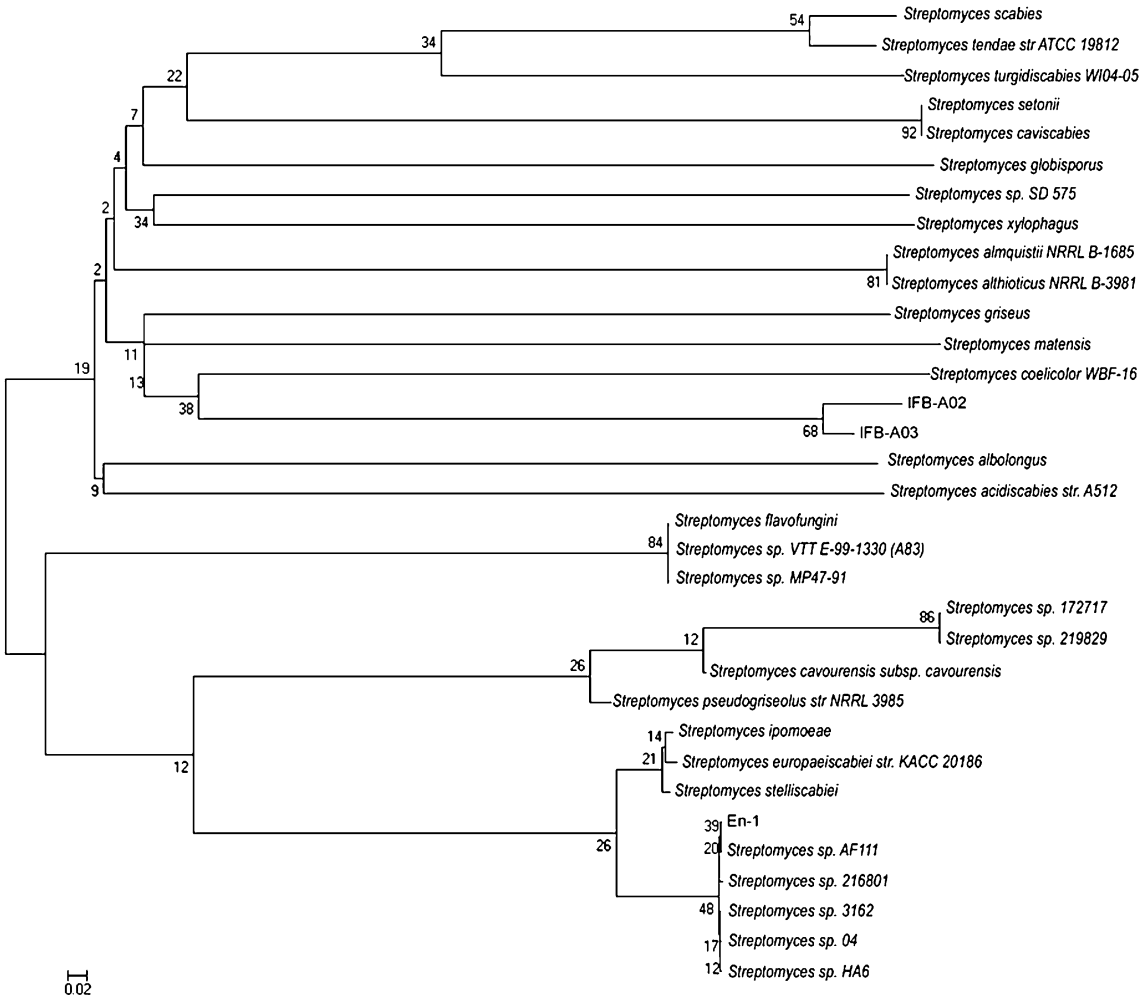

Fig. 1 Phylogenetic relationship of actinomycete isolates of IFB-A02, -A03, and En-1 with representative streptomycete species based on full length 16S rDNA sequences constructed using the neighbor-joining method. The sequence data of the known actinomycete species included in the tree were retrieved from the Genbank sequence database. The accession numbers for the sequences are as follows: *Streptomyces scabies*, GI 971124; *S. turgidiscabies* WI04-05A, GI 220681900; *S. acidiscabies* starin A512, GI 290467510; *S. coelicolor* WBF-16, GI 323690646; *S. stellisacbiei*, GI 32967132; *S. caviscabies*, GI 7715013; *S. setonii*, GI 971124; *S. griseus*, GI 32967142; *S. ipomoeae*, GI 32967131; *S. albolongus*, GI 90960241; *S. cavourensis subsp. cavourensis*, GI 90654239; *S. flavofungini*, GI 146759931; *S. globisporus*, GI 90959882; *Streptomyces* sp. VTT E-99-1330 (A83), GI 16611983; *Streptomyces* sp. MP47-91, GI 161137727; *S. europaeiscabiei* strain KACC 20186, GI 32967136; *Streptomyces* sp. 3162, GI 110348028; *S. almquistii* NRRL B-1685, GI 66379348; *S. althioticus* NRRL B-3981, GI 66379357; *S. matensis*, NR_041088.1; *S. pseudogriseolus* strain NRRL 3985, X 80827.1; *Streptomyces* sp. 04, EF 06345.1; *Streptomyces* sp. 172717, HQ 992742.1; *Streptomyces* sp. 216801, GI 209865555; *Streptomyces* sp. 219829, GI 332647042; *Streptomyces* sp. AF111, GI 218137896; *Streptomyces* sp. HA6, GI 310787797; *Streptomyces* sp. SD575, GI 169666106; *S. tendae* strain ATCC 19812, GI 219846281; *S. xylophagus*, AB184526.1. The bootstrap values from 1,000 pseudoreplications are shown at each of the *branch* points on the *tree*. Bar, 0.02 nucleotide (nt.) substitution per nt. position

were unable to biosynthesize IAA via IAM. Moreover, another IAM pathway gene *iaaH,* coding for the enzyme IAM hydrolase, was examined for its expression in the Trp-supplemented cultures of En-1 by reverse-transcriptase PCR (RT-PCR), in comparison with those of IFB-A02. Figure 3b illustrated that at 72 h post-treatment of Trp, transcriptional accumulation of *iaaH* occurred in En-1 as did in the positive control *S. coelicolor*, but no detectable transcripts were recorded for IFB-A02, which serves as a negative control herein. Taken together, it is evident that IAM-mediated IAA biosynthesis pathway was active and functional in En-1.

The In-planta Colonization of Endophytic En-1

Microscopic studies of the inoculated *Arabidopsis thaliana* stems and leaves (Fig. 4a, c) showed that endophytic streptomycete En-1 was predominantly found in intercellular

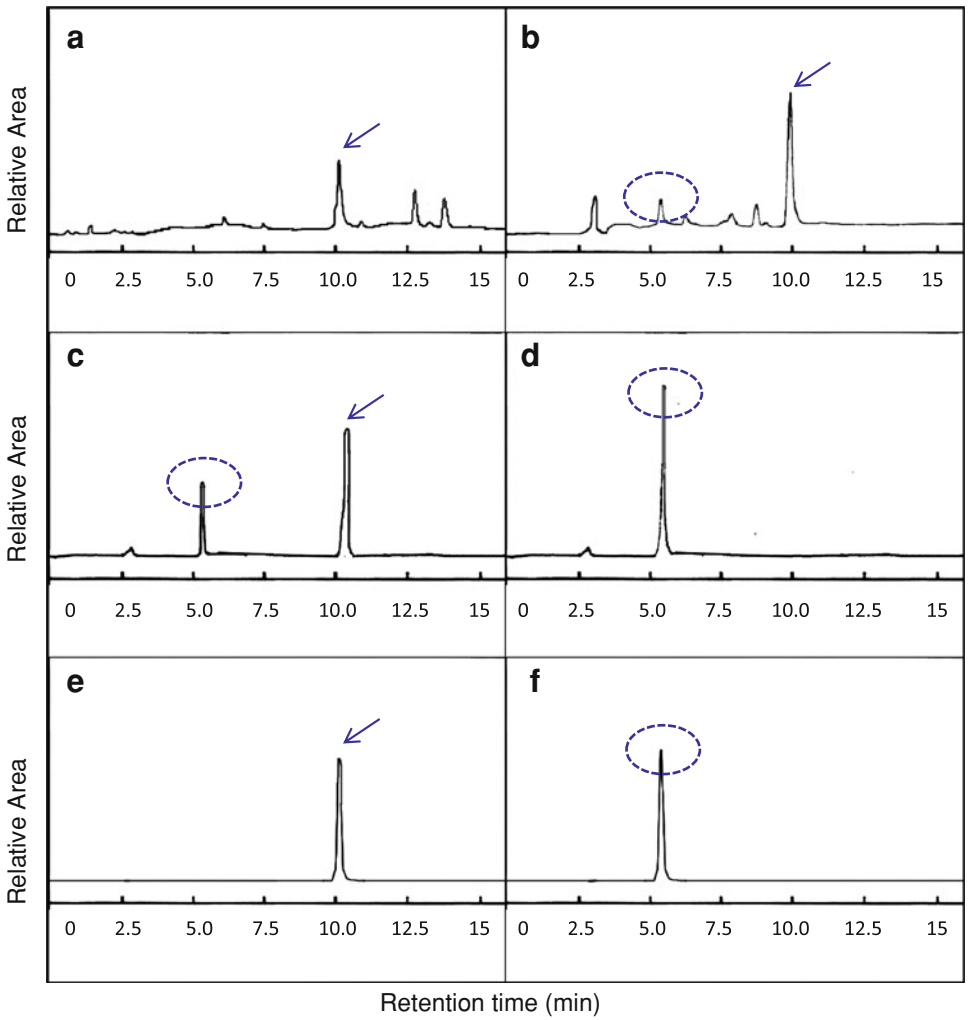

Fig. 2 Reverse-phase (RP) HPLC chromatograms of extracts of culture filtrates of endophytic *Streptomyces* sp. En-1 grown without Trp (**a**), with 5 mM Trp (**b**), with 5 mM IAM (**c**) in comparison to those of endophytic IFB-A02 amended with 5 mM IAM (**d**). The pure IAA at 0.1 mg ml^{-1} (**e**) and IAM at 1 mg ml^{-1} (**f**) were analyzed by RP-HPLC as standards. The IAA (as indicated by *arrows*) was eluted with retention time of 10.2 min, the peak of which was separated from that of IAM with retention time of 5.6 min (as indicated by *dashed circles*)

spaces, whereas no internal or surface microbial colonization were discerned in the sectioned stem and leave tissues of un-inoculated aseptic seedlings (Fig. 4b, d). The distribution of endophytic strain En-1 implies a putative in-planta spreading pattern of endophytes via apoplastic route upon their entry into plant. This proposition is in accordance with the previous findings that soil-living rhizobacteria enter the plant roots, mostly through the fissure of lateral root initiation, and later they spread systematically via apoplastic connections and develop intimate endophytic relationship with host plants [21].

Plant Growth Promoting Effects of Endophytic En-1

A gnotobiotic system was adopted to investigate the interactions between endophytic En-1 and *Arabidopsis thaliana* to exclude other unknown external factors interfering with this experimentation as occurred in the open environment. As shown in Fig. 5 and Fig. S1, the administration of En-1 inoculum resulted in a significantly increased biomass in treated *Arabidopsis* plantlets as compared to the un-treated control (35.1 ± 0.346 versus 26.2 ± 0.374 mg, $P < 0.001$), indicating the distinct phytostimulating effects of endophytic En-1; however, *Arabidopsis* plantlets administrated with either IFB-A02 or IFB-A03 did not displayed significantly improved growth as compared to control. Considering that both IFB-A02 and -A03 are non-IAA-producers, while En-1 has been shown to be an IAA-producing strain, IAA of endophytic En-1 origin may play a role in endophyte-plant mutual interactions exemplified by plant growth promotion herein.

Discussion

Despite earlier studies about IAA production in *Streptomyces* spp., most of which are soil-dwelling [13], few data are available concerning the biosynthesis of IAA in streptomycetes that are endophytic to medicinal plants. Here we demonstrate that an endophytic *Streptomyces* sp. En-1 may

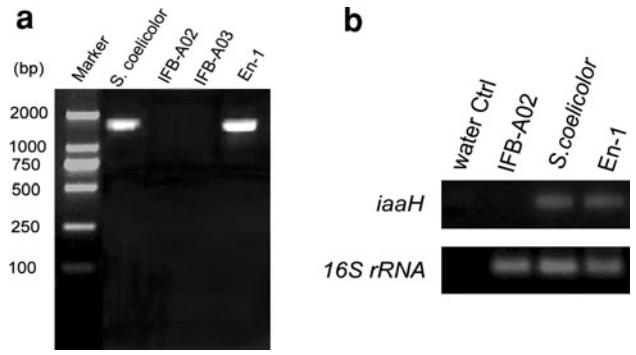

Fig. 3 Genetic examination of IAA biosynthetic genes in endophytic strains in contrast to *Streptomyces coelicolor* A3(2), the latter of which was used as the positive control because it is a known IAA producer. **a** Detection of *iaaM* in the genomes of endophytic strain *IFB-A02*, *-A03*, and *En-1* versus *S. coelicolor*. **b** Reverse-transcriptase PCR (RT-PCR) analyses of *iaaH* transcripts in endophytic strain En-1 as compared to those of non-IAA-producing strain *IFB-A02* and *Streptomyces coelicolor* A3(2), respectively. cDNA was generated from DNase-digested RNA that was extracted from 72 h cultured microbial cells supplemented with 5 mM Trp. Water control (*water Ctrl*) was set as an experimental negative control where PCR reactions were performed with equal volume of water as the template instead of cDNA. The 16SrRNA gene was used as a loading control

produce phytohormone IAA, putatively via IAM pathway. This finding not only expands the existence of microbial IAM-mediated IAA synthetic route to endophytic streptomycetes but also suggests that there is no definite boundary concerning IAM-mediated IAA biosynthetic pathway between pathogenic and endophytic streptomycetes, which seems opposite to the previously-reported tendency that phytopathogenic microbes would use the IAM route to produce IAA in contrast to plant-beneficial microbes using the IPyA pathway. As a fact, the occurrence of IAM-mediated IAA synthesis has been observed in symbiotic bacteria such as *Azospirillum* spp. *Rhizobium* spp. and *Bradyrhizobium* spp. [8, 18, 29].

Since gene products involved in IAA biosynthesis of endophytic streptomycetes are poorly understood, this study conducted a genetic approach. Here we utilize the documented conservative region of IaaM protein coding sequences derived from *S. coelicolor* and *S. scabies*, to design *iaaM* gene-specific primers for PCR, thereby facilitating genomic screening of *iaaM* in endophytic streptomycetes IFB-A02, -A03, and En-1. For verifying the operation of IAM pathway, we performed RT-PCR

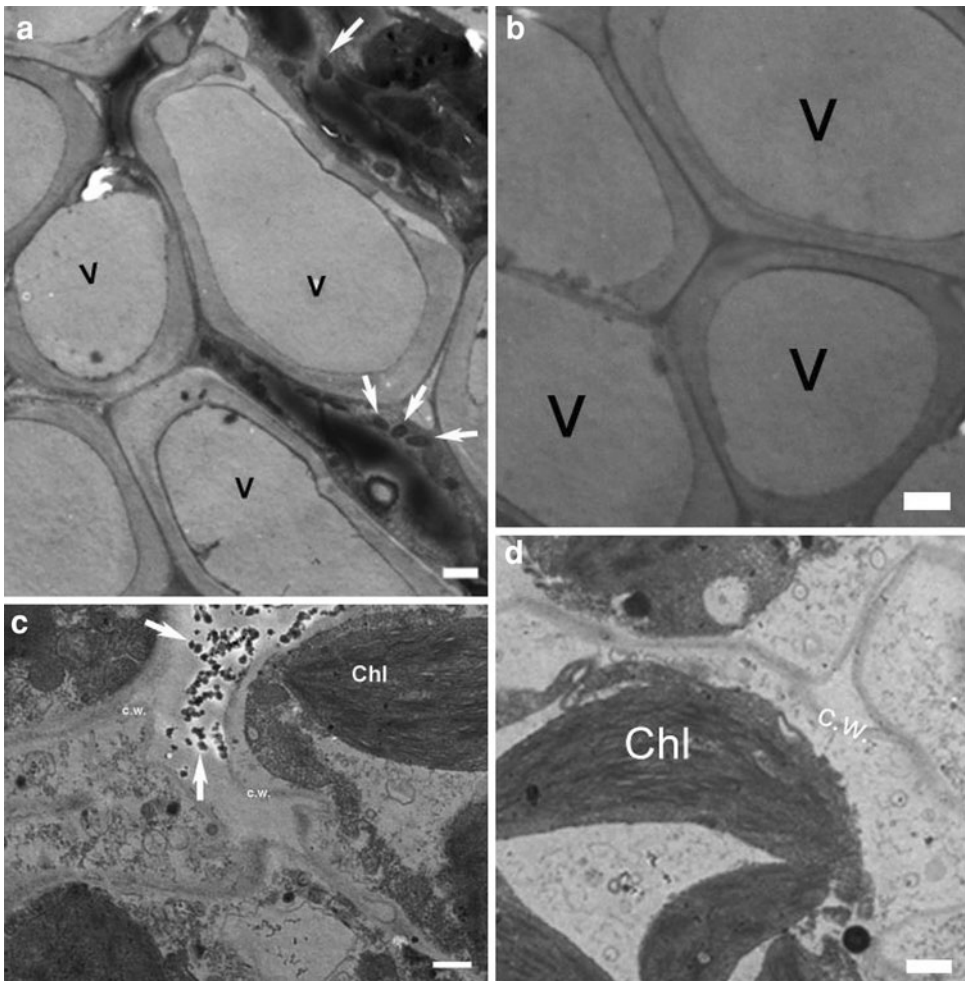

Fig. 4 Transmission electron micrographs of ultra-thin sections from endophyte-inoculated *Arabidopsis* stem (**a**) and leaf (**c**) tissues as compared to the un-inoculated counterparts (**b**, **d** respectively). The colonization of endophytic En-1 in the intercellular space of plant cells was indicated by *arrows*. *V* vessels, *Chl* chloroplasts, *c.w.* cell wall. The *scale bar* is 0.5 μm in **a** and **d**, 1 μm in **b** and **c**

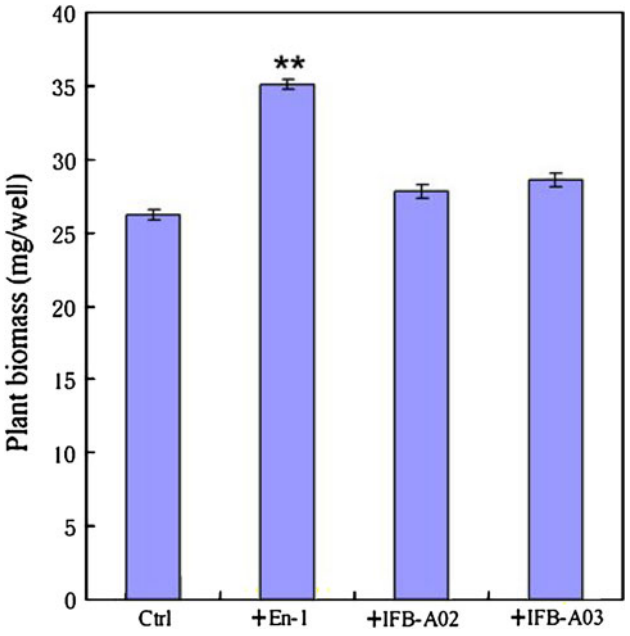

Fig. 5 Effects of endophytic culture on *Arabidopsis thaliana* growth, as indicated by plant biomass (fresh weight in milligrams per well) at 4 week. Data were expressed in mean ± SD. $^{**}P < 0.001$ versus control

analysis using the *iaaH* gene-specific primers originated from the conserved coding sequences for IaaH of *S. coelicolor* and *S. scabies*. The results demonstrated that the IAM pathway is functional in En-1, but not in IFB-A02 and -A03. To the best of our knowledge, for the first time we show the potential of IAA production of an endophytic *Streptomyces* sp. from a precious medicinal plant species.

As a spore-bearing endophytic streptomycete, IAA-producing strain En-1 would serve as a promising candidate for enhancing plant growth as exemplified in the model plant *Arabidopsis*. In light of that En-1 might grow simultaneously with the germinating seed and developing seedling, En-1 inoculants applied to seeds would facilitate its distribution throughout the mature plant and thus exert its beneficial actions.

This study demonstrates the presence of IAM-mediated IAA biosynthesis in endophytic *Streptomyces* sp. En-1 and that the endophytic IAA producing capacity is largely dependent on the supply of the precursor Trp. Actually, Trp has been detected as the predominant compound in some plant exudates [35]. Furthermore, endophytic IAA may exert phytostimulating effects on host plant while the plant provides favorable niches for endophytic colonization, as evidenced by the microscopic examination in this study. Taken together, it suggests that endophyte-produced IAA may play an important role in mutual interaction of endophytes and host plant.

Previous studies regarding endophytic streptomycetes from wheat revealed that they might promote plant health by activating the host defense signaling pathways mediated by phytohormone salicylic acid or jasmonic acid/ethylene using *Arabidopsis thaliana* as their alternative host [27]; however, the levels of the relevant phytohormone had not been measured via chemical analysis in those endophytic isolates. Several other investigations indicate that endophytic streptomycetes could produce anti-microbial agents to increase plant disease resistance [36, 37] and some non-streptomycete *Actinomyces* spp. such as *Amycolatopsis kentuckyensis* and *Micromonospora arenae* sourced from Upper Amazonian rainforest might possess antimicrobial activity against six test microorganisms, possibly owing to multiple compounds secreted by the endophytes [38]. Collectively, in the context of plant growth enhancement and health strengthening, endophytic actinomycetes represent a predominant reservoir.

Further work is necessary to elucidate the full picture of IAA biosynthetic pathways in endophytic streptomycete En-1, for example, whether endophytic IAA production proceeds solely via IAM pathway or any redundant IAA synthetic pathways co-exist. More investigations should also be directed to signaling transduction pathways modulated by IAA of endophytic origin during the endophyte-host plant interactions.

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