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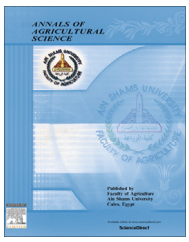

Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss.)

Amr Hamza Fouda [1], Saad El-Din Hassan [*], Ahmed Mohamed Eid [2], Emad El-Din Ewais [3]

Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt

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Abstract Fungal endophytes associated with medicinal plants have potential role to promote plant growth through different mechanisms. However, the biological and ecological roles of fungal endophytes still totally unexplored. In this study, three different fungal endophytes were isolated from the medicinal plant of *Asclepias sinaica* and identified as *Penicillium chrysogenum* Pc_25, *Alternaria alternata* Aa_27 and the third fungal strain was described as sterile hyphae Sh_26. It was recorded that, these endophytes had various ability to produce several extracellular enzymes including amylase, pectinase, cellulase, gelatinase, xylanase and tyrosinase. Their antimicrobial activities against different specific test organisms were investigated as well. In addition, both endophyte isolates i.e. Sh_26 and Aa_27 were found to promote root growth higher than Pc_25 and control treatments. These fungal isolates had a considerable impact on plant growth parameters including root elongation as a result of ammonia and IAA production.

Introduction

Endophytes are defined as microorganisms including bacteria, fungi, and actinomycetes that inhabit intra- and intercellular plant tissues for all or part of their life cycle. Endophytes have the ability to colonize internal plant tissues of healthy leaves, petioles, stems, twigs, bark, root, fruit, flower, and seeds without causing any apparent harm or pathogenic infection to their host plants. Endophytic fungi are ecological and polyphyletic group of highly diverse fungi, mostly belonging to ascomycetes and anamorphic fungi (Arnold, 2007). Approximately, it has been estimated that more than one million different endophytic fungal strains inhabit about 300,000 various plant species. The hyperdiversity of endophytic fungi derives from that each individual plant species can be colonized with one or more fungal strains (Huang et al., 2007). Fungal endophytes produce

* Corresponding author. Tel.: +20 1023884804.
E-mail addresses: amr.fouda83@gmail.com (A.H. Fouda), Saad.el-din.hassan@umontreal.ca (S.E.D. Hassan), medoahmedwave@yahoo.com (A.M. Eid), Ewais_e@yahoo.com (E.E.-D. Ewais).
[1] Tel.: +20 1113351244.
[2] Tel.: +20 1223360234.
[3] Tel.: +20 1119636529.

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bioactive metabolites that mediate in the plant–endophyte interaction (Strobel, 2003). In addition, fungal endophytic metabolites are useful resources for natural products which effectively have wide range of application in medicine, agriculture, and industry (strobel and Daisy 2003; Selim et al., 2012).

Fungal endophytes have the ability to produce numerous extracellular enzymes; such as pectinases, cellulases, lipases, amylases, laccases, and proteinases. These fungal enzymes play the key role in biodegradation and hydrolysis processes which are significantly important mechanisms against pathogenic infection and to obtain their nutritional need from the host plants (Sunitha et al., 2013). The ability of fungal endophytes to produce different enzymes has been reported by Choi et al. (2005) and Sunitha et al. (2013), however further quantitative assays for fungal endophytic enzymes are required to understand the ecological role of these fungal endophytes.

Many bioactive metabolites are originated from microbial organisms, fungi are the core important groups of eukaryotic organisms that have wide capacity to produce numerous metabolites with antimicrobial activities and possess potential application as drugs. Several bioactive compounds including antifungal and antibacterial agents have been isolated from fungi (Suryanarayanan et al., 2009). However few endophytic fungal isolates have investigated for their biological applications including their ability for antimicrobial activity; thus, it seems that screening the antimicrobial activity of fungal endophytes is valuable to discover novel antimicrobial producers.

Promotion of plant growth is the major contribution of fungal symbiosis (Hassan et al., 2013), however fungal endophytes promote plant growth through the production of ammonia and phytohormones, particularly indole acetic acid (IAA) (Bal et al., 2013). Generally, IAA acts as plant growth promoter which enhances both cell elongation and cell division, and is essential for plant tissues differentiation (Taghavi et al., 2009). The ability of soil microorganisms to involve in the synthesis of IAA in pure culture and in soil has been recorded (Spaepen and Vanderleyden, 2011); however, endophytic microorganisms isolated from various plants have showed high IAA production level compared to those isolated from root-free soil (Spaepen et al., 2007). The functional role of IAA in plant growth in addition to the capacity of fungal endophytes to produce IAA has gained great attention due to their impact on the concentration and distribution of IAA in plant tissues.

Little is known about the biology and ecology of fungal endophytes; subsequently, isolation and characterization of fungal endophytes that colonize different plant species of various habitats and ecosystem is potentially useful. *Asclepias sinaica* is a medicinal plant from the Sinai desert is widely used in traditional Bedouin medicine to treat some cancer diseases (El-Seedi et al., 2013). *A. sinaica* plant may host useful microbial community that might be potentially have several biological and ecological role in their environment. However, information about the biological and ecological role of fungal endophytes community of *A. sinaica* plant is still unknown. Therefore, the aim of this study was to isolate and identify fungal endophytes survived inside the leaves of *A. sinaica* plant, to determine the antimicrobial and extracellular enzymatic activities of these fungal endophytes, to assay the capacity of these endophytes to produce ammonia and IAA, and finally to estimate the effect of fungal inoculation on the growth of plant roots.

Materials and methods

Plant sampling and study area

Plants of *A. sinaica* were collected from Ain Shakaya (28.543386 N, and 33.933071 E), Saint Katherine, South Sinai, Egypt. The plant materials were carefully placed in sterile polyethylene bags and brought to the laboratory in portable cool chambers (4 °C). The botanical identification of these plants has been carried out at the herbarium of Botany and Microbiology Department of Al-Azhar University, and specimen of the plant herbarium is deposited in the herbarium of Botany and Microbiology Department of Al-Azhar University under the registration name of *A. sinaica* (1_As). Plant picture is shown in Plate 1.

Isolation of fungal endophytes

The plant leaves were washed by running tap water and sterilization of leaves surfaces was achieved by subsequent soaking them in series of solutions as follows: sterile distilled water for 1 min, ethanol 70% for 1 min, sodium hypochlorite 2.5% for 4 min, ethanol 70% for 30 s and finally washed in sterile distilled water for 3 times. The last washing water was plated onto bacterial, fungal, and actinomycetales culture media of Nutrient agar, Czapek Dox agar, and Starch nitrate agar, respectively. The success of surface sterilization method was confirmed by the absence of any microbial growth onto the cultural media from the plating of last washing water.

The sterilized plant leaves were cut by sterilize knife into 5 mm segments. Twenty leaf segments were placed in Petri dishes (9 cm) containing Czapek Dox agar and Malt extract agar media and incubated at 28 ± 2 °C. Another part of sterilized leaves segments were crushed in sterile saline solution by sterile homogenizer. One ml of sterilized crushed samples was serially diluted till 10^{-3} and 0.1 ml was spread onto Czapek Dox agar and Malt extract agar media and incubated at 28 ± 2 °C (Arora et al., 2014). Regular observations were done from the second day onwards for a period of 3–4 weeks for fungal growth (Bills and Polishook, 1991). The fungal

Plate 1 *Asclepias sinaica* (Bioss.).

growth from internal tissues or crushed segments was checked for purity, transferred to fresh cultural slants and stored at 4 °C for further study.

Identification of fungal endophytes

Identification was done on the basis of morphological, taxonomic characteristics and direct microscopic examination of the fruiting bodies and spores using standard manuals. (Moubasher and Moustafa, 1970; Pitt and Hocking, 1997 for *penicillium sp*., Domsch et al., 1980 for sterile hyphae; Woudenberg et al., 2013, for *alternarea sp*.).

Antimicrobial activity of fungal endophytes

Preparation of endophytes extracts

To test the antimicrobial activity of fungal endophytes, the isolated fungal strains were cultured in Malt extract liquid medium for 10 days at 28 °C on a shaker at 180 rpm. Crude fermentation broth was blended thoroughly and centrifuged at 4000 rpm for 5 min. Liquid supernatant was extracted with an equal volume of ethyl acetate thrice. The organic solvent extract was then evaporated under reduced pressure using rotary evaporator. The crude extracts were dissolved in dimethyl sulfoxide (DMSO) and used for antimicrobial screening (Lv et al., 2010).

Antimicrobial assay of fungal endophytes extracts

The crude extracts of fungal endophytes were dissolved in DMSO and used for antimicrobial activity assay. Antimicrobial activity of secondary crude metabolites was tested by well diffusion method according to the protocol of Yadav et al. (2010).

The coded test organisms used for antimicrobial assay were Gram-positive bacteria: *Staphylococcus aureus*, ATCC 6538 and *Bacillus subtilis*, ATCC 6633; Gram-negative bacteria: *Escherichia coli*, ATCC 8739; *Pseudomonas aeruginosa*, ATCC 9027 and *Salmonella typhimurium* ATCC 14028; and yeast strain of *Candida albicans*, ATCC 10231. Test organisms inoculated in Petri dishes containing Muller-Hinton agar medium for bacteria, and sabouraud agar medium for yeast.

Three wells of 1 cm diameter were made in the test organism growing media using a sterile cork borer and filled with 40 µl of fungal endophytic extract. Negative control wells were filled with 40 µl of DMSO without endophytic fungal extracts. The plates were kept in refrigerator at 4 °C for 4 h for complete diffusion of antimicrobial compounds, and then incubated at 35 ± 2 °C for 24 h for bacteria and 28 ± 2 °C for yeast. The presence of inhibition zones around the wells was measured to determine the antimicrobial activity of fungal endophytic secondary extracts. All antimicrobial activity assays were performed in triplicate.

Screening the extracellular enzymatic activities of fungal endophytes

The production and activity of extracellular enzymes by fungal endophytes were assessed by growing them on Yeast-Malt (YM) agar media (YM: 10 g/L glucose, 5 g/L peptone, 3 g/L yeast extract, 3 g/L malt extract, 1.5% agar, pH 6.7) (Molina et al., 2012) and placing 5 mm fungal plugs on the YM agar media supplemented with dissolved and specific indicative substrates. After incubation for 3–5 days depending on the growth rates of fungal endophytes at 28 °C, the appearance of clear zone surrounding the fungal colony was measured after adding specific reagent and used as indicator for extracellular enzymatic activities. All assays were performed in triplicate.

Amylolytic activity

Amylase activity was assessed by growing the fungal isolates on YM agar medium supplemented with 1% soluble starch. After incubation, the plates were flooded with 1% iodine. The appearance of clear zones around fungal growth was measured to determine the amylolytic activity.

Proteolytic activity

The YM agar medium containing 1% gelatine was used to determine the fungal protease enzyme activity. After incubation, the degradation of gelatine was seen as clear zoon around the colonies by using acidic mercuric chloride as an indicator.

Cellulase activity

The appearance of clear zone around the fungal colony grown on YM medium supplemented with 1% cellulose or carboxymethylcellulose (CMC) was measured, in order to assess the fungal cellulolytic activity after adding iodine solution as indicator.

Pectinolytic activity

Pectinolytic activity was determined by growing the fungi in 1% Pectin-YM containing medium. After the incubation period, the plates were flooded with 1% aqueous solution of hexadecyl trimethyl ammonium bromide. A clear zone formed around the fungal colony indicated the activity of pectinase enzyme.

Xylanolytic activity

Yeast-Malt agar medium supplemented with 1% xylan of corn cobs was used in order to measure the fungal xylanolytic activity. After incubation period, screening the xylanase activity was appeared as a clear zone around the fungal colony as a result of using absolute ethyl alcohol to indicate the xylan biodegradation.

Tyrosinase activity

The tyrosinase activity of endophytic fungal strains was assessed by growing fungi on YM agar supplemented with 1% tyrosine. After 5 days of incubation period, the mixture of 0.1% ρ-cresol and 0.05% glycin was overlaid on the surface of colony. The appearance of reddish brown colour around the fungal colony indicated the activity of tyrosinase enzyme (Hankin and Anagnostakis, 1975).

IAA Production by fungal endophytes

The ability of fungal endophytes to produce IAA was determined, where the endophytic fungal strains were inoculated in Czapek Dox (CD) broth at 28 °C. One disc (1 cm diameter) of each inoculum was added to 20 ml of CD liquid medium

containing different concentration of tryptophan (1, 2 and 5 mg/ml) or without tryptophan and incubated for 10 days.

Five ml of each culture was collected from the incubating broth after 10 days and centrifuged at 6000 rpm for 30 min. One ml of the supernatant was mixed with 1 drop of orthophosphoric acid and 2 ml of Salkowski's reagent (300 ml concentrated Sulphuric acid; 500 ml distilled water; 15 ml 0.5 M $FeCl_3$). Development of a pink colour indicated IAA production as the optical density was measured at 530 nm using Spectrophotometer (Jenway 6305 UV spectrophotometer). The amount of IAA produced was estimated by a standard IAA graph (Ahmad et al., 2005). All the IAA production and determination were performed in triplicate.

Ammonia production

The ability of Endophytic fungal strains to produce ammonia was assessed, where fungal strains were grown in peptone water for 72 h at 28 °C. The addition of 1 ml Nessler's reagent in peptone liquid media was used to determine the ammonia production by endophytes. Where, the colour change to faint yellow indicates the minimum ammonia production and colour change into the deep yellow to brownish colour indicates the maximum ammonia production by endophytes (Singh et al., 2014).

Effect of fungal endophytes on root growth

The effect of endophytic inoculation on root growth was investigated on maize (*Zea mays* L.) plant. The seeds of maize (*Zea mays* L.) were surface sterilized by soaking in 2.5% sodium hypochlorite for 3 min and then they were washed by sterile distilled water for 5 times. Endophytic fungal strains were inoculated in CD broth at 28 ± 2 °C for 5 days. Sterilized seeds were incubated with 50 ml of each fungal strain broth medium at room temperature for 24 h. The CD broth without fungal inoculation was used as control treatment. After 24 h incubation, the soaked seeds were placed in sterilized cup containing wet sterilized filter paper and they were incubated at room temperature for 5 days in dark to measure the root growth.

Statistical analysis

All results presented are the means of three independent replicates. Data were subjected to statistical analysis by a statistical package SPSS v17. The mean difference comparison between the treatments was analysed by analysis of variance (ANOVA) and subsequently by Tukey HSD test at $P < 0.05$.

Results

Isolation and identification of fungal endophytes

Three fungal endophytes Pc_25, Sh_26, and Aa_27 were isolated from *A. sinaica* leaves, these fungal strains were identified as *Penicillium chrysogenum*, one fungal strain was described as *Sterile hyphae*, and *Alternaria alternata*, respectively.

Morphological description of Pc_25 (Fig. 1 and Table 1) showed that the colonies on MEA had 25–40 mm diameter, usually plane, low and velutinous, occasionally floccose centrally or somewhat granular; mycelium inconspicuous; conidial production moderate to heavy, greyish turquoise to dull green; reverse pale, yellowish, yellow brown. Conidiophores are

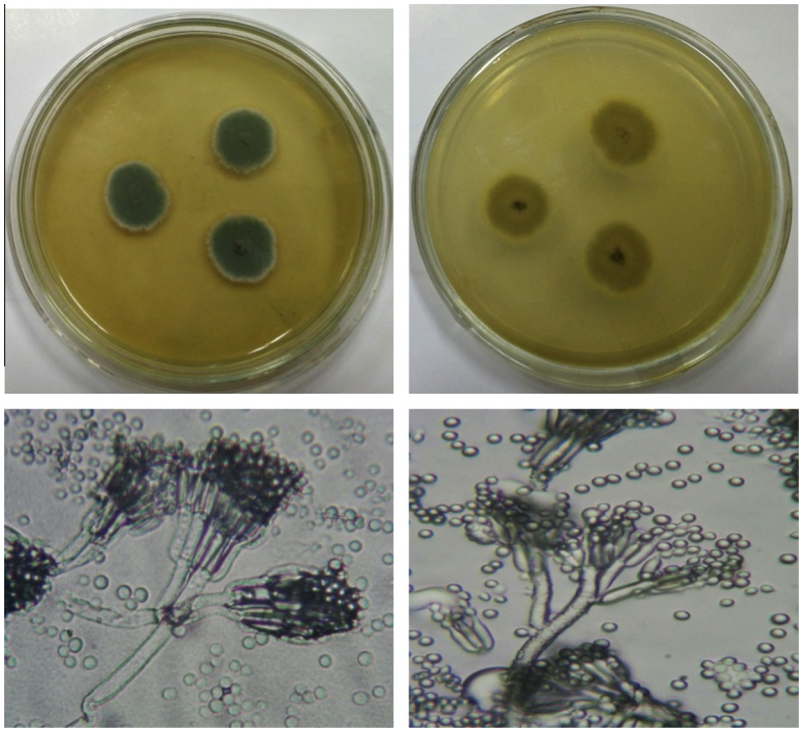

Fig. 1 Cultural morphology and microscopic picture of fungal endophyte Pc_25 grown on CYA and 2% MEA media ($X = 800$).

borne from surface or subsurface hyphae, stipes commonly 200–300 μm long, with thin smooth walls, penicilli typically terverticillate, with 1–2 rami, either terminal and appressed or sometimes subterminal and divergent, in that case appearing biverticillate; phialides ampulliform, 7–8(–10) μm long; conidia ellipsoidal to subspheroidal, 2.5–4.0 μm long, smooth walled, borne in long, irregular columns.

The morphology of second fungal strain, of Sh_26 (Fig. 2) showed that the colonies appeared on MEA medium reached to 45–65 mm diameter, dense to floccose white mycelium, reverse uniformly pale or white centrally, colourless at the margins, branched nonseptated mycelium.

Fig. 3 shows that colonies of Aa_27, grown on MEA were 50–70 mm diameter, or covering the whole Petri dish, plane, of deeply floccose off-white to grey brown mycelium converting to low and dense, dark olive to dark in old culture; reverse brown to nearly black. Conidia are blown out from the apices of undistinguished conidiophores as short, irregularly branched chains of up to 10 units, and then septating both laterally and longitudinally, with up to six transverse and two to three longitudinal or oblique septa, usually of clavate or pyriform shape overall, tapering towards the apices, forming a short beak, in culture usually 20–40 and 8–12 μm, with walls smooth to conspicuously roughened.

Table 1 Morphological characters of fungal endophyte Pc_25 identified as *Penicillium chrysogenum*.

Conidia diameter	2.5–4 μm
Conidial ornamentation	Smooth
Phialide length	7–8 (10) μm
Metulae length	7–8 (10) μm
Stipe width	2–3 μm
Stipe ornamentation	Smooth
Conidiophore branching pattern	Terverticillate
Macromorphology on MEA	
Colony diameter	25–40 mm
Colour obverse	Dull green
Colour reverse	Pale yellow, yellowish brown

Antimicrobial activity

The antimicrobial activities of fungal endophytes isolates were evaluated. Analyses of variance (ANOVA) revealed that there were significant differences and variations among the three fungal endophytes to inhibit the growth of the coded tested microorganisms (Fig. 4).

Multi-comparison analysis of the differences showed that the crude extract of Sh_26 significantly inhibited the growth of *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. Typhimurium*, compared to control and Pc_25 and Aa_27 endophyte extracts ($F_{3,8}$ = 366.75, 3481.0, 16.616, 968.0, and 27.848; $P \leqslant 0.001$) (Fig. 5). However, the Sh_26 did not significantly inhibit the growth of *C. albicans* compared to control treatment ($F_{3,8}$ = 2703.9; P = 1.00).

Inhibition zone of secondary metabolites from fungal endophyte of Aa_27 against *P. aeruginosa* and *S. aureus* was significantly higher ($P \leqslant 0.001$) than those observed for the control and Pc_25 endophytic treatments. Moreover, the only endophytic fungal extract which inhibited the growth of tested microbe of *C. albicans* was Aa_27 endophyte (Fig. 5). Results also showed that Endophyte of Aa_27 did not show

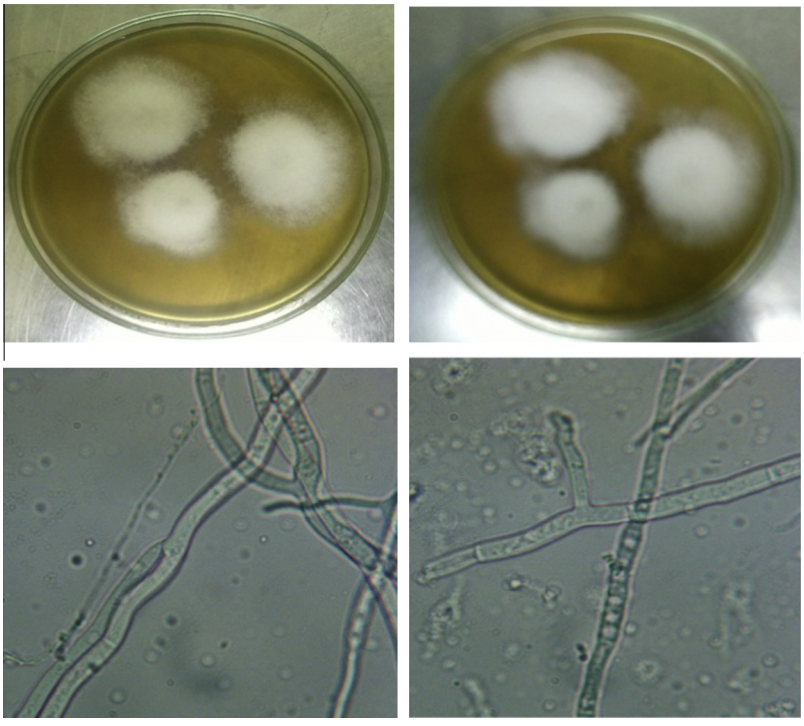

Fig. 2 Cultural morphology and microscopic picture of fungal endophyte Sh_26 grown on CYA and 2% MEA media (X = 800).

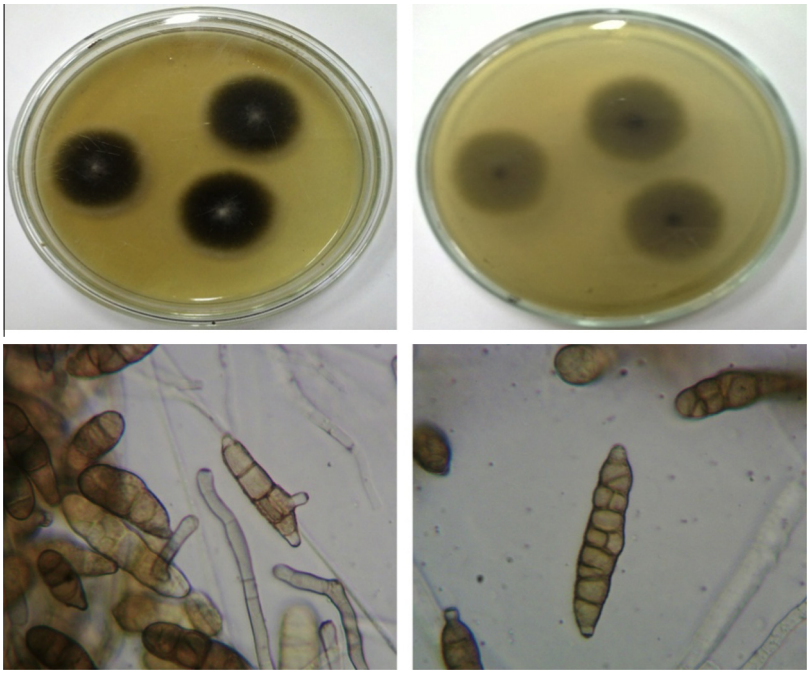

Fig. 3 Cultural morphology and microscopic picture of fungal endophyte Aa_27 grown on CYA and 2% MEA media ($X = 800$).

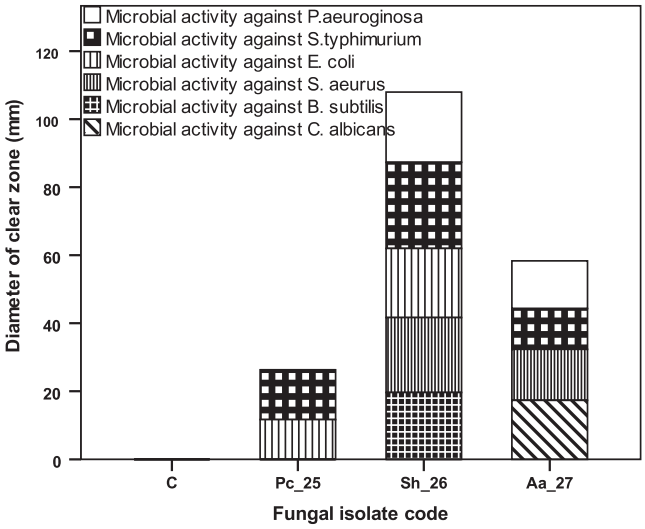

Fig. 4 Antimicrobial activities of fungal endophytes isolated from *Asclepias sinaica*. C, control (without fungal inoculation); Pc_25, *Penicillium chrysogenum*; Sh_26, *Sterile hyphae*; Aa_27, *Alternaria alternata*.

any antimicrobial activity against the pathogenic microbes of *B. subtilis* and *E. coli*.

Analysis of variance for the antimicrobial activity showed that Pc_25 endophyte significantly inhibited the pathogenic colonies of *S. typhimurium* and *E. coli*, compared to control and Aa_27 endophytic treatments ($P \leqslant 0.001$). Data also showed that, there are no any inhibition zones have been detected around the pathogenic microbial colonies of *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *C. albicans* due to the application of fungal metabolites of endophyte isolate Pc_25.

Extracellular enzymatic activities

The recorded results revealed that Aa_27 endophyte had the highest ability to produce all tested extracellular enzymes, but this endophytic strain was recorded as negative tyrosinase producer. The endophyte of Pc_25 exhibited extracellular activity for amylase, pectinase, cellulase and xylanase; whereas, the Sh_26 strain showed negative extracellular activity for amylase, xylanase, and gelatinase (Table 2).

Maximum amylase activity was recorded in Pc_25; however, there is no significant difference ($P = 0.121$) in amylase activity was detected between Pc_25 and Aa_27. Sh_26 endophytic strain did not show any amylase activity (Table 2).

The difference between pectinase activities of Pc_25 and Sh_26 ($P = 1.35$) and between Pc_25 and Aa_27 ($P = 1.354$) did not reach significant level. However pectinase activity of Aa_27 was significantly higher as compared to those detected by Sh_26 strain ($P = 0.025$).

Although maximum extracellular cellulase activity was recorded in Pc_25 followed by Aa_27 and Sh_26 endophytic strains, ANOVA analysis showed that variations between different endophytic strains to hydrolyze cellulose and CMC substrates ($P > 0.05$) did not reach significant level.

Among the tested endophytes, Sh_26 strain showed negative xylanase activity, whereas Aa_27 significantly exhibited high xylanolytic activity compared to Pc_25 ($P \leqslant 0.001$).

Proteolytic activity of Aa_27 exerted the highest value, compared to the other tested strains ($P \leqslant 0.001$).

The production of extracellular tyrosinase was positive in case of Aa_27 and Sh_26, and negative in case of Pc_25.

IAA production

Results presented in Fig. 6 showed that all the fungal endophytes are able to produce IAA without tryptophan or by

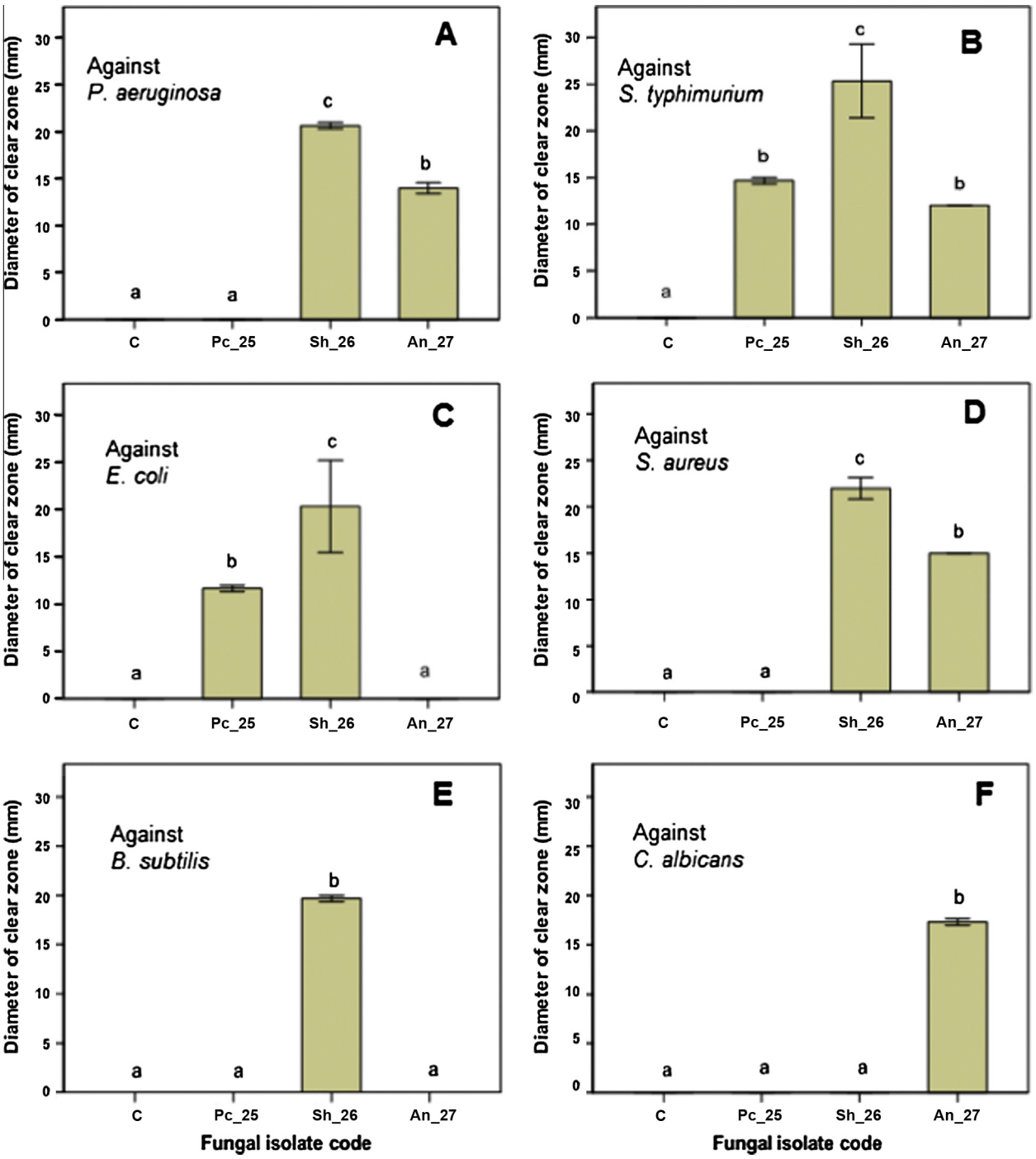

Fig. 5 Antimicrobial activities of fungal endophytes against some test organisms. C, control (without fungal inoculation); Pc_25, *Penicillium chrysogenum*; Sh_26, *Sterile hyphae*; Aa_27, *Alternaria alternata*. Error bars are ±SE ($n = 3$). Different letters on bars denote that mean values are significantly different ($P \leqslant 0.05$) by Tukey LSD test ($n = 3$).

Table 2 Extracellular enzymatic activities of fungal endophytes using solid media.

Fungal isolate code	Diameter of clear zones (mm)						Tyrosinase
	Amylase	Pectinase	Carboxy-methylcellulase	Cellulase	Xylanase	Gelatinase	
Control	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	−ve
Pc_25	38.33 ± 1.66^{b}	41.67 ± 1.66^{bc}	41 ± 1^{b}	38 ± 1.15^{b}	25.33 ± 0.33^{b}	0 ± 0^{a}	−ve
Sh_26	0 ± 0^{a}	40.67 ± 0.66^{b}	40 ± 1.15^{b}	38 ± 1^{b}	0 ± 0^{a}	0 ± 0^{a}	+ve
Aa_27	32 ± 3.03^{b}	45.68 ± 0.67^{c}	$38.67 \pm .66^{b}$	36.33 ± 1.2^{b}	36.66 ± 0.65^{c}	33.3 ± 1.65^{b}	+ve

In a column, values are the means ± SE followed by different letters are significantly different ($P \leqslant 0.05$) by Tukey LSD test ($n = 3$).

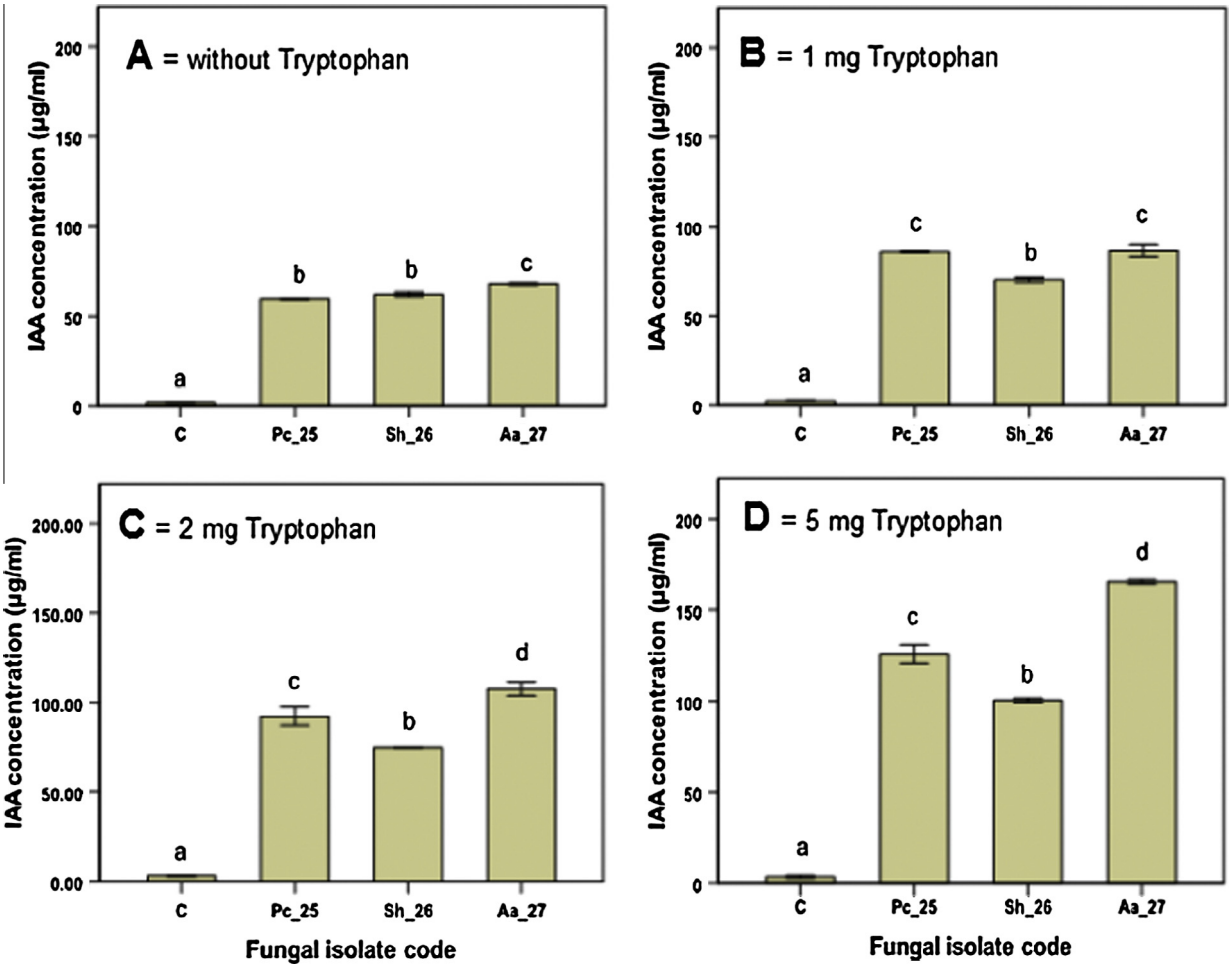

Fig. 6 IAA production by fungal endophytes isolated from *Asclepias sinaica* at different concentrations of tryptophan. C, control (without fungal inoculation); Pc_25, *Penicillium chrysogenum*; Sh_26, *Sterile hyphae*; Aa_27, *Alternaria alternata*. Error bars are ±SE ($n = 3$). Different letters on bars denote that mean values are significantly different ($P \leqslant 0.05$) by Tukey LSD test ($n = 3$).

using different concentrations of tryptophan as a precursor. Accordingly, increase the tryptophan concentration in the growth media resulted in the enhancement of IAA production, being maximum with 5 mg/ml of tryptophan ($P \leqslant 0.05$).

Analysis of variance revealed that there was significant variation in IAA production between the different endophytic inoculations. At growth media without tryptophan, the IAA production significantly differed between different fungal endophytes ($F_{3,8} = 1422.9$; $P \leqslant 0.001$), where Pc_25 and Sh_26 endophytes produced similar IAA contents ($P = 0.19$), which were significantly lower than those produced by Aa_27 ($P \leqslant 0.01$). At 1 mg/ml tryptophan-growth media, multi-comparison analysis ($F_{3,8} = 423.9$; $P \leqslant 0.001$) showed that Pc_25 and Aa_27 were similar IAA producers ($P = 0.996$) and significantly higher than Sh_26 endophyte ($P \leqslant 0.005$) (Fig. 6). At growth media with tryptophan concentrations of 2 mg/ml and 5 mg/ml, the analysis of variance was ($F_{3,8} = 203.6$; $P \leqslant 0.001$) and ($F_{3,8} = 682.4$; $P \leqslant 0.001$), respectively. In these media, the maximum IAA production was recorded for Aa_27 followed by Pc_25, and then Sh_26 endophyte ($P \leqslant 0.05$) (Fig. 6).

The results showed also that all three fungal endophytes had the ability to produce ammonia in the growth media (Table 3).

Table 3 Root growth of maize plants and levels of ammonia production as affected by inoculation with the three fungal endophytic isolates.

Fungal isolate code	Root length (cm)	Ammonia production
Control	9 ± 0.23^{a}	−ve
Pc_25	13.4 ± 0.24^{b}	High
Sh_26	14.6 ± 0.25^{c}	Low
Aa_27	14.4 ± 0.24^{c}	High

In a column, values are the means ± SE followed by different letters are significantly different ($P \leqslant 0.05$) by Tukey LSD test ($n = 5$).

Effect of fungal inoculation on root growth

Analysis of variance showed that fungal endophytic inoculation significantly affected root growth of maize plant ($F_{3,16} = 90.22$; $P \leqslant 0.001$) (Plate 2). The results revealed that inoculation with Sh_26 and Aa_27 had similar effect on root growth ($P = 0.937$), however both of these endophytes significantly increased root growth, compared to control and Pc_25 endophytic treatments ($P \leqslant 0.05$). In addition, inoculation

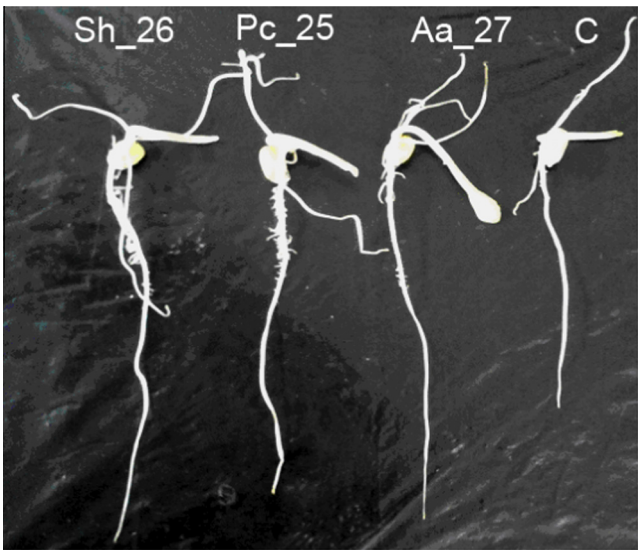

Plate 2 Effect of fungal endophytic inoculation on maize root growth. C, control (without fungal inoculation); Pc_25, *Penicillium chrysogenum*; Sh_26, *Sterile hyphae*; Aa_27, *Alternaria alternate*.

with Pc_25 endophyte enhanced the root growth comparing with control treatment ($P \leqslant 0.001$) (Table 3).

Discussion

Three fungal endophytes were isolated from *A. sinaica* plant, a medicinal native plant species in South Sinai- and identified as *P. chrysogenum* Pc_25, *A. alternata* Aa_27, and unidentified strain. Nonsporulating strain of fungal endophyte which failed to sporulate was described as sterile hyphae Sh_26. This is the common problem concerning with the identification of fungal endophytes (Gamboa and Bayman, 2001; Promputtha et al., 2005). These fungal endophytes have various biological and biochemical properties potentially useful.

In the present study, the antimicrobial activity of the three fungal endophytes isolated from *A. sinaica* plant revealed a significant inhibitive effect against the selected bacterial and fungal strains. The most antimicrobial activity was recorded for Sh_26 endophyte which inhibits the growth of most coded test organisms; however, Pc-25 and Aa_27 had inhibitory specific action against *C. albicans* and *E. coli*. Thereby, our investigations suggest that these fungal endophytes can be used as producers of metabolites with broad and specific antimicrobial activity. Kjer et al. (2009) isolated *Alternaria* sp. with antimicrobial activity from the mangrove plant, also Bertinetti et al. (2009) recorded endophytic *Penicillium* sp. as antifungal metabolite producer. Studies on endophytes for pharmaceutical and biotechnological purposes are fundamental for the discovery of new substances for human therapeutics including antibiotics, antimicotic, and anticarcinogenics (Bi et al., 2011). Endophytes isolated from medicinal plant *A. sinaica* showed bioactivity for broad spectrum of pathogenic microorganisms. Similarly, Devaraju and Sathish (2011) assayed the bioactivity of the endophytic microorganisms isolated from the medicinal plant *Mirabilis jalapa* L.

The three fungal endophytes isolated in this study have ability to produce various enzymes regarding to degrade starch, gelatine, cellulose, pectin, xylan, and tyrosine. Aa_27 endophyte had high ability to produce most of the tested extracellular enzymes, unlike to Sh_26 endophyte which only able to degrade cellulose and pectin. Cellulolytic and pectinolytic activities were prominent in all fungal endophytes where xylanolytic activity was found in Pc_25, and Aa_27 endophytes; similarly, Venkatesagowda et al. (2012) and Sunitha et al. (2013) recorded high activity of cellulase and pectinase enzymes in endophytic fungi isolated from oil-seeds and medicinal plants. Production of hydrolytic extracellular enzymes by fungal endophytes in particular cellulase and pectinase acts as a bioactivity to obtain nutrients from hosts and bio-resistance action against microbial pathogenic infection. In the present study, Pc_25, and Aa_27 endophytes exhibited maximum amylolytic activities, and Aa_27 was the only endophyte isolated in our study was able to produce gelatinase enzyme. Amirita et al. (2012) have been described 72% of endophytes isolated from some medicinal plants as good amylase and protease producers. Our finding supports that extracellular enzymatic activity of endophytes plays potential role to degrade polysaccharides and protein during the plant senesce. In another view regarding to biotechnology, amylolytic and proteolytic enzymes of endophytes are being investigated to improve industrial processes for polysaccharides and protein biodegradation. The production of extracellular enzymes by endophytes may imply a resistance strategy of the host plant against pathogenic microorganisms, and in another side to improve plant nutritional status (Choi et al., 2005).

In the present study, fungal endophytes isolated from *A. sinaica* plant possess different plant growth promoting activities including ammonia and IAA production. Different levels of IAA production were reported with various tryptophan concentrations. Although the obtained results also revealed the ability of these fungal endophytes to produce IAA in the absence of tryptophan i.e. the rate of IAA production was related to the increase of tryptophan concentration. Our findings of IAA production in the presence and absence of tryptophan are in agreement with those of other researchers (Ahmad et al., 2005). The three fungal endophytes showed difference in their capacity to produce ammonia and IAA; moreover, inoculation with these fungal isolates significantly enhanced the root growth. In addition, the effect of endophytic inoculation on root growth significantly differed with different endophytes, suggesting that the ability of these fungal endophytes to enhance plant root growth may be mediated by their effectiveness to produce ammonia and IAA. Zhang et al. (2011) reported that different endophytes having different ability for IAA production.

Conclusion

In the present investigation, three different fungal endophytes were isolated from medicinal *A. sinaica* plant and identified as *P. chrysogenum* Pc_25, *A. alternata* Aa_27 and Sterile hyphae Sh_26. These fungal endophytes were found to have antimicrobial activity against various coded test organisms, and have extracellular enzymatic activities to biodegrade different polysaccharides and gelatine. In addition, these fungal strains

have the capacity to produce ammonia and IAA which potentially influence the plant growth through various strategies. For biotechnological and ecological applications of plant growth promoting through fungal endophyte inoculation, various molecular and biochemical studies are required to investigate the role of endophytes to promote plant growth.

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