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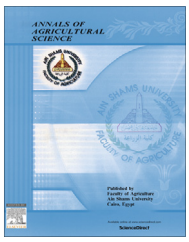

Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss.)

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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.

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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

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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.).

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