Characterization of plant growth-promoting activities of endophytic fungi isolated from Mongolian medicinal plants

Ph.D. dissertation

Adiyadolgor Turbat

Supervisors:

Dr. András Szekeres, senior research fellow

Prof. Dr. Csaba Vágvölgyi

Doctoral School of Biology

Department of Microbiology

Faculty of Science and Informatics

University of Szeged

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2 LIST OF ABBREVIATIONS

CAS	Chrome azurol S
EF	Endophytic fungi
EtOAc	Ethyl acetate
HPLC	High Performance Liquid Chromatography
IAA	Indole-3-acetic acid
IAAld	Indole-3-acetaldehyde
IAM	Indole-3-acetamide
IAN	Indole-3-acetonitrile
ILA	Indole-3-lactic acid
IOL	Indole-3-ethanol
IPDC	Indole-3-pyruvate decarboxylase
IPyA	Indole-3-pyruvate
ITS	Internal Transcribed Spacer
LB	Luria-Bertani broth
MeOH	Methanol
MM9	Minimal Media 9
MPKV	Modified Pikovskaya's Agar
MSBM	Murashige and Shook basal medium
MRM	multiple reaction monitoring
NB	Nutrient broth
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PCR	Polymerase chain reaction
PIPES	Piperazine-N, N-bis (2-ethanesulfonic acid)
PKV	Pikovskaya's Agar
rpm	Revolutions per minute
TAM	Tryptamine
TOL	Tryptophol
TSO	Tryptophan side-chain oxidase

3 INTRODUCTION

3.1 Characterization of endophytic fungi

Plant endophytes consist of both fungal and bacterial communities that live inside plants and were discovered for the first time by Freeman in *Persain darnel* (Freeman et al. 1904). Most species of vascular plants were found to be harbor of both endophytic fungi (EF) and endophytic bacteria (Rodriguez et al. 2009). According to Huang et al., over one million species of endophytes exist (Huang et al. 2007). There have been several reviews as well as studies on EF, in which their concept have been defined in many forms. EF are mainly the members of the Ascomycota division, but they can also be found in the taxa of Basidiomycota, Zygomycota or Oomycota (Zheng and Jiang 1995, Sinclair and Cerkauskas 1996). EF live in the tissues of plants without causing any symptoms of disease in their host (Gouda et al. 2016). Their specific ecological niche with the continual metabolic interactions between the endophytic microorganism and the host plant seems to serve a remarkable strong evolutionary pressure and improves the possibilities of the synthesis of secondary metabolites of endophytes with novel properties (Schulz et al. 2002). The diversity and population compositions of EF in plants are highly variable. The variation of these fungal communities under different conditions, such as different host plant species and genotypes, location of host, growth conditions, host developmental stages have been profoundly investigated (Balint et al. 2013, Lamit et al. 2014, Gazis and Chaverri 2010, Tejesvi et al. 2010, Langenfeld et al. 2013, Mei et al. 2014, Zimmerman and Vitousek 2012).

EF can be low in number in plants compared to endophytic bacteria, but they are equally beneficial to the host plants (Nath et al. 2015). Previous investigations of these microorganisms indicated that they are excellent producers of compounds that can be exploited for agrochemical or medicinal purposes due to their biological activity (e.g., antiviral, antimicrobial, anticancer, insecticidal, immunosuppressive and antioxidant effects) (Strobel and Daisy 2003). It was discovered that the produced compounds are occasionally the same as those which were produced by the respective hosts, which were exclusively isolated from higher plants (Kusari and Spiteller 2012) including the cardiotonic digoxin (*Digitalis lanata*) (Kaul et al. 2013), ginkgolides (*Ginkgo biloba*) (Cui et al. 2012), the antidepressant and antimicrobial hypericin (*Hypericum perforatum*) (Vigneshwari et al. 2019), the anticancer pro-drug podophyllotoxin (*Juniperus communis*) (Kusari et al. 2009) and the anticancer paclitaxel and its metabolites (*Taxus baccata*) (Kusari et al. 2014).

3.2 Relationship between fungal endophytes and their host

Plant-associated endophytes, such as EF are extensively distributed in nature. During the mutualistic interactions between the hosts and the EF, these plants could maintain vats and diverse niches for their microorganisms (Saikkonen et al. 2010). Host plants without endophyte-fungal association are devastated by the waves of extreme temperature, drought, salinity and pathogens (Saikkonen et al. 2010, Schulz and Boyle 2005, Waller et al. 2005, Rodriguez et al. 2012). The distribution of EF from the same regions presented a high degree of similarity in terms of species taxonomy (D'Amico et al. 2008). In the recent years, it is gradually recognized that EF or endophytes have played a fundamental role in affecting the quantity and quality of the crude drugs through a particular fungal-host interaction. This indicates that more understanding on the particular relationships between EF and medicinal plants is required for promoting crude drug production (Faeth and Fagan 2002). The knowledge of relationship between EF and their hosts are still not fully discovered. Understanding and exploring of this relationship will boost the ideal production of profound drugs via wielding the growth conditions of medicinal plants, for instance, adding a promising group of EF to the plants in order to boost the quantity and quality of a particular drug (Firáková et al. 2007).

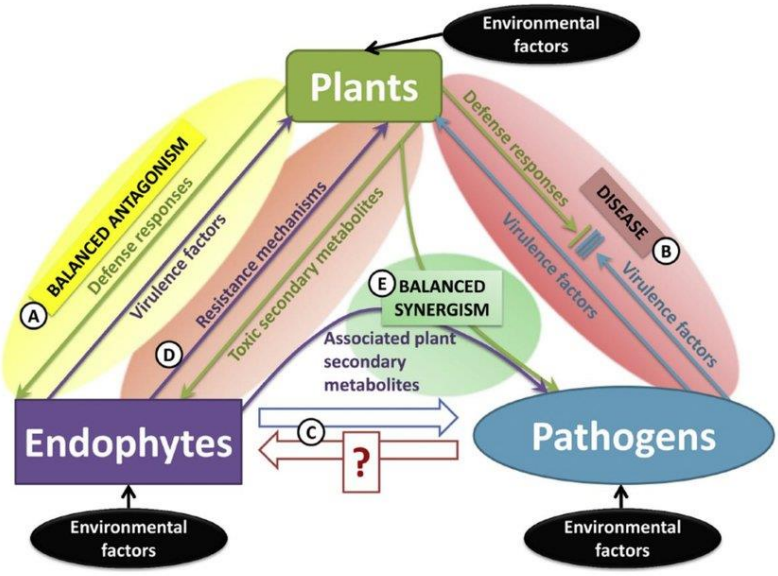

Figure 1. Schematic interpretation of fungal-plant cost-benefit interactions with emphasis on EF: (A) Balanced antagonism hypothesis, (B) plant disease caused by pathogenic fungi, (C) endophyte-pathogen reciprocity, (D) endophyte survival strategy, (E) balanced synergism (Kusari et al. 2012).

During the long period of co-existence and evolutionary alters, different relationships have been entrenched between EF and their host plants through a particular fungal-host interaction, which was recognized as: a continuum of mutualism, antagonism and neutralism (Figure 1).

The balanced antagonism hypothesis (Figure 1A) was designed to address how an EF avert to stimulate the host defenses, protects self-resistance before being immobilized by the harmful metabolites of the host, and maintain to grow within its host by not provoking visible infection or disease (Arnold 2007, 2008, Schulz and Boyle 2006). Both EF and pathogens acquire many virulence factors that are responded by plant defense mechanisms. If the plant defense mechanisms utterly neutralize the EF virulence factors, the EF vanish (Figure 1B). On the contrary, if the plant ceases to the virulence of the fungi, a relationship between plant and pathogen would attend to plant disease (Arnold 2008). Sometimes EF could be underlying pathogens (Figure 1C), they might be altered by certain intrinsic or environmental factors to explicit factors that direct to pathogenicity (Arnold 2008). The interaction between plant and EF might not just be equilibrium between virulence and defense, but a more complex and accurately controlled interaction (Figure 1C). For example, the plant *Camptotheca acuminata* produces camptothecin (anticancer compound) that suppresses topoisomerase I via binding and maintaining the covalent complex of topoisomerase I-DNA (Kusari and Spiteller 2012a). The mutual interrelation between EF and their host plants can impose certain effects on formulation of ceratain types of bioactive compounds that can be used by human. Endophytes are most likely to have advantages for the host plants in terms of production of plant growth regulating hormones, solubilization of minerals and their antagonistic behavior against plant pathogens as well as pests (Berg 2009, Jia et al. 2016).

3.3 Importance of endophytic fungi

3.3.1 Role of endophytic fungi in agriculture

Up until now, almost 100 million tons of chemical fertilizers have been used in order to increase plant production, but the adverse effects of using that chemical on environment and the expense of their high production makes them disadvantageous. During the last decade, EF have been studied as one of the vital microorganisms in a field of agriculture. The study conducted by Barka and coworkers (2002) has revealed that EF are essential components of sustainable agriculture in the view of their ability to enrich plant growth, crop yield and enhance plant fitness through providing both biotic and abiotic stress tolerance (Barka et al. 2002). A

considerable fact is that these fungi are agriculturally crucial as they can boost the growth of their host, have positive effects to increase nutrition and phytohormone production (Son et al. 2006, Chuang et al. 2007, Vassilev et al. 2007).

The mineral solubilization is the indirect way of plant growth promotion via obtaining nutritional elements such as nitrogen and phosphorus or by mobilizing metals which are useful for plants (Zhang et al. 2006, Hartley and Gange 2009, Kajule et al. 2010). The direct way of plant growth promotion is to produce phytohormones, particularly gibberellins and indole-3-acetic acid (IAA), which are able to improve plant growth and reduce the adverse impacts of abiotic stress. They also play crucial roles in plant physiology including cell division and elongation, tissue differentiation, root initiation and phototropic response (Khan et al. 2011, Khan et al. 2012, Khan et al. 2008). Sustainable agriculture requires practicable strategies in order to enrich and maintain the current rate of food production while declining devastation on the environment. In other words, EF along with other plant growth promoting microbes solitary or in consortium could potentially be used as future commercial bio-inoculants and bio-control agents on crops, which grow under normal as well as abiotic stress conditions. There are many examples of reports about bio-control agents originated from EF (Saikkonen 2004, Wang et al. 2015). Taking ryegrass as an example, it had been widely studied in order to find out the chemical basis of resistance towards insects in plant-endophyte mutualisms (Pennell et al. 2005).

According to Strobel et al. (1999), *Pezicula cinnamomea* and *Cryptosporiopsis quercina* were isolated from the medicinal plant *Tripterygium wildfordii* which exhibited antifungal activity against *Candida albicans*. Certain EF, *Trichoderma*, *Nigrospora* and *Curvularia* isolated from *Rauwolfia serpentina* – the fundamental plant of Assam – showed antagonistic activity against *Fusarium oxysporum* and *Phytophthora* spp. (Li et al. 2000). In addition, secondary metabolites produced by EF are involved in deterrence of herbivory (Pannaccione et al. 2014), protection against fungal (Soliman et al. 2015) or bacterial pathogens (Mousa et al. 2016) and amelioration of plant abiotic stress (Hamayum et al. 2017). Interestingly, the tolerance to biotic stress was associated with the bioactive compounds in several cases, which was emanated from EF (Saikkonen et al. 1998, Tan and Zou 2001, Zhang et al. 2006) that could have also antimicrobial activity on microbial pathogens (Gunatilaka, 2006). Some compounds produced by EF were either toxic or repellent to insects by protecting the hosts from the attacks of insects (Hartley and Gange 2009). For example, certain alkaloids produced by the EF in the genus *Neotyphodium* could deter insects of the host plants by enhancing their survival ability

from insect attacks. With the enhanced stress tolerance, host plants living together with EF could outcompete the native plants with no fungal interaction, and thereupon, these host plants could become even invasive (Tofern et al. 1999, Clement et al. 2005).

3.3.2 Role of endophytic fungi as a source of bioactive metabolites

Medicinal plants and EF isolated from them are quite promising sources of fundamental bioactive chemicals as well as secondary metabolites, which contribute to more than 80% of the natural drugs (Singh and Dubey 2015). In order to make human life better and longer by curing diseases, decades of medicinal plant usage lead scarcity of many plant species on our planet. If this process continues for a decade or two, not only medicinal plants will disappear from the flora, but the biodiversity of the whole kingdom Plantae will also be reduced (Chen et al. 2016). A number of well-known plants have been investigated for the diversity and the production of secondary metabolites of their EF. The discovery of novel bioactive compounds gathered from many different types of endophytic microorganisms is crucial alternative to overcome the enriching levels of drug resistance to various pathogenic organisms (Godstime et al. 2014). Several bioactive compounds including camptothecin ($C_{20}H_{16}N_2O_4$), diosgenin ($C_{27}H_{42}O_3$), hypericin ($C_{30}H_{16}O_8$), paclitaxel ($C_{47}H_{51}NO_{14}$), podophyllotoxin ($C_{22}H_{22}O_8$) and vinblastine ($C_{46}H_{58}N_4O_9$) are produced by different EF which all are agriculturally and pharmaceutically important (Zhao et al. 2011b, Molina et al. 2012, Nicoletti and Fiorentino 2015, Vigneshwari et al. 2019). EF have recently generated profound interest in the community of microbial chemistry because of their significant potential in order to contribute to new bioactive compound discoveries. Many anticancer compounds isolated from EF have been used in medicine including a novel anticancer agent, ergoflavin ($C_{30}H_{26}O_{14}$) which was detected from EF isolated from the leaf of the medicinal plant, *Mimusops elengi*, or vincristine ($C_{46}H_{56}N_4O_{10}$), which was isolated from the mycelia of EF and is mainly used as a chemotherapeutic regimen in acute lymphoblastic leukemia and nephroblastoma (Yang et al. 2004).

The main chemical components that are derived from EF are from the groups of alkaloids, steroids, terpenoids, flavonoids, glycosides, quinones, phenylpropanoids, lignans and lactones (Zhang et al. 2006). These secondary metabolites are produced by EF and are associated with medicinal plants which can be exploited for curing diseases (Tejesvi et al. 2007). One of the most promising health care applications of EF metabolites is their antimicrobial activity. For instance, 1-tetradecene ($C_{14}H_{28}$), 8-octadecanone ($C_{18}H_{36}0$), 8-pentadecanone ($C_{15}H_{30}0$) and

10-nonadecanone ($C_{19}H_{38}0$) which were produced by *F. solani* isolated from *Taxus baccata* performed antibacterial as well as antifungal activity (Tayung et al. 2011). Chemical compounds 1-hydroxy-5-methoxynaphthalene ($C_{11}H_9NO_4$) and 1,5-dimethoxy-4-nitronaphthalene ($C_{12}H_{11}NO_4$) isolated from *Coniothyrium* sp. have potential effect on *Bacillus megaterium* and *Escherichia coli* bacteria (Tayung et al. 2011). A *Pestalotiopsis microspora* strain was isolated from rainforest and was able to produce ambuic acid ($C_{19}H_{26}O_6$), which has significant potential towards fungi and oomycetes (Zhang et al. 2018). Another considerable research was carried out by the Varughese research group (2012) that cordycepsidone A ($C_{17}H1_40_7$) isolated from *Cordyceps dipterigena* is capable of antifungal activity for inhibiting the plant pathogenic fungi *Gibberella fujikuroi* (Varughese et al. 2012). The study revealed and reported that *Phomopsis* sp. produced a crucial secondary metabolite phomopsichalasin ($C_{32}H_{41}NO_4$), which has high antibacterial activity against *Bacillus subtilis, Salmonella enterica* and *Staphylococcus aureus* (Horn et al. 1995). Similarly, flavipucine ($C_{12}H_{15}NO_4$) isolated from *Phoma* sp. of the host plant *Salsola oppositifolia* also has potential against *B. subtilis, S. aureus* and *E. coli* in agar plate assay by giving inhibition zones of 16, 17 and 11 mm, respectively (Loesgen et al. 2011). Cryptocandin ($C_{51}H_{82}N_8O_{17}$) is a lipopeptide isolated from *C. quercina* and has potential against many plant pathogenic fungi including *Sclerotinia sclerotiorum* and *Botrytis cinerea* (Strobel et al. 1999).

3.4 Endophytic fungi as plant growth promoter

3.4.1 Siderophore production by endophytic fungi

Siderophores are vital micro-nutrients, which are required for metabolism of many plants because of their diverse role in biosynthesis of chlorophyll, reactions of redox and several physiological activities (Briat et al. 1995). In general, siderophores are extracellular and small (low molecular weight, <1000 Da) compounds, which selectively bind iron (Fe^{3+}). Now it is known, that siderophores are mainly generated from microorganisms and the production of these compounds usually occur in bio-control agents (Kloepper et al. 1980, Sayyed et al. 2005). Their production by pathogenic fungi is typically associated with virulence (Sayyed et al. 2005). The first report about siderophore production was from *Ustilago sphaerogena* (Neilands 1952), then it was gradually revealed that many EF as well as endophytic bacteria are also able to produce these compounds (Leong 1986). In addition, Aramsirirujiwet et al. (2016) demonstrated in their study that seven EF which belong to the *Colletotrichum*, *Lasiodiplodia*

and *Fusarium* genera showed potential siderophore clear halo, which was more than 30 mm on Chrome azurol S (CAS) agar test. EF *Glomerella cingulata, Colletotrichum gloesporioides, C. truncatum, Lasiodiplodia pseudotheobromae* and *Fusarium* species performed orange halo (more than 30 mm) on CAS agar (Yaovapa et al. 2016). According to Sirinivas et al. (2020), 60% of the EF isolates presented positive results for their ability to produce siderophore in which *Penicillium chrysogenum, Aspergillus sydowii* and *A. terreus* were able to produce more siderophore compared to other species (Sirinivas et al. 2020). Hordt et al. (2000) also noted that *P. chrysogenum* isolated from soil secreted siderophores (Hordt et al. 2000).

3.4.2 Phosphate mobilization potential of endophytic fungi

Phosphorus mineral is considered as an essential nutrient for the growth of plants which compound generally encourages growth and affects the structure of plants on cellular level, (Hameeda et al. 2008, Sharma et al. 2013). Although, most soils lack enough P availability for plants (Richardson 2001). The availability of soil P can be conducted by EF, such as *A. niger*, *P. sclerotiorum*, *P. chrysogenum* and *F. oxysporum*, which were isolated from *Camellia sinensis* that grows in Assam, India (Hasan 1996, Nath et al. 2015). Plants associated with P solubilizing microorganisms have enriched the use of efficiency of P source, because this association could improve the sustainability of production of agriculture in a system through the reuse of P residues (Shrivastava et al. 2011). Another similar report concluded that an EF, *Trichoderma pseudokoningii*, isolated from roots of tomato in central Himalaya, exhibited plant growth-promoting activities through the solubilization of phosphates as well as synthesis of auxins, siderophores and ammonia (Chadha et al. 2015). Many bacterial, fungal, yeast and actinomycetes species have ability of solubilizing P (Halder et al. 1991, Abd-Alla 1994, Whitelaw 2000, Goldstein 1986). Among the EF groups, mainly the *Aspergillus* and *Penicillium* genera show high P solubilizing activity (Wakelin et al. 2004, Souchie et al. 2006). In addition, Prajapati's group isolated four different P solubilizing fungi from soils and established that *A. niger* and *A. terreus* possess the highest P solubilizing activity (Prajapati et al. 2012). *Aspergillus, Penicillium* and *Fusarium* species were also noted for their significant activity to solubilize P (Gour 1990, Simine et al. 1998, Whitelaw 2000). *A. niger* has also a great ability to solubilize aluminium beside of P (Lopes-Assad et al. 2010). Many EF genera such as *Woolsia pungens, Epacridaceae, Hymenoscyphus ericae* and *Oidodendron maius* were reported that they could dissolve P (Van leerdam et al. 2001, Martino et al. 2003). Sterflinger

(2000) also reported that *A. niger, P. simplicissimum, P. expansum* and *Scopulariopsis brevicaulis* gave positive results for P solibuliziation.

3.4.3 Phytohormone production of endophytic fungi

Several research groups have focused on phytohormone production of EF for a long time. These producer organisms can promote plant growth directly through releasing many different plant hormones (Nath et al. 2015). EF are directly associated to their phytohormone production in plants, pointedly in the production of auxin and gibberellins that ensure important functions of the host plants, while they benefit greater colonization being inside of the plant (Navarro et al. 2006). Among phytohormones, auxin is crucial to influence different cellular functions and impact plant growth. In response to light and gravity, they help in comforting the growth of root and shoot as well as differentiating vascular tissues and initiating lateral rootgrowth (Datta and Basu 2000, Tsavkelova et al. 2007). Some EF, such as *A. fumigatus*, *Paecilomyces* sp., *Penicillium* sp., *Phoma glomerata*, *Chrysosporium pseudomerdarium* and *P. formosus* are known to produce gibberellic acid and IAA. These fungi play fundamental roles in promoting rice growth through increasing both their shoot length and chlorophyll content as well as positively influencing their biomass (Waqas et al. 2014a, b). *F. proliferatum* isolated from *Physalis alkekengi* have presented vast plant growth promoting activity due to its ability to release gibberellins (Rim et al. 2005). *Phoma* and *Penicillium* species produced gibberellins as well as IAA, and strongly promoted both the shoot and growth attributes of rice during stress conditions, such as salinity and drought (Waqas et al. 2012). *F. tricinctum* and *Alternaria alternate* isolated from potato also released IAA and increased the growth of rice plants (Waqas et al. 2012). EF *Piriformospora indica* discovered from the rhizosphere of *Prosopis juliflora* and *Ziziphus nummularia* promoted growth during its symbiotic relationship with a broad spectrum of plants (Varma et al. 1999).

3.4.4 Auxin biosynthetic pathway in bacteria and fungi

IAA is a widely produced phytohormone in plants and plays a crucial role in plant physiology including cell division, elongation, tissue differentiation, root initiation and phototropic response. Both plants and microorganisms have been reported as IAA producers (Spaepen et al. 2007, Apine and Jadhav 2011, Sun et al. 2014). L-tryptophan is known to serve as a general precursor of IAA synthesis, but it can also be synthesized via a Trp-independent pathway (Spaepen et al. 2007) (Figure 2). According to present studies, there are five Trp-

dependent IAA biosynthesis pathways that have been discovered in microorganisms, namely the indole-3-acetamide (IAM), indole-3-pyruvate (IPyA), tryptamine (TAM), indole-3-acetonitrile (IAN) and tryptophan side-chain oxidase (TSO) pathways (Spaepen et al. 2007, Spaepen et al. 2011). Regarding IAN pathway, the intermediates of the IAN pathway are indole-3-acetaldoxime, indole-3-acetonitrile, indole-3-acetamide. This pathway starts from Trp then converted to indole-3-acetaldoxime via glucobrassicin and then converted to indole-3-acetonitrile. From here it converted to indole-3-acetamide via nitrile hydratase activity and finally it is converted to IAA by IAM hydrolase activity.

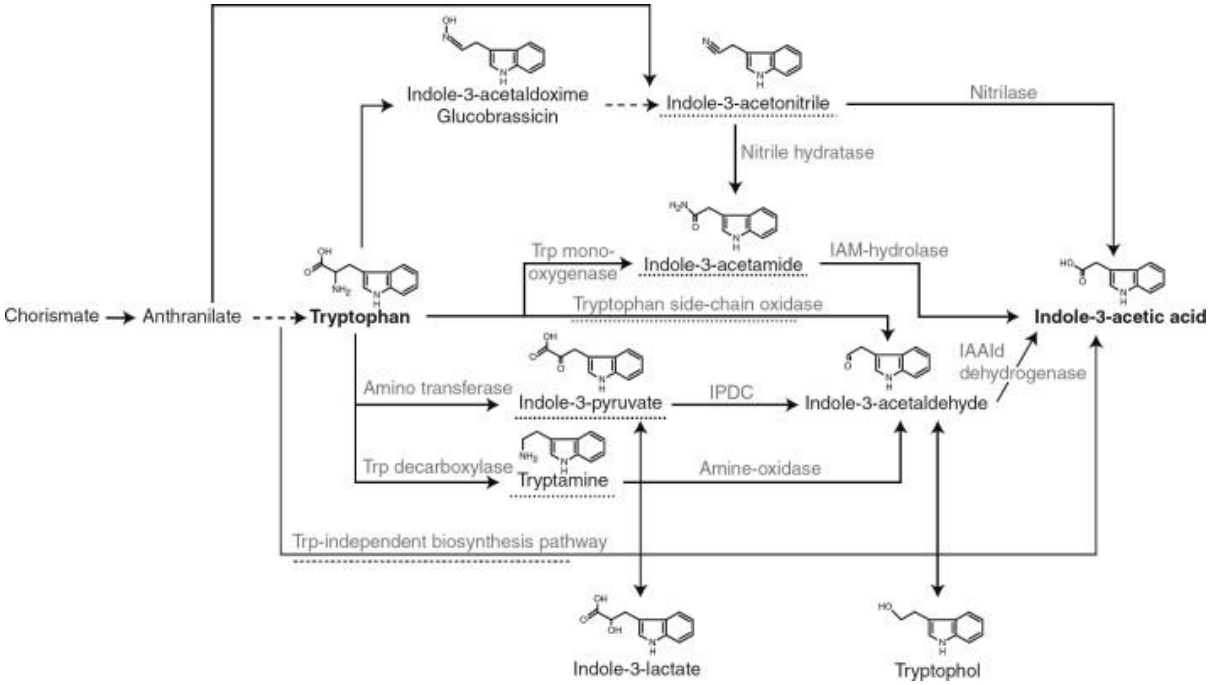

Figure 2. Overview of the different pathways of IAA synthesis in bacteria (Remans et al. 2006).

Based on the literature fungi could convert IAN to IAA and for example *F. graminearum* could use both TAM and IAN as intermediates (Yamada et al 1990).

In the case of IAM pathway, tryptophan is first converted to IAM by tryptophan-2-monooxygenase then IAM is converted to IAA by IAM hydrolase activity. IPyA pathway differs from IAM pathway. The first step is tryptophan conversion to IPyA by aminotransferase activity, and then IPyA is carboxylated to indole-3-acetaldehyde (IAAld) by indole-3-pyruvate decarboxylase (IPDC) activity. The last step is the oxidation of IAAld to IAA. During the TAM pathway, the first step is decarboxylation of tryptophan to TAM, which is then converted to

IAAld by amine oxidase. As the last step, IAAld is converted to IAA. The TSO pathway includes a step, where Trp is directly converted to IAAld prior to the oxidization of IAAld to IAA, while intermediates could be directly converted to IAA by nitrilase or converted to IAM prior to conversion of IAM to IAA by nitrile hydratase in the IAN pathway (Spaepen et al. 2007, Spaepen et al. 2011, Sakaoduoen et al. 2019). Bacteria mostly produce IAA through the IAM, IAN and IPyA pathways (Duca et al. 2014), but TSO pathway has only occurred in the bacteria *Pseudomonas fluorescens* (Oberhansli et al. 1991). Little is known about IAA biosynthesis in fungi, however, mostly fungi use the IAM and IPyA pathways for their IAA biosynthesis (Spaepen et al. 2007, Sun et al. 2014, Chung et al. 2003, Robinson et al. 1998, Chung et al. 2004, Shilts et al. 2005, Tsavkelova et al. 2012, Nutaratat et al. 2016). Despite that, it is significant to progress a better understanding of their IAA biosynthetic pathway in order to study a research field. IAA biosynthetic pathways in microorganisms were identified after the detection of indole intermediate compounds from the culture medium including IAM, IAN and TAM pathways. In spite of that, some intermediates such as IPyA and IAAld could be reversibly transformed to storage compounds. In view of the fact, indole-3-ethanol (IOL) and indole-3-lactic acid (ILA) were described as stable compounds and efficiently transformed from IPyA and IAAld (Spaepen et al. 2007, Sardar and Kempken 2018). An endophyte, *Colleotrichom fructicola* produced IAA through the IAM pathway, which was recognized via the tryptophan 2-monooxygenease activities of the fungal IAA production, while some EF, such as *C. gloeosporioides*, *F. proliferatum*, *F. verticillioides*, *F. fujikuroi* and *F. oxysporum* use the IAM pathway for their IAA synthesis (Tsavkelova et al. 2012, Robinson et al. 1998, Maor et al. 2004). IAM and IPyA pathways were found in *C. acutatum*, while the IPyA pathway was also confirmed from *P. indica*, *Rhodosporidium paludigenum*, *Rhizoctonia cerealis*, *R. solani*, *U. maydis* and *U. esculenta* (Chung et al. 2004, Reineke et al. 2008, Hilbert et al. 2012, Nutaratat et al. 2016, Furukawa et al. 1996, Kumla et al. 2020).

3.5 Medicinal plants used in this study

Medicinal plants and herbs are known for their significant potential source of treating diseases as well as they are also linked to various medicinal activities (Brown and Wright 2016). Since ancient times, they have been profound to be benefitial extensively in medicinal practices, and thus, they proved to be effective drugs in traditional system of medicine (Chen et al. 2015, Newman and Cragg 2016). EF, which live inside of medicinal herbs are found to be promising candidates for medically crucial compound sources as well as for agricultural applications. In

this study, 8 plant species were selected as medicinal plants to investigate and characterize their EF.

3.5.1 Sophora flavescens

The genus *Sophora* includes about 45 species among which *S. flavescens* (shrubby sophora) is the best known for its traditional medicinal value. *S. flavescens* is an endangered medicinal plant species growing only in a small area of Mongolia. In traditional Chinese medicine, this herb is connected to numerous medicinal activities, such as antipyretic, anthelminitic, antimicrobial, insecticidal, anticancer, antiviral and diuretic properties (He et al. 2015). The main chemical components of *S. flavescens* are alkaloids and flavonoids (Chen et al. 2004, Zhang et al. 2007).

3.5.2 Halerpestes salsuginosa

H. salsuginosa is a common, widespread and variable species in many parts of Siberia, Afghanistan, Pakistan, North India, Himalaya, Mongolia, China and Japan. They are found in damp alkaline and swampy meadows, rivers, springs, brook banks and lake coasts, often in water at shoals. *H. salsuginosa* leaves are 0.5-2.5 cm long, center-shaped, flowers are small, 6-8 mm in diameter and 3-4 mm long. Medicine name of the plant is water hyacinth seedling and it is commonly used for treating arthritis and edema (Harald and Yasin 1937).

3.5.3 Thermopsis dahurica

The stem of *T. dahurica* is 10-40 cm long, short or long appressed or divaricate hairy, leaflets are oblong-elliptic and 32-50 mm long. The flowers are yellow, the legumes are straight, oblong-linear and 50-60 mm long. *T. dahurica* has profound beneficial effects in the field of medicine and a long history in traditional use. *Thermopsis* plant species are used for treating phlegm and relieving cough. *T. dahurica* grows in both China and Mongolia. They are herbaceous perennials and are known as goldenbanners. This plant is widely distributed in the dessert area, alkaline steppe meadows, hollows in mountain-steppe belts as well as grass-lands (Yang et al. 2018).

3.5.4 Oxytropis glabra

Oxytropis is one of the three genera of plants known as locoweeds and are notorious for being toxic towards grazing animals. The plants have 8-32 flowers that rise from a scape. They are of a cream to yellowish color, but sometimes pink, blue, or purple, with usually black hair. *Oxytropsis* contains swainsonine, which possesses the function of inhibiting tumor cells' growth and metastasis (Dorling et al. 1980, Molyneux and James 1982). Locoweed species are used as anti-cancer therapy, against immune injury, to enhance the body's immune system, and to stimulate the proliferation of medullary cells (Klein et al. 1999, Rooprai et al. 2001, Sun et al. 2006, Liu et al. 2006, Jun et al. 2007).

3.5.5 Convolvulvus arvensis

C. arvensis is a species of bindweed belonging to the morning glory family that is rhizomatous and native to Europe and Asia. It is a climbing or creeping herbaceous perennial plant, which grows 0.5–2 m high. *C. arvensis* reproduces by producing seeds which may remain dormant in the soil for longer periods of time (20 years or more) (Holm et al. 1977, Americanos 1994). *C. arvensis* has a number of medicinal properties (traditional/folklore) (Abdel-Sater et al. 2021). The resins from the root may act as diuretics and laxatives and the tea made from leaves may be used to treat fevers or wounds (Abdel-Sater et al. 2021).

3.5.6 Astragalus melilotoides

The genus *Astragalus* consists of nearly 3000 species, which are considerable sources of herbal drugs. The main chemical components of these plants are polysaccharides, saponins and flavonoids. Medicinal use and importance of *Astragalus spp.* is their cardiovascular, antihypertensive, diuretic, choleretic effect, which can also be used as antimicrobial and antiviral agents. *A. melilotoides* is used for the treatment of fear in children as well as a diuretic (Stepashkina 1959).

3.5.7 Nitraria sibirica

Nitraria is a genus of flowering plants in the family *Nitrariaceae* which contains 9 species. *N. sibirica* is traditionally used in folk medicine in Mongolia and China. This plant is one of the dominant species in the central Asian deserts. *Nitraria* are 25-200 cm tall shrubs with spiny branches at the apex with simple serrated leaves. *Nitraria* contain various beneficial

components such as alkaloids, flavonoids and phenolic acid. This plant has numerous pharmacological effects including antifatigue, antitumor, antioxidant, antibacterial, antimutagenic, hypotensive and hypoglycemic properties (Du et al. 2015).

3.5.8 Sphaerophysa salsula

Sphaerophysa species are known as locoweed (Mo 2004) which are used as anti-cancer agents, or to treat immune injury, to enhance the body's immune system, to stimulate the proliferation of medullary cells (Klein et al. 1999, Rooprai et al. 2001, Sun et al. 2006, Liu et al. 2006, Jun et al. 2007) and to treat hypertension (Deng et al. 2011). It is a long-lived perennial herb growing up to 1.5 meters tall. It reproduces via seed as well as by sprouting vigorously from its creeping root system. The stems are coated in short white hairs. *S. salsula* is one of the most famous traditional Chinese medicines (Zhen et al. 2011), but currently, there are only few studies conducted on the EF living in *S. salsula*. Recent studies have demonstrated that the major bioactive components of *S. salsula* are flavonoid glycosides (Zhou 1997, Zhou et al. 1997). Flavonoid glycosides have many biological functions including anti-hepatitis (Shi et al. 2017), anti-aging (Li et al. 2016), anti-HIV-1 (Reutrakul et al. 2007) and anti-tumor (Nasri et al. 2016) activities.

4 OBJECTIVES

Over the last few years, EF have been reported as excellent candidates for producing various secondary metabolites. These compounds have a significant impact on the field of medicine as well as agriculture. It has been also proved that EF are able to enhance plant growth via producing plant growth regulating hormones and minerals.

Thereby, the aim of this study was to isolate, identify and investigate EF harbored in Mongolian plants. Furthermore, the study also aimed to contribute significant insights into the plant hormone producing capabilities of EF and reveal the antimicrobial activity of the secondary metabolites produced by the examined endophytic community.

For this purpose, the specific objectives were the following:

- Isolation and identification of EF from Mongolian medicinal plants
- Investigating of antibacterial and antifungal activity of these EF
- Test of plant growth promoting activities of the isolates
- Examination of IAA biosynthetic pathway of the IAA producer EF

5 MATERIALS AND METHODS

5.1 Culture medium used in this study

Basal liquid medium for related indole compounds assay: 1.5 g of (NH_4) H_2PO_4, 0.3 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of KH_2PO_4, 0.02 g of $CaCl_2$, 0.01 g of NaCl, 0.01 g of $FeCl_3 \cdot 6H_2O$, 0.001 g of thiamine HCl and 5.0 g of glucose, pH 6.0, 2.0 mg/mL Trp, 1000 mL distilled water.

Chrome azurol agar medium (CAS): (1). Blue Dye: Solution 1: Dissolve 0.06 g of CAS (Fluka Chemicals) in 50 mL of ddH_2O. Solution 2: Dissolve 0.0027 g of $FeCl_3$-6 H_2O in 10 mL of 10 mM HCl. Solution 3: Dissolve 0.073 g of Hexadecyltrimethylammonium bromide (HDTMA) in 40 mL of ddH_2O. Mix Solution 1 with 9 mL of Solution 2. Then mix with Solution 3. (2). Mixture solution: Minimal Media 9 (MM9) Salt Solution Stock: Dissolve 15 g KH_2PO_4, 25 g NaCl and 50 g NH_4Cl in 500 mL of ddH_2O. 20% Glucose Stock: Dissolve 20 g glucose in 100 mL of ddH_2O. NaOH Stock: Dissolve 25 g of NaOH in 150 mL ddH_2O. Casamino Acid Solution: Dissolve 3 g of Casamino acid in 27 mL of ddH_2O. (3). CAS agar Preparation: Add 100 mL of MM9 salt solution to 750 mL of ddH_2O: Dissolve 32.24 g piperazine-N, N′-bis (2- ethanesulfonic acid) PIPES. Add 15 g Bacto agar. Add 30 mL of sterile Casamino acid solution and 10 mL of sterile 20% glucose solution to MM9/ PIPES mixture. Slowly add 100 mL of Blue Dye solution along the glass wall with enough agitation to mix thoroughly.

Luria-Bertani broth (LB) medium: 10 g of peptone, 5 g of NaCl, 5 g of yeast extract, 0.1% of agar, 1000 mL distilled water.

Modified Pikovskaya's medium (MPKV): 5 g of $Ca_3(PO_4)_2$, 0.5 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of NaCl, 0.2 g of KCl, 0.003 g of $FeSO_4 \cdot 7H_2O$, 0.003 g of $MnSO_4 \cdot H_2O$, 2 g of lecithin, 10.0 g of glucose, 0.5 g of yeast extract, 15.0 g of agar, 1000 mL distilled water.

Murashige and Skoog basal medium (MSBM): 2.2 g of Murashige and Skoog salt, 5 g of sucrose and 8 g of agar, 1000 mL distilled water.

Nurtient broth medium (NB): 1 g of peptone, 15 g of sodium chloride, 6 g of yeast extract, 1000 mL distilled water.

Potato Dextrose Agar – PDA powder (VWR International Ltd., Hungary), distilled water

Potato Dextrose Broth – PDB powder (VWR International Ltd., Hungary), distilled water

Pikovskaya's medium (PKV): 0.5 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.02 g of NaCl, 0.02 g of KCl, 0.003 g of $FeSO_4 \cdot 7H_2O$, 0.003 g of $MnSO_4 \cdot H_2O$, 2 g of lecithin, 10 g of glucose, 0.5 g of yeast extract, 15 g of agar and 1000 mL distilled water.

PDB with Trp: 24 g of PDB, 0.1% (w/v) Trp, 1000 mL distilled water.

Yeast-YPD: 20 g of Peptone, 20 g of glucose, 10 g of yeast extract, 15 g of agar, 1000 mL distilled water

5.2 Collection of plant samples

To isolate EF, plant samples were randomly collected from 8 different plant species in the middle of the July of 2013 and 2014 in Mongolia (Table 1). The places from where the host plants were sampled are as follows: (1) Dornod province where, in winter, the average temperature is around from −27 to −32 ˚C with frequent snowfalls. In summer, the average temperature is 25–30 ˚C with cold nights of around 13 ˚C. The number of sunny days per year is 251–260 and the annual average precipitation level is 276.9 mm. (2) Uvs province is in the west part of Mongolia, where the third biggest lake is located with an altitude of approximately 1025 m above the see level, which is one of the coldest places in Mongolia. This terrain has a cold semi-arid climate with long, dry, freezing winter (−45 to −49 ˚C) and short, warm summer (+35 to 37 ˚C). Precipitation is very low with an annual average of 130 mm. Each collected species sample was placed in a sealed plastic bag and was labeled, numbered and noted with the date of collection and stored at +4 ˚C until its procession.

Table1. The name, taxon (family) and location of the 8 sampled plant species.

No	Plant name	Plant taxon (Family)	GPS	Location
1	*Sophora flavescens*	Fabaceae	49° 148'204" N, 114° 87'5597" E	Dornod province
2	*Oxytropis glabra*		50° 6′709″ N, 92°23886″ E	Uvs province
3	*Astragalus melilotoides*		50° 6′476″ N, 92°22′553″ E	
4	*Thermopsis dahurica*			
5	*Sphaerophysa salsula*		50° 2′666″ N, 92° 16′961″ E	
6	*Halerpestes salsuginosa*	Ranunculaceae		
7	*Convolvulus arvensis*	Convolvulaceae	50° 8′888″ N, 91° 38′901″ E	
8	*Nitraria sibirica*	Nitrariaceae	50° 9′153″ N, 92° 19′306″ E	

5.3 Isolation of endophytic fungi

Plant samples were exposed to a three-step surface sterilization procedure according to the method described by Vigneshwari et al. (2019). The portions of leaves, branches and roots were thoroughly washed in running tap water for 10 min to remove soil particles and adhered debris, then, they were allowed to dry on the paper towel. The plant tissues were then cut into small pieces of less than 1 cm, which were washed in 70% ethanol for 1 min, then in 50% sodium hypochlorite solution for 3 min and 70% ethanol for 30 sec. After drying, each piece was cut to expose their inner tissue and placed on potato dextrose agar (PDA) or corn meal malt extract agar supplemented with 50 mg/L chloramphenicol to suppress bacterial growth. All the plates were incubated at 25 ˚C for up to 7-10 days and were checked daily for the growth of fungal colonies. Pure fungal isolates were obtained by picking individual hyphal tips from the PDA plates and placed on fresh PDA plates to incubate at 25 ˚C for 10 days. Each fungal culture was checked for purity and transferred separately to PDA slants and finally maintained at 4 ˚C. All isolates were also deposited into the Szeged Microbiological Collection (SZMC, Hungary; http://szmc.hu/).

5.4 Molecular identification of endophytic fungi

For DNA isolation, fungal isolates were grown in PDB for 5 days at 25 ˚C. Isolation of the genomic DNA from the mycelia was performed using E.Z.N.A. Fungal DNA Kit (Omega Bio-tek) according to the manufacturer's instructions. The internal transcribed spacer (ITS) region of the rDNA was amplified using the primers ITS1 and ITS4 as described previously (White et al. 1990). Sequencing of the amplified DNA fragments was performed on an ABI 373A DNA sequencer (Applied Biosystems Inc., USA) using dye dideoxy terminator reaction chemistry. The sequences were first analyzed by Basic Local Alignment Search Tool (BLAST) similarity search at the website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST) and the species were identified based on their identity values (>97%).

5.5 Bioactivities of endophytic fungi

5.5.1 Preparation of samples to the bioactive assays

Isolated EF were cultured for 7 days at 25 ˚C in 50 mL PDB medium. The extraction was carried out according to Kusari et al. (Kusari and Spiteller 2012) with minor modifications. The mycelia were separated from the broth by filtration through a cheese cloth, then dried overnight in an oven until constant weight, which was recorded. Then 25 mL distilled water was added to the dry material, which was then sonicated for 20 min after the addition of an aliquot of liquid nitrogen to maintain the chilled condition. This aqueous solution was extracted then three times, first, with 25 mL ethyl acetate (EtOAc), then with 25 mL chloroform-methanol (MeOH) (4:1) and finally the extracts obtained with the same solvent were pooled. Fifty mL of the ferment broth was also extracted three times sequentially with 50–50 mL of EtOAc and chloroform-MeOH (4:1), respectively, and the extracts were also pooled. The organic solvents from each pooled extract were removed by a rotary evaporator (IKA HB10 basic, VWR International Ltd., Hungary) in vacuum at 30 ˚C. The resulted four dry samples per each isolate were stored at −20 ˚C and resuspended in 1 mL of high performance liquid chromatography (HPLC) grade MeOH (VWR International Ltd., Hungary) prior to use.

5.5.2 Antibacterial activity assay

For testing the antibacterial potential of the crude extracts, 400 µL of the MeOH extracts were transferred into new Eppendorf tubes, evaporated and dissolved in 1 mL 10% MeOH. In the antibacterial activity assay, the following bacteria were applied: *E. coli* (SZMC 0582), *Pseudomonas aeruginosa* (SZMC 21886), *S. aureus* (SZMC 14532), *B. subtilis* (SZMC 14624), *Micrococcus luteus* (SZMC 6207) and *Streptomyces albus* (SZMC 0282), which were obtained from the Szeged Microbiological Collection (SZMC, http://www.szmc.hu, Szeged, Hungary) according to the M07-A10 CLSI guideline (Clinical and laboratory standards institute).

For this assay, the suspensions of the microbes were prepared from overnight broth cultures that were cultivated in ferment broth (bacteria-LB; yeast-YPD) at 37 ˚C. Their concentrations were set to 4×10^5 cells/mL using sterile media. Then, 96-well plates were prepared by dispensing 100 µL of suspension containing the bacterial cells or yeast cells, then 100 µL of the extracts, which is dissolved in 10% of MeOH, were added into each well, which were then incubated for 24 hours at 37 ˚C. The mixture of 100 µL broth and 100 µL of 10% MeOH was used as the blank sample for the background correction, while 100 µL of microbial

suspension supplemented with 100 µL of 10% MeOH was applied as the negative control. The positive control contained ampicillin (100 µg/mL, Merck Ltd., Hungary) for bacteria and nystatin (10 µg/mL, Merck Ltd., Hungary) for fungi. The inhibitory effects of each derivative were spectrophotometrically determined after incubation at 620 nm, and the inhibition rate was calculated as the percentage of the positive control after blank correction.

5.5.3 Antifungal activity assay

For these experiments, evaporated 400 µL of crude extracts were dissolved in 1 mL 10% MeOH. To determine the potential antifungal activity of the fungal extracts against plant pathogenic fungi, agar plate diffusion assay was applied. For this purpose, four holes were bored into PDA plates in the diameter of 8 mm at the same distances around to centre of the plate. Then precultured (PDA media, 25 °C, 7 days) *F. culmorum* (SZMC 11039), *R. solani* (SZMC 21048), *A. niger* (SZMC 0851) and *C. albicans* (SZMC 1533) strains were inoculated using agar plugs in the centre of plates. After that, 100 µL of extracts were pipetted into each hole and 10% MeOH was used as solvent control. The mycelial plug inoculated without any extracts was used as an untreated control. Antifungal activity of the samples was determined by the size of the observed inhibition zone.

5.6 In vitro plant growth-promoting assays

5.6.1 Siderophore production assay

Siderophore detection was carried out according to the description of Schwyn and Nieland (1987). For this purpose, the isolated endophytes were precultured on PDA for 7-10 days at 25 °C. Agar plugs (3 mm in diameter) from the edges of young colonies were introduced to Chrome Azurol A (CAS) agar medium. The plates were then incubated in the dark at 25 °C for 48 hours. The color change of blue colored medium to yellow or orange indicates the positive result of siderophore production.

5.6.2 Phosphate solubilization assay

At first step, the cultures of EF were grown on PDA medium for 7-10 days at 25 °C. Screening of fungal isolates for phosphate solubilizing activity was done on PKV agar and on MPKV agar (Cao Y et al. 2018, Neiwolak 1980). After inoculation, the strains were grown at

28 ˚C for 48 hours in three replicates. After the incubation period, the clear zones around the colonies were checked.

5.6.3 Auxin production by agar plate assay

For this assay, the isolated EF were precultured on PDA medium supplemented with tryptophan (Trp, 0.1% (w/v)) and were incubated at 25 ˚C for 7 days. Then, each strain was transferred with toothpicks aseptically onto fresh plates containing the same medium at 1.5 cm distance from each other and the plate was overlaid with an 82-mm-diameter disk membrane Whatman 540 filter paper. After 24 hours of incubation at 25 ˚C, when the colonies reached 0.5 to 2 mm in diameter, the membrane was removed from the plates and saturated using Salkowski reagent by immersing the cultures directly in the Petri dishes. The tests were carried out at room temperature separately with two different formulations of the reagents (Reagent A: 2% 0.5 M $FeCl_3$ in 35% $HClO_4$; Reagent B: 1.2% $FeCl_3$ in 37% H_2SO_4.) in three replicates. The reaction was allowed to proceed until the adequate color appeared and the fungi producing IAA were identified by the formation of a characteristic red halo within the membrane surrounding the colony (Bric et al. 1991).

5.6.4 Confirmation of auxin production by HPLC-MS

The isolates were cultured in three replicates for 7 days at 25 ˚C in 30 mL potato dextrose broth (PDB) in the presence (0.1 g/L) and absence of Trp, shaken on an orbital shaker at 200 rpm. Then, the extraction was carried out according to Shah Sujit et al. (2018) with minor modifications. The mycelia were separated from the broth by filtration through a cheese cloth. The oven-dried mycelia were extracted three times with 15 mL ethyl-acetate, while the ferment broth (20 mL) was extracted sequentially three times with 20 mL ethyl-acetate. The organic solvents from both pooled extracts were removed by a rotary evaporator (IKA HB10 basic, VWR) in vacuum at 30 ˚C. The resulted four dry samples per each isolate (mycelia and broth, with and without Trp) were stored at −20 ˚C and resuspended in 1 mL of HPLC grade MeOH prior to use. The analytical measurements were conducted on a Nexera XR HPLC system (Shimadzu Corporation, Kyoto, Japan) composed of a quaternary pump (LC-20ADXR), an auto sampler (SIL-20AXR), a column oven (CTO-10-ASVP) and a degasser (DGU-20A5R) coupled to a TSQ Quantum Access (Thermo Fischer Scientific, USA) triple quadrupole mass spectrometer (MS/MS).

Chromatographic separations of samples (5 µL) were performed at 40 ˚C using Phenomenex Gemini NX C18 50 mm × 2 mm, 3 µm column (Gen-Lab, Budapest, Hungary) coupled with a guard column with same stationary phase using gradient elution. Eluent A was H_2O and eluent B was acetonitrile both supplemented with 0.1% formic acid. The mobile phase flow rate was 0.3 mL/min. The gradient program started with eluent B at 20% for 1 min changing to 27% until 3.04 min, which was increased to 95% until 3.2 min with the value being held for five min, then decreased to the initial 20% in 0.2 min in the end which was kept constant for further 4 min, resulting in a run of 12.4 min in total. The MS ion source was operated in positive electrospray ionization (ESI) with the following ion source parameters: Spray Voltage, 4000 V; Vaporizer Temperature, 379 ˚C; Sheath Gas Pressure, 20; Sweep Gas Pressure, 2; Aux Gas Pressure, 55. The Capillary Temperature, Capillary Offset and Tube Lens Offset parameters were 250 ˚C, 35 and 70, respectively.

The analysers worked in MRM mode with 0.015 sec Scan Time and 2.4 mTorr Collision Pressure applying the *m/z* 176→130 and *m/z* 176→103 transitions for the IAA at Collision Energies of 13 V and 31 V, respectively. The instrument control, data acquisition and evaluations were carried out with Xcalibur 1.7 (Thermo Fischer Scientific, USA) and Trace Finder 2.6 (Thermo Fischer Scientific, USA) software. For the quantitative determination, the standard stock solution (1 mg/mL) of IAA (Sigma, Hungary) was prepared in MeOH. A series of calibration levels (n = 7) ranging in concentration from 0.05 to 5 µg/mL were prepared by appropriate dilution of the stock solution with MeOH.

5.7 Determination of intermediates of auxin biosynthesis

5.7.1 IAA production from intermediate indole compound cultivation

In order to evaluate the IAA biosynthetic pathway of the EF, all fungi were grown in 100 mL Erlenmeyer flasks in 25 mL of basal liquid medium supplemented with 0.1 g/L of L-Trp at 25 ˚C under shaking conditions at 120 rpm for one week. Then, the extraction was carried out according to Kumla et al. (2020) with minor modifications. The mycelia were separated from the broth by filtration through a cheese cloth. Then the oven-dried mycelia were extracted three times with 15 mL EtOAc, while the ferment broth (20 mL) was extracted sequentially three times with 20 mL EtOAc. The organic solvents from both pooled extracts were removed by a rotary evaporator (IKA HB10 basic, VWR) in vacuum at 30 ˚C. The resulted dry samples were stored at −20 ˚C and resuspended in 1 mL of HPLC grade MeOH prior to use. Cultured filtrates were

extracted and the crude extracts were examined in terms of IAA and intermediate indole compound production by HPLC techniques. The cultivations were carried out in three replications.

5.7.2 Detection of IAA related indole compounds by HPLC

The members of the IAA biosynthetic pathway were detected by HPLC-HESI-MS measurements including a Nexera XR HPLC (Shimadzu Corporation, Japan) coupled with a TSQ Quantum Access triple quadrupole MS (Thermo Scientific, USA) based on the method described by Lin et al. (2015). The separation was carried out on a Gemini-NX (3µ, C18, 150 × 2 mm) column and the gradient solvent-delivery system consisted of two solvents, A was 0.1% formic acid in water and B was 0.1% formic acid in MeOH. The gradient elution was applied at a flow rate of 200 µL/min and was started with 20% of eluent B for 1 min and increased linearly to 50% at 4 min and to 90% at 16 min, which value was held for 4 min then decreased to the initial 20% in 0.2 min and kept constant until the pressure stabilized ending the run of 27.5 min in total. The injection volume was 10 µL.

The MS measurements were carried out with heated electrospray ionization (HESI) ion source in positive polarity. The ionization parameter and multiple reaction monitoring (MRM) transitions for the target analytes were obtained by direct infusion of their individual standard solutions with a concentration of 1µM. The spray voltage was +4000 V, the capillary temperature was 270 ˚C. Nitrogen was used as the sheath and auxiliary gas at optimal values of 30 and 5 units (arbitrary units), respectively. The argon collision gas pressure was set to 1.0 mTorr, while the collision energy and the scan time were 25 eV and 15 ms at each transition. The MRM transitions were the followings (quantification-, confirmatory transition): TAM (161 > 144, 161 > 115), IAM (175 > 130, 175 > 103), IPyA (204 > 130, 204 > 158), ILA (206 > 130, 206 > 118), TOL (162 > 144, 162 > 115), IAA (176 > 130, 176 > 103), IAAld (160 > 130, 160 > 118) and IAN (157 > 130, 157 > 117).

5.8 *In vivo* bioactivity assay of the extracts on *Arabidopsis thaliana*

To study the effect of the extract of IAA producing EF isolates on plants, *A. thaliana* (Col-0 ecotype) seeds were planted in plastic vertical Petri dishes (90 × 17 mm) on 0.5 × MSBM (0.8%) (Horváth et al. 2015) with the addition of 0.5% sucrose (w/v) (pH adjusted to 5.5 with NaOH), five seeds per Petri dish in one line. The experimental setup was structured as

described by Marik et al. (2019). Briefly, seeds were surface sterilized with 70% ethanol for 1 min, treated with 4% hypochlorite for 15 min and washed with sterile distilled water. After vernalization at 4 °C for 24 hours, seeds were sown onto the agar plates. The plants were then placed in a greenhouse with a photoperiod of 12 hours of light and 12 hours of darkness with a light intensity of 300 μmol/m^2/s and a temperature of 25 ± 1 °C. After the three-day of germination, plates were placed at an angle of 50° to allow the root of the plants to develop downwards. Five mm holes were bored with a sterile cork borer 0.5 cm away from the root tips of the 5-day-old *Arabidopsis* seedlings, which were filled with 50 μL of MeOH solutions of mycelial and broth extracts. For the assays, the IAA were tested in five concentrations (100, 10, 1, 0.1 and 0.01 μg/mL) and the ferment broth extracts were diluted also to same concentration levels based on their original IAA contents. For the controls, the untreated and MeOH (50 μL) treated plants were used. Primary root growth was measured every 24 hours for 4 days. The fresh weights of the plants from each plate were measured, and photosynthetic pigments were quantified as described by Faragó et al. (2018).

5.9 Statistical analysis

The statistical analysis of the plant growth promoting assays of the extracts on *Arabidopsis* (primary root growth, biomass of the plant and pigment contents) was performed using GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, CA, USA, 2016). In the bioactivity tests, the significant differences of the plant fitness parameters were determined by one-way ANOVA analysis of variance with Bonferroni's multiple comparison tests. Values throughout the study unless specified are means of three replicates ± standard deviations (SD).

The data visualization of the graphs prepared from the bioactivity of the isolates (antibacterial and antifungal activity) were carried out using R version 3.5.1; The R Foundation for Statistical Computing and RStudio Desktop software, ggplot2 packages (Wickham 2016) as well as libraries tidyverse, hrbrthemes and viridis. Visual representation of siderophore and phosphate activities was performed using R and ggplot2/ggthemes package as an average of the replicates ± standard deviation. Values throughout the study unless specified are means of three replicates ± standard deviations (SD).

6 RESULTS AND DISCUSSIONS

6.1 Isolation and identification of endophytic fungi

In this study, plant samples were collected from 8 species of medicinal herbs randomly from two different locations in Mongolia. The leaf, stem, root and flower parts were separated, and these parts were examined for their EF content. Altogether, 62 pure endophyte strains were isolated from *A. melilotoides* (7), *C. arvensis* (11), *H. salsuginosa* (4), *N. sibirica* (4), *O. glabra* (9), *S. flavescens* (15), *S. salsula* (9) and *T. dahurica* (3), which harbored in the leaves (12), stems (18), flowers (10) and roots (22) of these plants (Figure 3 and 4). This suggests that the presence of EF in plant tissues is not the same and do not spread randomly, which was also obtained previously in the work of Stovall (1987) and Sunariasih (2014). They also report that the existence of the type and number of the EF on each part of the plant is different.

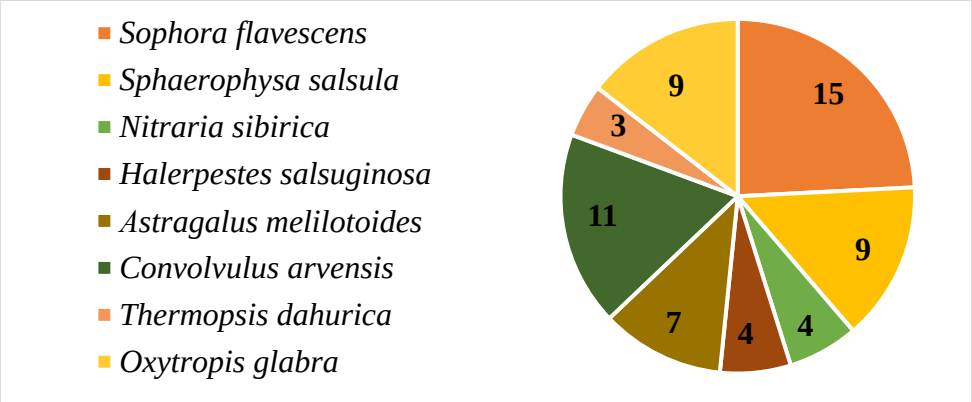

Figure 3. Number of the isolates from eight different plants.

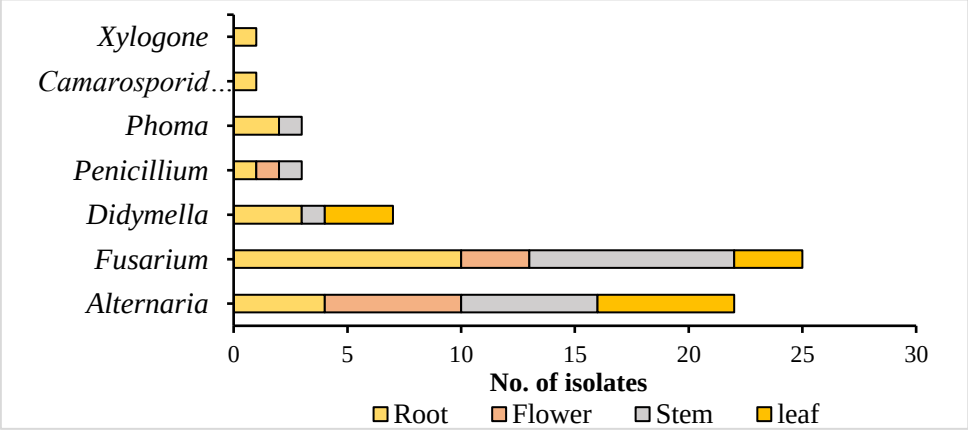

Figure 4. Distribution of EF isolated from the host at genus level.

Molecular identification of the EF was carried out by comparative sequence analyses of the standard fungal sequence marker ITS rDNA. Based on the similarity of PCR products to Blastn hits of the NCBI database, EF were assigned into 22 taxons. Among the 62 isolates, 44 isolates were identified at species level, eighteen isolates at genus level. The isolates belonged to 7 genera involving *Fusarium* (24), *Alternaria* (21), *Didymella* (9), *Penicillium* (3), *Phoma* (3), *Camarosporidiella* (1) and *Xylogone* (1), the accession numbers to the GenBank database are listed in Table 2.

Table 2. Identification of the 62 fungal isolates using ITS sequence analysis.

Collection code	Closest relative based on sequence homology	Similarity (%)	GenBank accession No	Host plants
SZMC 26647	*Didymella glomerata*	100.0	MT218409	*S. flavescens*
SZMC 26648	*Didymella glomerata*	100.0	MT218408	
SZMC 26649	*Didymella glomerata*	100.0	MT218413	
SZMC 26661	*Xylogone sphaerospora*	98.4	MT218400	
SZMC 26660	*Fusarium* sp.	99.6	MT218399	
SZMC 26650	*Didymella glomerata*	100.0	MT218407	
SZMC 26651	*Alternaria alternata*	100.0	MT218406	
SZMC 26652	*Alternaria alternata*	100.0	MT218412	
SZMC 26653	*Alternaria alternata*	100.0	MT218411	
SZMC 26654	*Fusarium sporotrichioides*	99.8	MT218410	
SZMC 26655	*Didymella glomerata*	100.0	MT218401	
SZMC 26656	*Fusarium* sp.	98.9	MT218405	
SZMC 26657	*Fusarium tricinctum*	99.6	MT218404	
SZMC 26658	*Fusarium tricinctum*	99.6	MT218403	
SZMC 26659	*Fusarium armeniacum*	99.6	MT218402	
SZMC 26975	*Alternaria* sp.	99.0	MT134965	*H. salsuginosa*
SZMC 26976	*Alternaria* sp.	98.0	MT134966	
SZMC 26977	*Alternaria* sp.	100.0	MT134964	
SZMC 26978	*Fusarium proliferatum*	100.0	MT134967	
SZMC 26979	*Fusarium redolens*	99.0	MT134968	*S. salsula*
SZMC 26980	*Fusarium armeniacum*	99.0	MT134969	
SZMC 26981	*Didymella glomerata*	97.0	MT134970	
SZMC 26982	*Alternaria tenuissima*	99.0	MT134971	
SZMC 26983	*Fusarium oxysporum*	99.0	MT134972	
SZMC 26984	*Fusarium tricinctum*	96.0	MT134973	
SZMC 26985	*Alternaria* sp.	99.0	MT134974	
SZMC 26986	*Alternaria* sp.	99.0	MT134975	

Collection code	Closest relative based on sequence homology	Similarity (%)	GenBank accession No	Host plants
SZMC 26987	*Penicillium chrysogenum*	94.0	MT134976	
SZMC 26988	*Alternaria tenuissima*	99.0	MT134977	
SZMC 26989	*Fusarium proliferatum*	100.0	MT134978	
SZMC 26990	*Fusarium verticillioides*	88.0	MT134979	
SZMC 26991	*Didymella* sp.	97.0	MT134980	
SZMC 26992	*Alternaria tenuissima*	99.0	MT134981	*O. glabra*
SZMC 26993	*Alternaria* sp.	91.0	MT134982	
SZMC 27024	*Alternaria alternata*	95.0	MT134983	
SZMC 27025	*Alternaria tenuissima*	99.0	MT134984	
SZMC 26996	*Alternaria* sp.	99.0	MT134985	
SZMC 26997	*Alternaria tenuissima*	99.0	MT134986	
SZMC 26998	*Penicillium chrysogenum*	98.0	MT134987	
SZMC 26999	*Fusarium chlamydosporum*	97.0	MT134988	
SZMC 27000	*Fusarium chlamydosporum*	99.0	MT134989	
SZMC 27001	*Fusarium oxysporum*	96.0	MT134990	
SZMC 27002	*Alternaria alternata*	99.0	MT134991	*C. arvensis*
SZMC 27003	*Alternaria alternata*	100.0	MT134992	
SZMC 27004	*Alternaria* sp.	99.0	MT134993	
SZMC 27005	*Phoma* sp.	99.0	MT134994	
SZMC 27021	*Didymella* sp.	99.0	MT134995	
SZMC 27033	*Fusarium* sp.	99.0	MT145329	
SZMC 27036	*Phoma* sp.	99.0	MT145332	
SZMC 27034	*Paraphoma chrysanthemicola*	99.0	MT145330	
SZMC 27006	*Penicillium rubens*	96.0	MT134996	
SZMC 27012	*Alternaria tenuissima*	99.0	MT134997	*A. melilotoides*
SZMC 27035	*Camarosporidiella moricola*	99.0	MT145331	
SZMC 27007	*Fusarium verticillioides*	95.0	MT134998	
SZMC 27041	*Fusarium tricinctum*	100.0	MT134999	
SZMC 27037	*Fusarium incarnatum*	99.0	MT145333	
SZMC 27038	*Fusarium proliferatum*	93.0	MT145334	*T. dahurica*
SZMC 27039	*Fusarium* sp.	99.0	MT145335	
SZMC 27016	*Fusarium oxysporum*	97.0	MT135004	
SZMC 27017	*Fusarium* sp.	99.0	MT135005	*N. sibirica*
SZMC 27018	*Alternaria* sp.	96.0	MT135006	
SZMC 27019	*Didymella glomerata*	95.0	MT135007	

Based on the phylogenetic analysis of the ITS sequences of the strains, majority of the isolates proved to belong to *Alternaria* and *Fusarium*. In contrast, *Camarosporidiella, Xylogone, Penicillium* and *Phoma* species were found the least (Figure 4). Most of the EF sequences presented 95-100% homology with related fungi. However, some strains such as SZMC 26990, 26993 and 27038 showed less than 95% identity with MT134979, MT134982 and MT145334, respectively, which were retrivied from the NCBI Genbank database (Table 2). These EF, were characterized as *F. verticillioides*, *Aspergillus* sp. and *F. proliferatum*, respectively.

Previous studies on EF inhabiting the species *S. flavescens* comprised only two fungal genera, *Aspergillus* and *Penicillium* (He et al. 2013, Yu et al. 2014, Zhang et al. 2017). Here this study presents the results of the screening for the presence of EF in various plant organs of the host. Based on the examination, the fungal isolates represented the genera of *Alternaria, Didymella, Fusarium* and *Xylogone*, which has not been previously reported as the EF of *S. flavescens*.

In this study, 3 EF were isolates from the root parts of *T. dahurica* and three of them belonged to *Fusarium* species. However, earlier studies on EF inhabiting the species *T. dahurica* comprised four fungal genera, *Alternaria, Aspergillus, Cladospora* and *Fusarium* (Yang et al. 2018).

Two *Fusarium*, one *Alternaria* as well as one *Didymella* EF species inhabited the *N. siberica* (Table 2). Cui et al. (2018) isolated twenty-four EF belonging also to *Alternaria* and *Fusarium* among 22 other genera, however, *Didymella* species were not found earlier in this plant (Lu et al. 2012). Lin et al. also isolated strains from the roots of *N. siberica* in China and among these strains a novel species within the genus of *Devosia*. They proposed the name for this isolate as *Devosia nitraria* (Lin et al. 2017).

According to Gao et al., the members of ten endophytic fungal genera (*Hysterium, Asterodon, Oospora, Ascochyta, Schizophyllum, Sclerotinia, Fusarium, Colletotrichum, Embellisia* and *Undifilum*) have been isolated from *Oxytropsis* species, meanwhile, Gao et al. (2012) cultured *Undifilum oxytropis* from *O. glabra* (Lu et al. 2017). Furthermore, *Embellisia oxytropis* was isolated from *O. kansuensis* and *O. flabra* (Li et al. 2007, Lu et al. 2009, Yu et al. 2009a, Yu et al. 2009b). The isolates containe *Alternaria* (6), *Fusarium* (2) and *Didymella* (1) genera as well, which (best of our knowledge) is the first time to determine *Alternaria* and *Fusarium* species as EF of *Oxytropsis*.

Currently, there are very few studies conducted on EF of *Astragalus*, *Alternaria*, *Fusarium* and *Aspergillus* species were only isolated from *A. variabilis* (Lu et al. 2009). Based on the result of this present study, *Phoma*, *Penicillium* and *Camarosporidiella* were also found to be the EF of *Astragalus*.

S. salsula has not been studied for its EF. Although, *S. salsula* was nodulated with symbiotic bacteria belonging to *Mesorhizobium, Rhizobium* and *Sinorhizobium*, mainly *Mesorhizobium gobiense,* nonsymbiotic endophytes cohabited universally with the microsymbionts in the nodules with bacteria (Deng et al. 2011). Whereas, 9 EF were isolated from the root, leaf and flower parts of *S. salsula* representing the *Alternaria, Fusarium, Phoma* and *Didymella* species (Table 2).

In case of *C. arvensis* collected from El-Kharga Oasis in Egypt, 85 EF were isolated from the root as well as the leaf of the plant (Abdel-Sater et al. 2021). The EF that was isolated from the root were *A. alternata* (1), *A. flavus* (1), *A. parasiticus* (1), *F. oxysporum* (36), *F. solani* (1), *Macrophoma phaseolina* (1) and *S. kilience* (2). EF that was isolated from the leaves of the plant were *Acremonium rutilum* (1), *A. sclerotigenum* (3), *A. creber* (2), *A. terreus* (1), *F. oxysporum* (33), *F. solani* (1) and *V. fungicola* (2) (Abdel-Sater et al. 2021). In this study, 11 EF were isolated from the stem and the root of *C. arvensis*. EF that was isolated from the stem were *A. tenuissima* (1), *P. chrysogenum* (1) and *F. chlamydosporum* (2), while from the root of the plant, *F. oxysporum* (1), *F.* sp. (1), *A. alternata* (2), *Alternaria*. sp. (1), *Phoma* sp. (1) and *Didymella* sp. (1).

Up to date, *Halerpestes salsuginosa* has not been studied for its endophytic content, this work is the first, which provides information about the EF of these plant species, which were *Alternaria* sp. and *Fusarium proliferatum* (Table 2.).

6.2 Antibacterial and antifungal activity of the isolates

Altogether 372 extracts were tested against six microorganisms. In general, the ferment broth extracts of the isolates showed higher inhibitory activity on these bacteria than the mycelial extracts (Figure 4 and 5). *S. aureus* and *S. albus* were the most sensitive against both mycelia and ferment broth extracts based on 287 and 260 extracts of the isolates with the ranges of 80% and 96.35%, respectively (Figure 4 and 5). Most of these isolates belong to *Fusarium* followed by *Alternaria* and *Didymella* species. Among three solvents, both ferment broth and

mycelia extract of hexane showed the lowest inhibitory activity on the tested bacteria (Table S1-S6).

Among the applied three organic solvents, the EtOAc extracts showed the highest antibacterial activities (Figure 4 and 5). *F. tricinctum* (SZMC 27041) and *F. armeniacum* (SZMC 26659) proved to be the best candidates of all 62 isolates with the highest antimicrobial activity on all six bacteria.

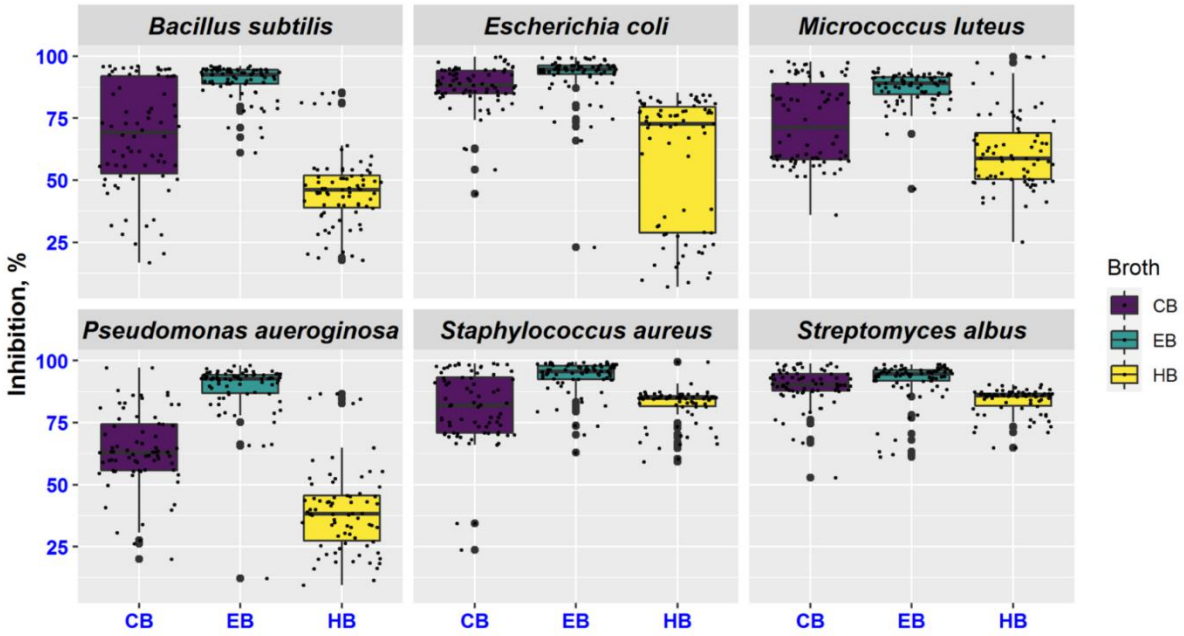

Figure 4. Antibacterial activity of the extracts from ferment broth of the isolates against six bacteria. (CB: Chloroform-Ferment broth, EB: EtOAc-Ferment broth, HB: Hexane-Ferment broth). The boxplot represents the median (interquartile range (IQR) of the replicates with upper and lower quartiles. Outliers are shown as dots.

Moreover, EtOAc extracts of nine *Alternaria* (SZMC 26975, 26976, 26977, 26985, 26986, 27096, 26992, 27025, 27097), two *Didymella* (SZMC 26991, 26981) and five *Fusarium* (SZMC 26978, 26979, 26990, 27099, 27001) strains displayed inhibition activities of 90 - 94.9% on *S. aureus, S. albus* and *E. coli*, while chloroform extracts of ferment broth of seven *Fusarium* (SZMC 26978, 27038, 26659, 26639, 26637, 26641, 27007) and a *D. glomerata* (SZMC 26647) presented the potential of 80 - 94% against *P. aueroginosa, M. luteus* and *B. subtilis* (Figure 5).

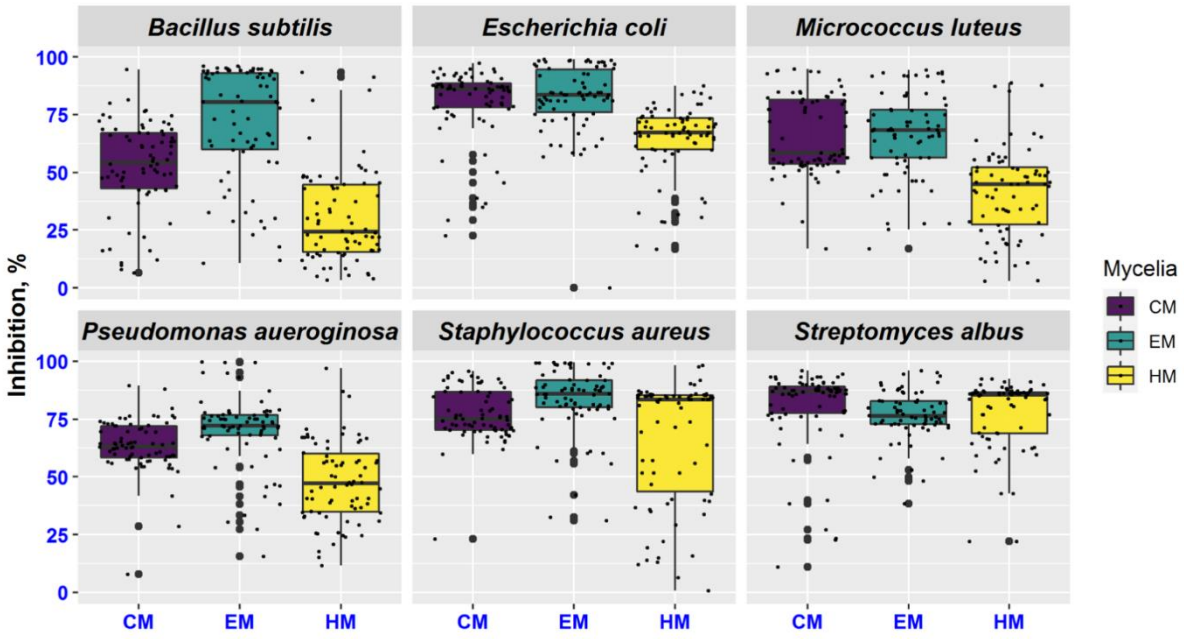

Figure 5. Antibacterial activity of the extracts from mycelia of the isolates against six bacteria. (CM: Chloroform-Mycelia, EM: EtOAc, HM: Hexane-Mycelia). The boxplot represents the median (interquartile range (IQR) of the replicates with upper and lower quartiles. Outliers are shown as dots.

During the antifungal activity tests, all 62 isolates were screened against the pathogenic *C. albicans* and three filamentous fungi, *A. niger*, *F. culmorum* and *R. solani* (Table S7, S8). Based on these tests, EtOAc extract of *D. glomerata* (SZMC 26649) showed the highest inhibition zone on *C. albicans* by giving a significant inhibition activity of 23 mm, which was followed by the chloroform-MeOH extract of the same isolate (22 mm).

D. glomerata (SZMC 26648, 26650, 26649), *Aspergillus* sp. (SZMC 26977), *A. tenuissima* (SZMC 27025) and *F. redolens* (SZMC 26979) were found to be the most active against all tested fungi, meanwhile, *Fusarium* sp. (SZMC 26656, 26660), *F. tricinctum* (SZMC 26657, 26984), *A. alternata* (SZMC 26651) and *P. chrysogenum* (SZMC 26987) isolates were active against three out of the four tested fungi (Figure 6).

In total, 64 extracts of the fungal isolates - most of them belong to *Fusarium* and *Alternaria* genera were found to show potential against *C. albicans* (Figure 6). It was observed that EtOAc extracts have significant inhibition against *A. niger* and *R. solani* by involving 38 and 41 extracts of the isolates, respectively (Table S7, S8). On the contrary, only 21 extracts were active against *F. culmorum* (Figure 6). In general, chloroform-ferment broth extracts provided

the most active solutions (44 extracts of the isolates) by giving significant potential against all tested fungi, while extracts of hexane-broth have the lowest inhibition against the 4 tested fungi. (Table S7, S8).

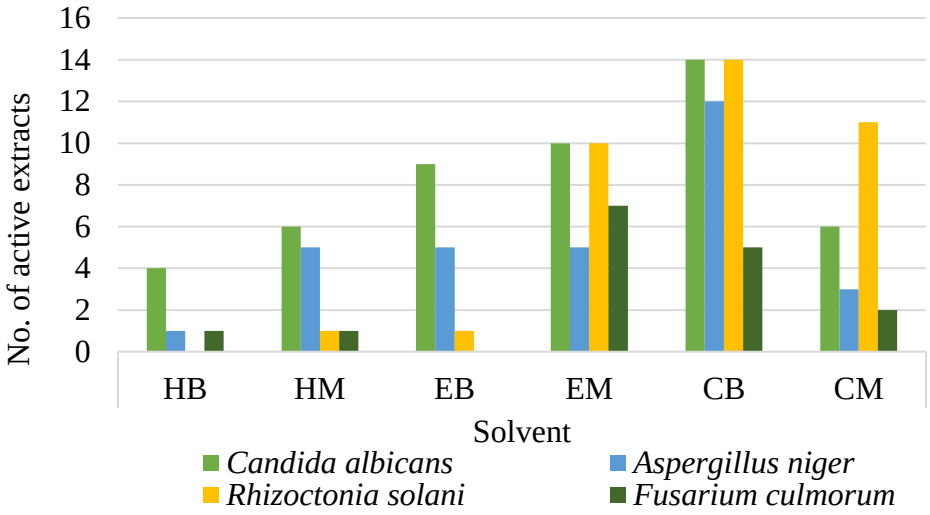

Figure 6. The result of the antifungal activity assay against 4 test organisms. HB-hexane ferment broth extract, HM-hexane mycelia extract, EB-ethyle-acetate ferment broth extract, EM-ethyle-acetate mycelia extract, CB-chloroform ferment broth extract, CM-chloroform mycelia extract.

6.3 Plant growth promoting activities

6.3.1 Plant growth-promoting ability of endophytic fungi

In order to test the plant promoting activity of the isolates, three different assays were applied. One of them measures the direct promotion activity of the endophytes through the plant hormone secretion (IAA production), while two of them belong to the indirect plant promoting trait including mobilization of phosphates and production of siderophores. In general, twenty isolates (*Alternaria* 5, *Didymella* 2, *Fusarium* 11, *Phoma* 1 and *Penicillium* 1) were found to be the most efficient in the assays by producing all applied plant promoting compounds (Table S9).

All the isolates were proved to produce the plant hormone IAA either in the presence or in the absence of Trp or in both cultivation condition (Figure 7). This was detected after observing red ring formation in the membrane, which surrounded the colony after the treatment of

Salkowski reagents. There was no difference between the results of the perchloric and sulfuric acid containing reactions. Most of the active isolates were *Fusarium* including 23 strains, followed by 21 strains belonging to the *Alternaria* genus. (Table S9).

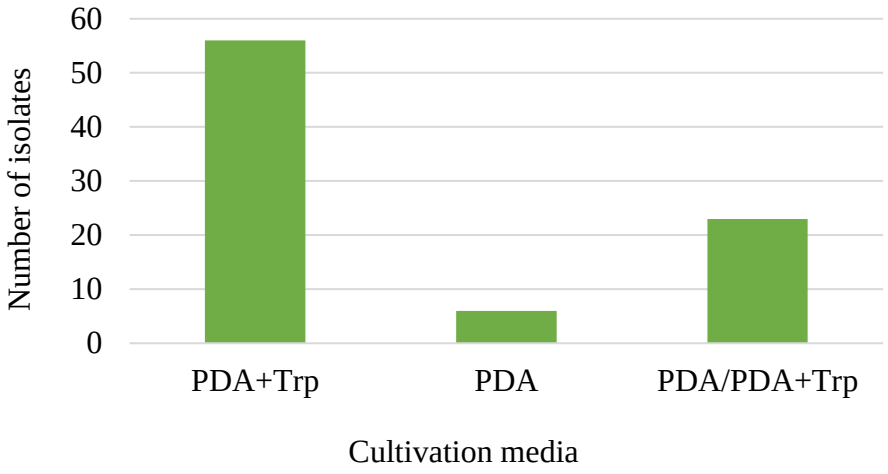

Figure 7. IAA activities of EF isolated from medicinal plants by agar plate assay. The PDA+Trp group produced IAA only in the presence of Trp; the potato dextrose agar (PDA) group produced IAA only without Trp; the PDA/PDA+Trp group showed IAA production in both cultivation conditions.

Furthermore, two *Didymella* (SZMC 26648 and 26650), eight *Fusarium* (SZMC 26660, 26654, 26657, 26990, 27039, 27017, 27016 and 27001) and five *Alternaria* (SZMC 26652, 26985, 26982, 27004 and 27003) isolates were able to produce auxin on Trp supplemented media. The two *Didymella* (SZMC 26647 and 26649), five *Alternaria* (SZMC 26651, 26653, 26977, 26996 and 26993) and two *Fusarium* (SZMC 26659, 27038) strains showed IAA production on both Trp and non-Trp containing media, meanwhile the two *Alternaria* (SZMC 26975 and 27002), the *F. proliferatum* (SZMC 26978), the *P. chrysogenum* (SZMC 26998), the *Phoma* sp. (SZMC 27036) and the *C. moricola* (SZMC 27035) endophytes presented positive IAA results only on that media, which was not supplemented with Trp (Figure 9c and Table S9).

During the siderophore detection assay, 34 endophytes caused orange/yellow zones around their colonies on CAS agar plates as a result of their siderophores sequestering and binding of iron from the medium (Figure 9B). As a consequence, 9 *Fusarium* as well as to 9 *Alternaria* showed positive results. The largest zone (32 mm) appeared on the plate of the *F. tricinctum* (SZMC 26658) strain, but also high siderophore productions were detected in the case of *F.*

tricinctum (SZMC 27019) (30 mm), *F. tricinctum* (SZMC 26984) (29.33 mm), *Didymella.* sp. (SZMC 26991) (29.33 mm) and *F.* sp. (SZMC 26656) (26 mm) (Figure 8).

Regarding the third examined plant grow promoting activity (Figure 9A), half of the isolates were capable to solubilize phosphate according to the observed zones on PKV agar medium (Figure 8). They were the member of the *Alternaria*, *Fusarium*, *Didymella*, *Xylogone*, *Camarosporidiella* and *Penicillium* genera with the highest activity of *F. tricinctum* (SZMC 26657) (40.33 mm) followed by *F. armeniacum* (SZMC 26981) (38.83 mm), *A. alternata* (SZMC 26651) (38.67 mm), *Paraphoma chrysanthemicola* (SZMC 27034) (36.67 mm) and *D. glomerata* (SZMC 26648) (34.67 mm) (Figure 8).

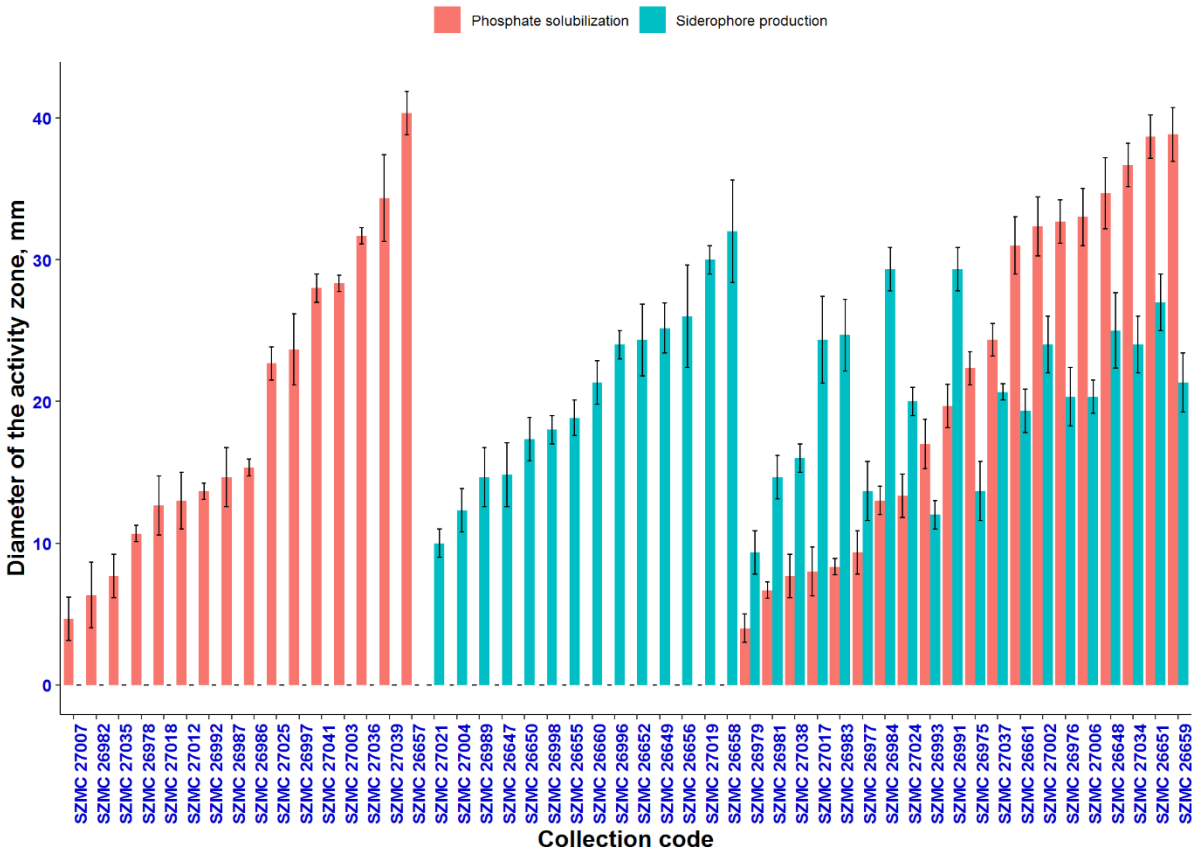

Figure 8. Phosphate solubilization (red bars) and siderophore production (green bars) of the fungal isolates examined by agar plate assays. Strains with activities of only siderophore or only phosphorus are grouped and in the order from the lowest to the highest values. The graph was performed as an average of 3 replicates ± standard deviation.

According to several reports, many fungal, bacterial, yeast and actinomycetes species have been discovered and isolated for their ability to solubilize soluble phosphate (Halder et al. 1991, Abd-Alla 1994, Whitelaw 2000, Goldstein 1986). *Aspergillus* and *Penicillium* species have been extensively noted for solubilizing disparate forms of inorganic P (Whitelaw 2000). Sterflinger (2000) established that *A. niger, P. simplicissimum*, *P. expansum* and *S. brevicaulis* are able to solubilize the insoluble potassium salts, such as potassium aluminosilicates. Milagrs et al. (1999) concluded that ascomycete, deuteromycete and zygomycete produced siderophores on CAS blue agar media, which concurs with this study present results that fungi have potential to produce siderophores on CAS agar medium.

Altogether 34 isolates out of 62 showed potential for both IAA and siderophore production, 36 strains had positive response to IAA production and phosphate solubilization, while 20 showed remarkable performance for both siderophore production and phosphate solubilization. It is important to mention that in all three experiments, the highest production level was detected from the isolates of *Alternaria* and *Fusarium* genera compared to the other isolates. From the 62 strains, half of the isolates produced siderophores with a measurable amount, while 34 showed phosphate mobilization activities.

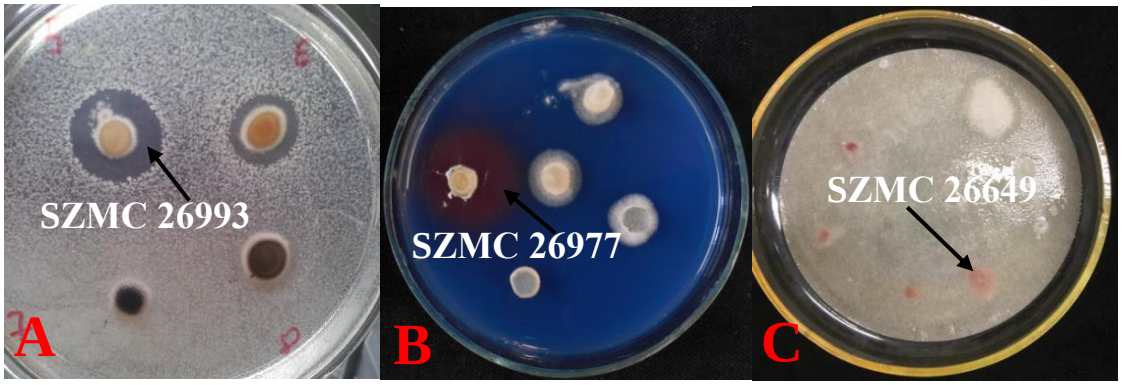

Figure 9. Examples of plant growth promoting experiments. (**A** - phosphate solubilization, **B** - siderophore detection, **C** - auxin detection)

All of the isolates proved to produce IAA on either Trp supplemented media or on simple broth media, from which 25 strains showed production only in the presence of Trp and 6 only in the absence of Trp as well as 6 in both cultivation conditions. It is important to emphasize that 20 isolates (*Alternaria* - 7, *Didymella* - 3, *Fusarium* - 8, *Xylogone, Paraphoma* - 1 and *Penicillium* - 1) were found to be the most efficient during the assays by producing all three-

plant growth-promoting compounds (Table S9). These strains were isolated from different plant organs including stem F. sp.(SZMC 27017), *Alternaria* sp.(SZMC 26976), *A. alternata* (SZMC 26651), *F. armeniacum* (SZMC 26659), leaf *Alternaria* sp. (SZMC 26993), *D.* sp. (SZMC 26991), *F. tricinctum* (SZMC 26984), *F. oxysporum* (SZMC 26983), *Alternaria* sp. (SZMC 26975), *D. glomerata* (SZMC 26648), root *D. glomerata* (SZMC 26981), *X. sphaerospora* (SZMC 26661), *F. redolens* (SZMC 26979), *F. proliferatum* (SZMC 27038), *F. incarnatum* (SZMC 27037), *P. rubens* (SZMC 27006), *Paraphoma chrysanthemicola* (SZMC 27034), *A. alternata* (SZMC 27002) and flower *A. alternata* (SZMC 27024), *Alternaria* sp. (SZMC 26977). Based on these results, it seems that the plant growth-promoting activities may be independent from the isolation source (plant organ) and the taxonomical position of the isolates.

6.3.2 Auxin production of isolates by HPLC-MS/MS

As IAA has a direct influence on plant growth, this activity of the isolates was also confirmed by HPLC-MS/MS analysis using MRM mode. The detection limit of the developed method was 5 µg/mg and 5 µg/mL for the mycelia and the ferment broth, respectively. In contrast to the plate assays, IAA production could be measured more specifically, because the mycelia and the ferment broth could be examined separately for the hormone content.

Similar to the results of the plate assays, each isolate showed remarkable IAA production either through the Trp-dependent or the Trp-independent pathway. In the case of mycelial extracts, two strains accumulated remarkable amount of IAA in PDB medium including *Alternaria* (SZMC 26993) and *Fusarium* (SZMC 26657) isolates. Meanwhile, 27 strains proved to be outstanding producers on PDB media supplemented with additional Trp source. These were five *D. glomerata* (SZMC 26647, 26648, 26649, 26650 and 27019), two *F. armeniacum* (SZMC 26980, 26659), a *F. tricinctum* (SZMC 26658), a *D.* sp. (SZMC 26991), a *F. incarnatum* (SZMC 27037), a *F. proliferatum* (SZMC 27038), two *F.* sp. (SZMC 26660, 26656) two *Alternaria* sp. (SZMC 26985 and 27004), two *A. tenuissima* (SZMC 26988, 26992), three *A. alternata* (SZMC 27024, 26652, 26653), a *F. oxysporum* (SZMC 26983), a *P. rubens* (SZMC 27006), a *C. moricola* (SZMC 27035), a *Phoma* sp. (SZMC 27005), two *F. verticillioides* (SZMC 27007 and 26990) and a *F. sporotrichioides* (SZMC 26654) isolates.

Furthermore, mycelial extracts of *Alternaria* sp. (SZMC 26993, 26996), *A. alternata* (SZMC 26651), *F. tricinctum* (SZMC 26984), 26657, *F. proliferatum* (SZMC 26989), *D. glomerata* (SZMC 26655) and *X. sphaerospora* (SZMC 26661) isolates were found to be

higher IAA producers than their ferment broth extracts on PDB medium without Trp source (Table 3). The ferment broth extracts of three isolates contained high IAA amounts on simple PDB medium which were produced by *F. tricinctum* (SZMC 27041), *D. glomerata* (SZMC 26655) and *A. alternata* (SZMC 26651). On Trp supplemented PDB medium, the highest IAA production was detected for *D. glomerata* (SZMC 26648), which was the same as observed for mycelial extract. Significant productions were also measured in case of *Alternaria* sp. (SZMC 26975, 26976), *F. verticillioides* (SZMC 26990), *A. alternata* (SZMC 27024), *Didymella* sp. (SZMC 27021), *Phoma* sp. (SZMC 27036), *F. incarnatum* (SZMC 27037), *F. proliferatum* (SZMC 27038) and *F. oxysporum* (SZMC 27016) (Table 3).

Based on these results, it could be concluded that IAA productions were generally improved after additional Trp in the media. IAA generaly could be detected in higher amounts from the ferment broth than from the mycelia, however, from seven strains higher amounts of IAA were detected from the cultivation media than the mycelial extracts.

Table 3. IAA amount in the ferment broth and mycelia of the isolates detected by HPLC-MS/MS. BDL, below detection limit; **Trp+**, the media supplemented with tryptohan; **Trp-**, the media do not contain tryptophan. Values are expressed as mean ± SD of three replications.

Collection code	**Ferment broth (µg/mL)**		**Mycelia (µg/mL)**	
	Trp+	**Trp-**	**Trp+**	**Trp-**
SZMC 26647	0.227±2.18	0.162±3.88	0.292±2.06	0.056±9.2
SZMC 26648	16.043±6.7	0.095±5.5	2.021±2.3	0.030±3.6
SZMC 26649	0.816±5	0.017±18.3	0.176±4.5	BDL
SZMC 26661	0.459±2.01	0.077±6.4	0.037±4.93	0.072±9.1
SZMC 26660	2.566±6.36	0.264±3.34	0.161±3.06	0.014±8.22
SZMC 26650	BDL	0.064±4.67	0.438±2.48	0.031±3.38
SZMC 26651	2.143±4.52	0.981±6.5	0.042±2.69	0.279±7.25
SZMC 26652	2.655±6.37	0.056±2.21	0.415±4.35	0.043±11.2
SZMC 26653	1.925±6.50	0.251±3.61	0.872±8.6	0.066±10.2
SZMC 26654	0.655±2.3	0.257±0.9	0.105±5.5	0.053±2.9
SZMC 26655	1.304±1.91	1.188±3.1	0.085±6.89	0.678±4.2
SZMC 26656	1.435±2.61	0.298±3.2	0.123±9.87	0.017±5.73
SZMC 26657	1.160±1.70	0.054±0.3	0.071±1.10	1.282±3.2
SZMC 26658	2.626±2.73	0.264±5.3	0.195±8.23	0.049±3.5
SZMC 26659	0.099±2.94	0.034±3.2	1.103±7.8	0.029±5.3
SZMC 26975	7.452	0.222	BDL	BDL
SZMC 26976	7.773	BDL	BDL	BDL
SZMC 26977	0.322±3.87	0.042	0.039	0.041

Collection code	Ferment broth (μg/mL)		Mycelia (μg/mL)	
	Trp+	Trp-	Trp+	Trp-
SZMC 26978	0.217±3.76	BDL	BDL	BDL
SZMC 26979	0.078±7.40	0.049±4.3	BDL	BDL
SZMC 26980	0.187±9	0.012±5.30	0.025±0.96	BDL
SZMC 26981	0.014±3.1	BDL	BDL	BDL
SZMC 26982	0.296±4.46	BDL	BDL	BDL
SZMC 26983	0.044±7.2	BDL	2.836±9.3	0.018±3.2
SZMC 26984	5.031±2.3	0.007±6.29	0.016±1.07	0.026±5.11
SZMC 26985	0.705±4.48	0.084±7.58	0.108±3.60	0.019±2.99
SZMC 26986	0.182±0.52	0.176±4.61	0.020±4.65	0.014±2.74
SZMC 26987	0.187±0.82	0.007±4.18	0.065±3.41	0.030±8.55
SZMC 26988	0.398±1.69	BDL	0.041±14.23	BDL
SZMC 26989	1.140±1.51	0.387±3.39	0.044±0.75	0.072±2.28
SZMC 26990	5.693±7.7	0.230±4.12	0.235±1.81	BDL
SZMC 26991	0.101±1.51	0.014±9.50	0.090±3.41	0.018±8.55
SZMC 26992	2.839±8.47	0.242±5.17	0.224±7.71	0.030±6.14
SZMC 26993	0.269±0.79	0.224±1.41	0.042±1.11	1.373±2.95
SZMC 27024	5.927±3.9	BDL	0.050±12.1	BDL
SZMC 27025	0.028±4.42	BDL	BDL	BDL
SZMC 26996	0.671±2.38	0.057±4.82	0.018±9.98	0.074±6.97
SZMC 26997	3.075±5.02	0.014±3.03	BDL	BDL
SZMC 26998	3.567±7.76	0.450±5.33	0.340±4.47	0.196±4.29
SZMC 26999	0.009±19.4	0.011±12.96	0.008±1.13	0.005±3.51
SZMC 27000	0.611±2.12	BDL	BDL	BDL
SZMC 27001	0.014±1.12	0.124±1.11	0.014±13.06	0.010±2.15
SZMC 27002	0.678±1.37	0.041±1.83	0.006±1.38	0.063±1.08
SZMC 27003	1.796±2	0.571±7.1	0.023±6.64	0.029±2.99
SZMC 27004	1.837±8.32	0.110±18.1	0.021±1.4	BDL
SZMC 27005	0.021±5.75	0.023±2.15	0.070±0.54	0.018±1.43
SZMC 27021	6.741±1.1	0.011±7.39	0.507±0.9	0.364±0.96
SZMC 27033	BDL	0.039±0.57	0.006±9.36	0.037±1.84
SZMC 27036	15.452±2.9	0.346±4.23	BDL	BDL
SZMC 27034	BDL	0.142±1.34	0.152±2.37	0.009±1.87
SZMC 27006	1.893±2.4	0.150±1.87	1.284±1.87	BDL
SZMC 27012	0.982±1.36	0.754±2.65	0.034±2.66	BDL
SZMC 27035	0.470±1.38	0.183±2.18	0.981±2.17	0.081±2.81
SZMC 27007	0.232±1.44	0.050±0.87	1.205±2.7	BDL
SZMC 27041	2.116±1.75	1.377±1.3	0.256±1.72	0.280±0.42
SZMC 27037	8.858±5.3	0.401±2.62	0.401±2.56	0.052±4.96
SZMC 27038	5.146±7.9	0.059±2.91	0.451±1.48	0.057±2.41
SZMC 27039	3.835±3.71	0.3421±2.67	0.33±10.9	0.021±3.13
SZMC 27016	5.162±5.9	0.730±4.7	0.198±1.53	0.109±1.27
SZMC 27017	1.326±9.76	BDL	0.124±4.18	BDL
SZMC 27018	0.558±0.59	0.030±6.12	BDL	BDL
SZMC 27019	0.014±3.12	BDL	0.022±4.23	0.009±5.49

Recently, a growing number of studies report the IAA production of EF. Waqas et al. (2012) isolated 18 endophytes from the roots of field grown cucumber plants, from which two strains produced varying levels of IAA in their culture filtrate. The range of IAA production of *D. glomerata* on media with or without Trp supplement was found to be 3.89 µg/mL in, while *Penicillium* sp. produced a significantly higher amount of IAA (29.8 µg/mL) on these medium. In the report of Khan et al. (2012), only an *Aureobasidium* sp. showed potential for IAA production using the Trp-independent pathway out of 17 endophytes isolated from *Boswellia sacra*, an economically important frankincense-producing tree, while using the Trp-dependent pathways, almost all of the fungal strains showed the potential to produce IAA. In that case, the *Aureobasidium* sp. showed the highest potential (544.8 ng/mL) for IAA production (Khan et al. 2016). Later, the production of a *Preussia* sp. isolated in that study was also determined, where the production was 1.64 µg/mL based on GC MS/SIM analysis (Al-Hosni et al. 2018). Khan et al. (2012) also published the IAA production of *Paecilomyces formosus* endophyte isolated from the roots of cucumber plant, where the quantity of IAA was 10.2 µg/mL. An *A. japonicus* endophyte strain isolated from the wild plant of *Euphorbia indica* was also able to produce IAA at the concentration of 19.19 µg/mL, which facilitated the growth of the host plant under both normal and heat stress conditions (Ismail et al. 2018). From arabica coffee, 27 EF isolates were obtained and only one isolate, a *C. fructicola* strain displayed positive IAA production after testing by colorimetric assay of Salkowski's reagent. This IAA production was confirmed by HPLC analysis as well which resulted in a level of 1205.58 µg/mL IAA under optimal conditions at 26 days after cultivation (Numponsak et al. 2018).

Therefore, previous reports have shown that the *in vitro* IAA level produced by EF ranged from 0.5 to 1205.6 µg/mL, while in this study, the IAA production to the ferment broth of the isolated endophytes ranged from 0.02 - 1.2 µg/mL and from 0.1 - 16.0 µg/mL in the absence and presence of Trp, respectively. The highest amount of IAA was produced by *Didymella* sp. (SZMC 26648) strain.

Furthermore, it is important to consider that the literature did not contain data about the IAA contents of the fungal mycelia, which were ranged in these experiments from 0.4 - 52.9 µg/mL and from 1.5 - 81.8 µg/g in dry weight, in the absence and presence of Trp, respectively, which were higher than the ferment broth IAA contents.

6.3.3 Plant growth assay of the extracts on *Arabidopsis thaliana*

In order to evaluate the effects of IAA produced by endophytes on plants, the ferment broth extracts of six (*D. glomerata* (SZMC 26648), *A. alternata* (SZMC 26652), *F. tricinctum* (SZMC 26658), *F*. sp. (SZMC 26660), *A. alternata* (SZMC 26651) and *A. alternata* (SZMC 26653) selected isolates, showing the highest IAA productions in the presence of Trp, were investigated for their growth promoting activities on the model plant *A. thaliana*. The extract was diluted to 100, 10, 1, 0.1 and 0.01 µg/mL based on their original IAA contents and impact of these solutions was compared to the standard IAA solutions and to the pure solvent (MeOH) as well as to the untreated plants. The solvent inhibited only slightly the primary root growth compared to the untreated control. The roots of control plants showed 2.16±0.46 mm, 3.12±0.69 mm, 3.56±0.46 mm and 4.08±0.58 mm growth, while the MeOH treated plants increased their roots up to 0.96±0.22 mm, 1.68±0.46 mm, 2.40±0.35 mm and 2.80±0.40 mm at the 6th, 7th, 8th and 9th days, respectively (Figure 10).

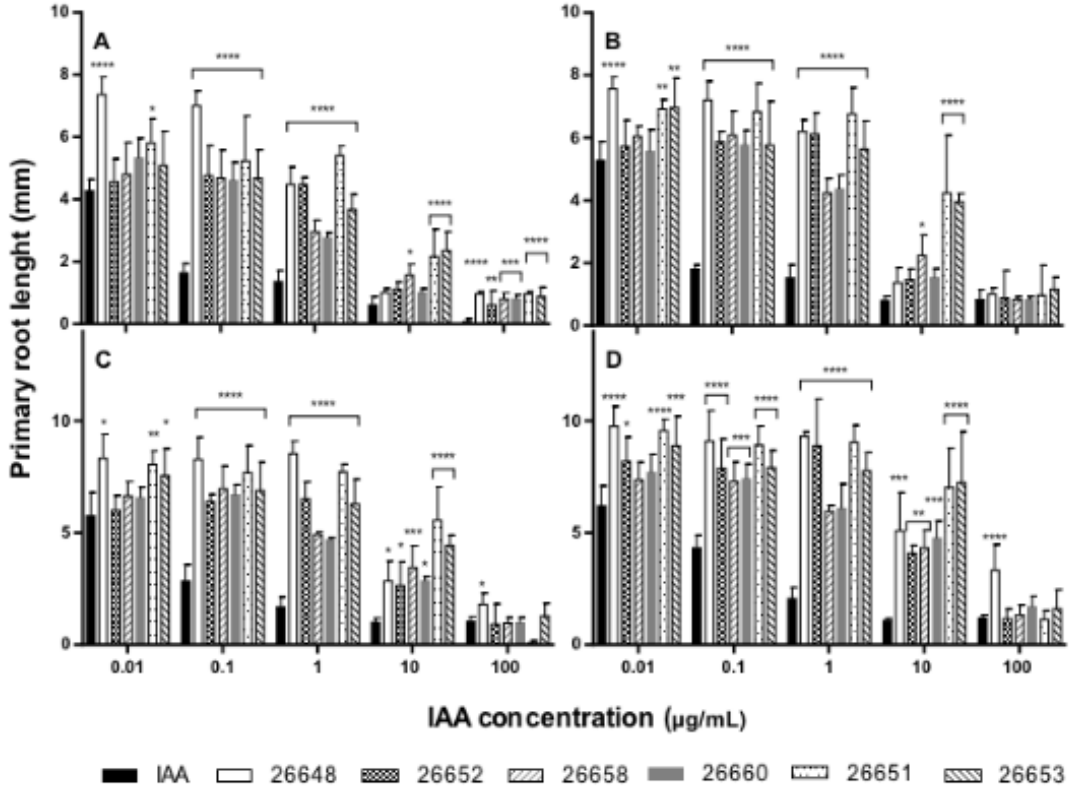

Figure 10. Primary root growth of 6 **(A)**, 7 **(B)**, 8 **(C)** and 9 **(D)** days old *Arabidopsis thaliana* plants after treatment with standard solutions and endophyte extracts at different IAA concentration levels. Significance is assessed based on P-values: *P ≤ 0.05; **P≤ 0.01; ***P ≤ 0.001 and ****P ≤ 0.0001.

The activities of the MeOH ferment broth extracts were compared to the IAA solutions, which were diluted in same organic solvent. The exogenous IAA influenced the root elongation with dose dependent manner according to the literature (Rahman A et al. 2007). At the higher concentration, the growth of primary root was inhibited (1 µg/mL, 10 µg/mL and 100 µg/mL), while it was stimulated at the lower levels of IAA (0.01 µg/mL and 0.1 µg/mL) compared to the MeOH control experiments. However, the lengths of all *Arabidopsis* roots treated with fungal IAA were significantly higher than those of the IAA treatments at concentrations of 0.1 and 1 µg/mL at any of the days measured (Figure 10). Therefore, the fungal extracts could cause higher growth promotion than the pure IAA solutions containing the same levels of plant hormone.

Furthermore, in the case of extracts of *Alternaria* sp. (SZMC 26651, 26653) and *F. avenaceum* (SZMC 26658) strains, these promoting effects remained at 10 µg/mL level with high significances at any of the days measured. At 100 µg/mL concentration level, remarkable differences were observed only on the 6th day between the effects of IAA solution and the fungal IAA extracts on the primary root growth, but later these disparities disappeared (Figure 10).

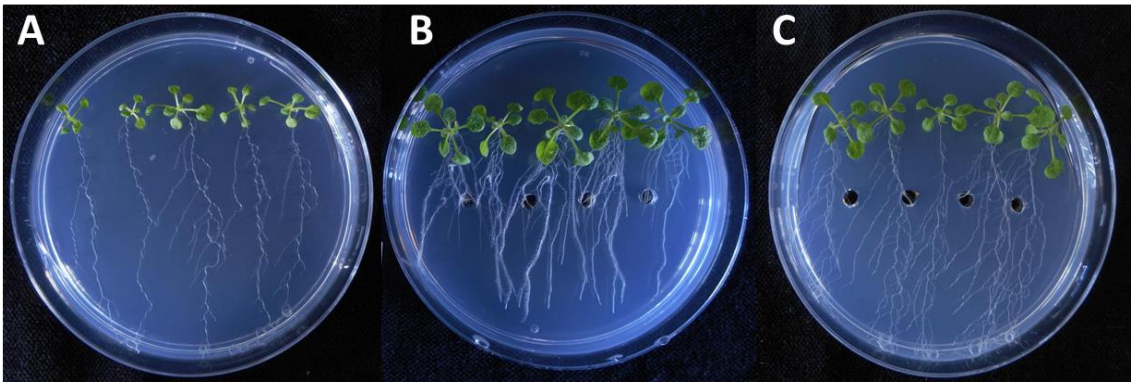

Figure 11. Effect of a selected fungal extract on the growth of *Arabidopsis* seedlings. Untreated control **(A)**, 0.01 µl/mL of IAA standard **(B)** and extract of SZMC 26648 broth contained 0.01 µl/mL of IAA **(C)**.

It is also important to consider that, on the 9th day of incubation, the fungal extracts promoted more efficiently the growth of the primary root than the standard IAA solution almost at all the applied concentration levels. In the experiment, both the pure IAA and the fungal extract treatments lead to a substantial increase in root proliferation and lateral root growth.

However, the application of the fungal extract in the plant assays showed a different type of growth that the plants developed longer but also thin root branches compared to the control ones, while the roots were thicker after the application of standard IAA (Figure 11).

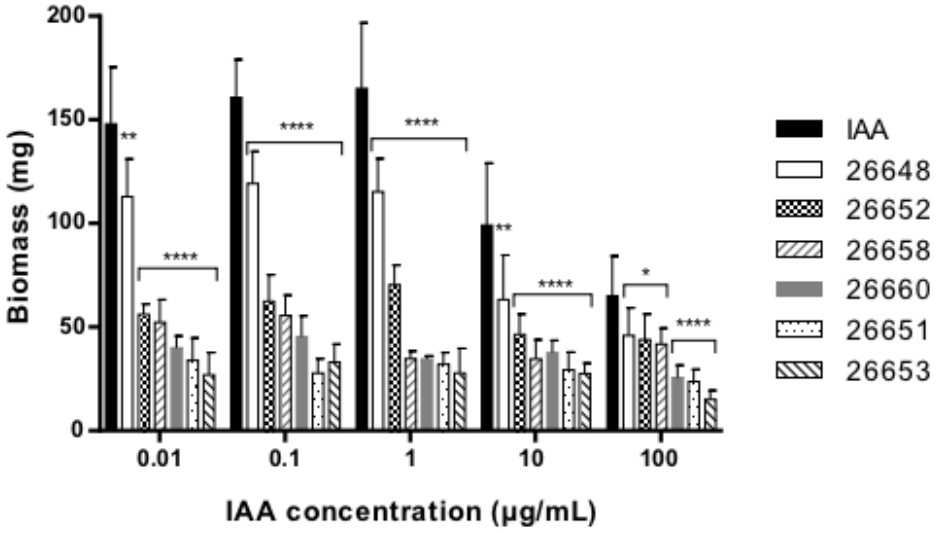

Figure 12. Biomass of 15-day-old *A. thaliana* plants after treatment with ferment broth extracts of selected endophytes diluted to five concentration levels based on their IAA content. IAA standard was used for the control plants. Significance is assessed based on P-values: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001 and ****P ≤ 0.0001.

The IAA treatment led to a remarkably higher level of biomass of the plants treated with fungal extracts at each concentration level (Figure 12). In the case of the untreated and the MeOH control experiments, the biomasses were 0.03 ± 0.01 mg and 0.02 ± 0.01 mg, respectively. Treatment with the fungal extracts at 0.01 µg/mL IAA level resulted in a similar rate of photosynthetic pigment production compared to the results of the treatment with IAA solution (Figure 13). Although, SZMC 26653 possesses increased amount of chlorophyll-a and total chlorophyll as well as carotenoids at this level. The leaves of plants treated with the extracts of strains *A. alternata* (SZMC 26651, 26653 (at 0.1 µg/mL); *F.* sp. (SZMC 26660), *A. alternata* (SZMC 26653) (at 1 µg/mL); and *F.* sp. (SZMC 26660) (at 10 µg/mL and 100 µg/mL) contained

higher chlorophyll-a, while *D. glomerata* (SZMC 26648) (at 0.1 μg/mL); *F.* sp. (SZMC 26660) (at 1 μg/mL); *D. glomerata* (SZMC 26648), *A. alternata* (SZMC 26652), *F. tricinctum* (SZMC 26658) and *F.* sp. (SZMC 26660) (at 10 μg/mL) as well as *A. alternata* (SZMC 26652), 2 *F. tricinctum* (SZMC 26658) and *F.* sp. (SZMC 26660) (at 10 μg/mL) comprised increased quantities of chlorophyll-b pigment compared to the pure IAA treated plants.

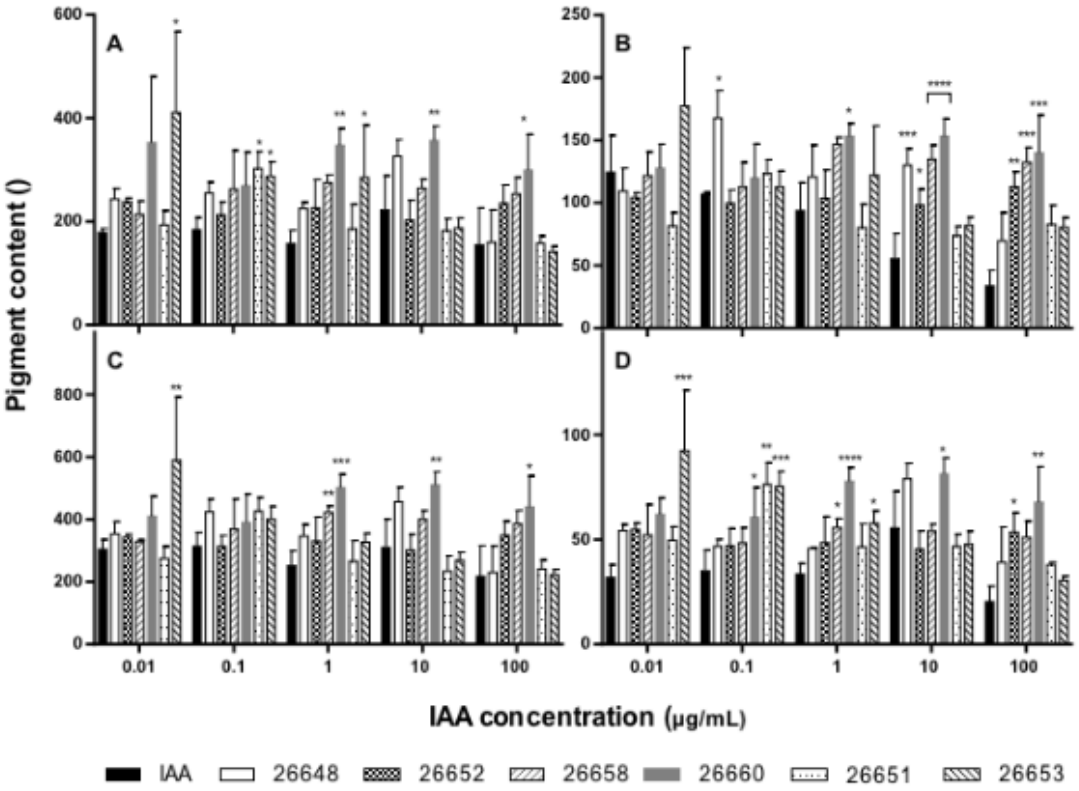

Figure 13. Photosynthetic pigment content of 15-day-old *A. thaliana* plants after the treatment with ferment broth extracts of selected endophytes diluted to five concentration levels based on their IAA content: chlorophyll-a **(A)**, chlorophyll-b **(B)**, total chlorophylls **(C)** and carotenoids **(D)**. IAA standard was used for the control plants. Significance is assessed based on P-values: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001 and ****P ≤ 0.0001.

The extracts of *F.* sp. (SZMC 26660), *A. alternata* (SZMC 26651), *A. alternata* (SZMC 26653) (at 0.1 µg/mL); *F. tricinctum* (SZMC 26658), *F.* sp. (SZMC 26660), *A. alternata* (SZMC 26653) (at 1 µg/mL), *F*. sp. (SZMC 26660) (at 10 µg/mL) and *A. alternata* (SZMC 26652), *F.* sp. (SZMC 26660) (at 100 µg/mL) induced the production of carotenoids in the plants. Pigment contents for control and MeOH-treated plants were the following: chlorophyll-a, 250.77 mg/g FW and 446.35 mg/g FW; chlorophyll-b, 114.79 mg/g FW and 207.32 mg/g FW; total chlorophylls, 365.55 mg/g FW and 654.67 mg/g FW; carotenoids, 58.79 mg/g FW and 103.22 mg/g FW, respectively. It seems to be that the collected data did not follow a specified trend, but it could be highlighted that pigments were found in increased amounts in the plants after the treatment with the extract of *F.* sp. (SZMC 26660) at 1, 10 and 100 µg/mL IAA concentrations.

A. thaliana has become a recognized model to analyze non-mycorrhizal plant-microbe interactions (Dovana et al. 2015). The root system extension of this organism could be changed considerably and significantly in plant–fungal co-cultures, including both decreasing and increasing effects. Furthermore, the presence of EF also affects the root architecture of *A. thaliana* such as the number of lateral roots and the root area (Dovana et al. 2015). The production of hormones by EF could play an essential role in the growth and development of the host plants. The application of IAA-producing endophytes on the host plant appreciably increased shoot length, leaf number, internodes and quantities of photosynthetic pigments (chlorophyll-a, -b and total carotenoids) (Khan et al. 2016). This is in agreement with previous reports, which showed that endophytic inoculation to the host plants resulted in the improvement of growth and stress tolerance (Khan et al. 2011, Khan et al. 2012, Khan et al. 2008). However, the elongation of rice, corn and rye coleoptile segments induced by the crude IAA extract of *C. fructicola* endophyte were not statistically different from those of the pure IAA treatments, but the values were significantly higher than the control (distilled water) treatment (Numponsak et al. 2018).

In this work, the crude fungal IAA of the isolated endophytes at concentrations of 0.1 and 1 µg/mL significantly promoted the elongation of the lengths of *Arabidopsis* roots, but these effects were not unequivocal at the higher IAA concentrations. Similarly, IAA production of EF including *Preussia* sp., *A. japonicus* and *F. oxysporum* stimulated rice and corn root growth (Al-Hosni et al. 2018, Numponsak et al. 2018, Mehmood et al. 2018). Interestingly, the biomasses of *Arabidopsis* plants were reduced due to the treatment of the fungal extracts, while the accumulation of photosynthetic pigments was increased only in certain cases.

6.4 Determination of auxin biosynthesis pathways

Firstly, the agar plate assay and HPLC-MS/MS measurements were applied to assess the capability of EF to produce IAA. The observed results proved that all the isolated EF were positive for IAA production and produced it at different levels. Then, to determine the background of the IAA production in the isolated endophytes, the possible members of the IAA biosynthetic pathway were measured and determined by HPLC techniques. These intermediates involved the TAM (2.3 min), IAM (7.9 min), IPyA (9.4 min), ILA (9.4 min), TOL (9.8 min), IAA (9.8 min), IAAld (9.8 min) and IAN (10.3 min) (Figure 14).

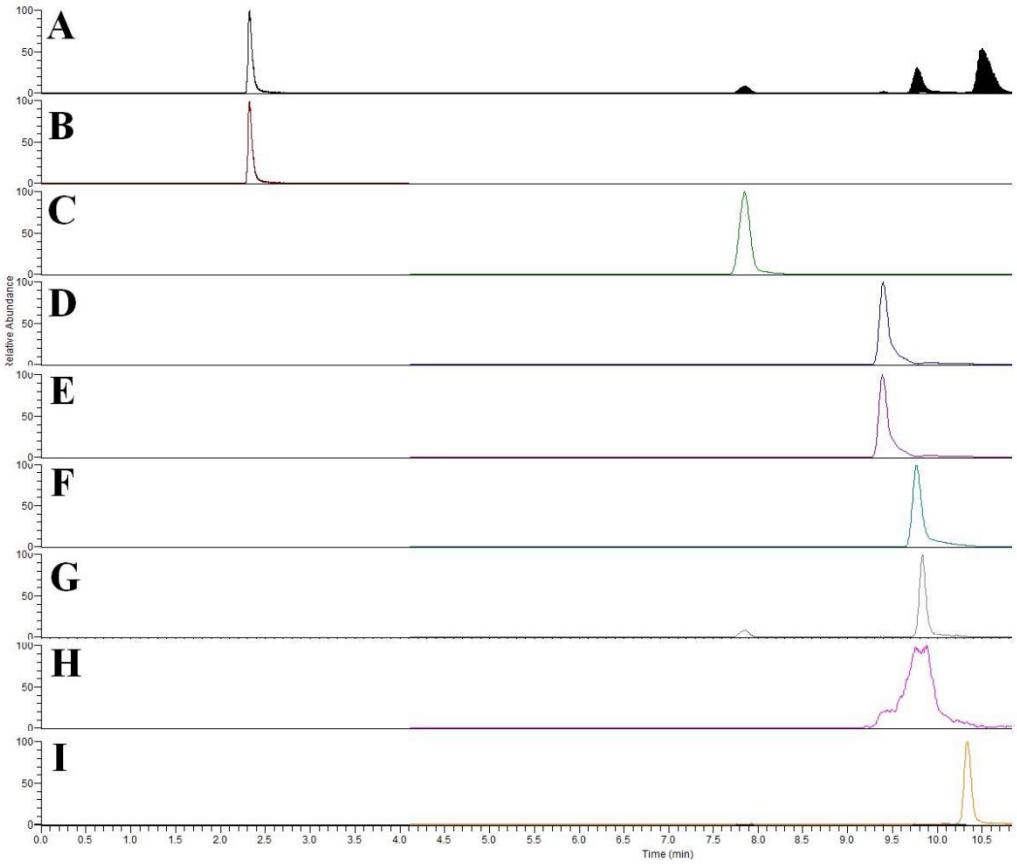

Figure 14. Representative MRM chromatograms of eight target indole-compounds (50 µg/ml of each). **A** – Total ion chromatogram, **B** - TAM (2.3 min, 161 > 144), **C** - IAM (7.9 min, 175 > 130), **D** - IPyA (9.4 min, 204 > 130), **E** - ILA (9.4 min, 206 > 130), **F** - TOL (9.8 min, 162 > 144), **G** - IAA (9.8 min, 176 > 130), **H** - IAAld (9.8 min, 160 > 130) and **I** - IAN (10.3 min, 157 > 130).

The IAA biosynthetic pathway in microorganisms can be established via the detection of indole intermediates in the culture medium, however, it is possible that some intermediate compounds could be reversibly converted to storage compounds (Spaepen et al. 2007, Sardar and Kempken 2018). An example is the reduction of IAAld to ILA. In plants, much of the IAA molecules are found covalently linked to sugars or amino acids, known as IAA-conjugates (Woodward and Bartel 2005). These compounds have diverse roles, such as protection against degradation, storage and transport, and are important to maintain the IAA homeostasis in cells (Cohen and Bandurski 1982, Seidel et al. 2006).

The microorganisms can produce IAA through five different pathways (IAM, IPyA, TAM, TSO and IAN) which was detected by the presence of the related intermediaries in the fungal culture extracts. In all cases, the detection of IAA showed the operation of the IAA Trp-dependent pathway. The presence of IAM means the working of IAM pathway, the co-occurrence of both IPyA and IAAld or both TAM and IAAld justify the operation of IPyA and TAM pathways, respectively, while the presence of IAAld alone supports the existence of the TSO pathway. Measurement of IAN or both IAN and IAM detected together from the samples mark the function of the IAN pathway (Spaepen et al. 2007, Bunsangiam et al. 2019). As it was mentioned, certain intermediates of IAA biosynthesis can be converted into storage compounds, e.g., the reduction of IPyA and IAAld to ILA and IOL or TOL. The physiological function of these compounds remains unknown. ILA is inactive as a phytohormone, but it can compete for auxin-binding sites in plants with IAA (Sprunck et al. 1995).

In the IAA producing isolates, three types of Trp dependent IAA biosynthesis were identified including IAM, IPyA and TAM pathways, moreover, the presence of TSO pathway could also be possible. IAN was not detected at any of the samples, and thus, the presence of the IAN pathway was excluded (Table 4). In general, most strains operated IPyA pathway (26 isolates) in their IAA biosynthesis, which was followed by IAM pathway (16 isolates) (Table 4). The IAA biosynthetic pathway could not be determined in two cases *Alternaria* sp. (SZMC 26986) and *Phoma* sp. (SZMC 27036) due to the absence of the related intermediates.

In the case of two isolates *Alternaria* sp. (SZMC 26986) and *Phoma* sp. (SZMC 27036), as a byproduct of IAAld, the presence of only TOL was detected, which could be originated from the IPyA, TAM and TSO pathways due to the loss of other indicator metabolites. Moreover, beside the IPyA and TAM pathways, the existence of the TSO pathway could not also be excluded in these isolates due to the overlapped intermediate profiles of these pathways.

Interestingly, most strains have conducted two or more biosynthetic pathways for producing IAA (Table 4). These were already described in the literature for bacteria (Zakharova et al. 1999) and fungi (Chung et al. 2003), where more than one pathway was detected also for IAA biosynthesis based on the different intermediates found.

Altogether, IAA production of sixteen EF functioned only one pathway (Table 4). The *F. armeniacum* (SZMC 26980), *D. glomerata* (SZMC 26981), *A. ternuissima* (SZMC 26982), *D. glomerata* (SZMC 26649) and *F.* sp. (SZMC 26660) isolates used IAM pathway, *Alternaria* sp. (SZMC 26975), *P. chrysogenum* (SZMC 26987), *Alternaria* sp. (SZMC 27004), *Paraphoma chrysanthemicola* (SZMC 27034), *Alternaria* sp. (SZMC 27018), *D. glomerata* (SZMC 27019), *A. alternata* (SZMC 26651), *A. alternata* (SZMC 26653), *D. glomerata* (SZMC 26655) and *F. chlamydosporum* (SZMC 27000) apply IPyA pathway, while *Alternaria* sp. (SZMC 26985) synthetized IAA via TSO pathway.

Table 4. Determination of indole intermediate compounds by HPLC techniques. Only those isolates (36 strains), which peaks of intermediates appeared are showed in this table. **IAM** - indole-3-acetamide, **IAAld** - indole-3-acetaldehyde, **ILA** - indole-3-lactic acid, **IPyA** - indole-3-pyruvic acid, **TAM** - tryptamine, **TOL** - tryptophol, **TSO** - possible occurrence of the TSO (tryptophan side-chain oxidase) pathway, **X** - the pathway could be not determined (TAM/IPyA/TSO). The values are in μg/ml and the standard deviations are in brackets.

SZMC number	Indole related compounds						Pathway
	IAM	IAAld	ILA	IPyA	TAM	TOL	
26648	-	-	15.30 (1.28)	-	0.21 (0.05)	-	**IPyA, TAM**
26649	0.35 (0.03)	-	-	-	-	-	**IAM**
26650	0.91 (0.03)	5.61 (0.49)	204.18 (11.94)	126.18 (2.41)	0.22 (0.02)	425.77 (15.99)	**IPyA, TAM, IAM, TSO(?)**
26651	-	-	0.96 (0.11)	-	-	-	**IPyA**
26652	1.08 (0.04)	-	47.17 (0.48)	47.30 (6.85)	-	166.08 (4.47)	**IPyA, IAM, TSO(?)**
26653		-	1.33 (0.12)	-	-	-	**IPyA**
26654	0.17 (0.01)	-	2.17 (0.34)	-	-	-	**IPyA, IAM**
26655	-	19.15 (3.45)	21.15 (0.57)	-	-	1678.73 (2.83)	**IPyA, TSO(?)**

SZMC number	Indole related compounds						Pathway
	IAM	IAAld	ILA	IPyA	TAM	TOL	
26660	0.25 (0.01)	-	-	-	-	-	**IAM**
26975	-	-	17.13 (4.51)	-	-	1389.73 (6.34)	**IPyA, TSO(?)**
26979	0.97 (0.06)	-	2.33 (0.27)	-	-	-	**IPyA, IAM**
26980	0.18 (0.02)	-	-	-	-	-	**IAM**
26981	7.02 (0.26)	-	-	-	-	-	**IAM**
26982	1.46 (0.05)	-	-	-	-	-	**IAM**
26983	0.12 (0.02)	-	7.67 (0.40)	-	0.31 (0.02)	-	**IAM, IPyA, TAM**
26985	-	5.71 (0.73)	-	-	-	288.13 (14.56)	**X**
26986	-	-	-	-	-	32.88 (1.80)	**X**
26987	-	-	2.45 (0.01)	-	-	-	**IPyA**
26988	0.12 (0.01)	-	5.05 (1.07)	-	-	47.64 (4.20)	**IPyA, IAM, TSO(?)**
26990	0.24 (0.01)	9.12 (0.56)	16.11 (3.26)	-	0.28 (0.02)	1600.73 (63.2)	**IPyA, IAM, TAM, TSO(?)**
26991	-	-	3.17 (0.15)	-	0.24 (0.04)	-	**IPyA, TAM**
26999	-	3.27 (0.56)	-	69.17 (3.16)	0.64 (0.09)	168.84 (12.50)	**IPyA, TAM, TSO(?)**
27000	-	-	2.01 (0.55)	-	-	31.63 (9.61)	**IPyA, TSO(?)**
27003	27.57 (2.39)	166.15 (15.16)	281.58 (24.66)	162.74 (15.40)		23509.00 (8.7)	**IPyA, IAM, TSO(?)**
27004	-	-	4.60 (0.12)	-	-	26.20 (2.13)	**IPyA, TSO(?)**
27012	-	2.03 (0.70)	3.31 (0.48)	-	-	158.08 (8.38)	**IPyA, TSO(?)**
27017	0.41 (0.07)	-	13.90 (0.15)	-	0.23 (0.03)	-	**IPyA, IAM, TAM**
27018	-	-	1.62 (0.06)	-	-	29.48 (2.22)	**IPyA, TSO(?)**
27019	-	-	0.83 (0.12)	-	-	26.27 (0.64)	**IPyA, TSO(?)**
27024	-	2.96 (0.13)	1.60 (0.02)	-	-	204.84 (11.30)	**IPyA, TSO(?)**
27034	-	-	1.84 (0.15)	-	-	26.82 (0.91)	**IPyA, TSO(?)**

SZMC number	Indole related compounds						Pathway
	IAM	IAAld	ILA	IPyA	TAM	TOL	
27035	-	13.94 (1.39)	23.43 (3.81)	-	-	1494.80 (7.06)	**IPyA, TSO(?)**
27036	-	-	-	-	-	30.09 (2.65)	**X**
27037	0.18 (0.01)	-	23.69 (1.87)	-	0.19 (0.01)	123.35 (8.98)	**IPyA, IAM, TAM, TSO(?)**
27038	-	37.59 (3.96)	40.72 (1.46)	-	-	3046 (4.14)	**IPyA, TSO(?)**
27041	0.99 (0.05)	-		-	-	21.39 (1.66)	**IAM, X**

The IAM pathway is the major route for IAA production in pathogenic bacteria (Spaepen et al. 2007). This study is in agreement with the previous work of Rajagopal et al. (1994) that the IAM pathway has also been identified based on the fungal production from IAM. This pathway has two steps, Trp is first transformed to IAM by the enzyme tryptophan-2-monooxygenase which eventually results in the transformation of IAM to IAA by an IAM hydrolase (Spaepen et al. 2007).

In the case of EF, sixteen strains (four *Alternaria*, three *Didymella* and nine *Fusarium* species) used this pathway for their IAA production. Similarly, previous reports noted the synthesis of IAA by IAM pathway in *C. fructicola*, *C. gloeosporioides* f. sp. *aeschynomene*, *F. proliferatum*, *F. verticillioides*, *F. fujikuroi* and *F. oxysporum* (Numponsak et al. 2018, Robinson et al. 1998, Tsavkelova et al. 2012, Maor et al. 2004). The production of IAA by the IAM pathway was also confirmed by Theunis et al. (2004) for *Rhizobium* sp. based on LC-MS detection of intermediates. It is vital to mention that this study established for the first time that *Didymella* and *Alternaria* species produce IAA via IAM pathway.

According to Spaepen et al. (2007), the first step in IPyA pathway is the conversion of Trp to IPyA via an aminotransferase. Then, it is decarboxylated to IAAld by the enzyme IPDC which in the final step is oxidized to IAA. Besides IPyA and IAAld intermediates, this pathway could also be detected by the presence of ILA and TOL. According to Toshiko at al. (1996), TOL was noted as a specific by-product of IPyA pathway. *R. solani, G. fujikuroi, V. nashicola* produced TOL via IPyA pathway, hence, IAA production via the IPyA pathway was established in *P. indica, R. paludigenum, R. cerealis, U. maydis, U. esculenta, Taphrina deformans* and *Physcomyces blakesleeanus* (Chung and Tzeng 2004, Reineke 2008, Hilbert et al. 2012, Yamada et al. 1990, Schramm et al. 1987, Ludwig-Muller et al. 1990).

Similarly, a couple of studies reported that ectomycorrhizae (ECM) fungi were observed to use IPyA pathway for IAA production (Krause et al. 2015, Kumla et al. 2020). Most IAA producing EF operated IPyA pathway through the production of two intermediates (IPyA and IAAldwhich were identified from *Alternaria* (10) and *Fusarium* (8) species.

There were eight isolates, where ILA was the only detected compound of this pathway (Table 4), but this metabolite can be originated solely from IPyA according to the recent knowledge about IAA biosynthesis (Figure 2). Its presence clearly indicates the operation of IPyA pathway. Furthermore, beside this pathway, the operation of TSO pathway could not also be excluded if the IAAld and/or TOL was also detected (Table 4). Altogether, six strains, four *Fusarium* (SZMC 26983, 26990, 27017 and 27037) and two *Didymella* (SZMC 26648 and 26991) were able to produce IAA from TAM. TAM pathway has also been found in bacteria, such as *B. cereus* (Perley and Stowe 1996).

As it was mentioned earlier, many fungal isolates were found to produce IAA using different intermediates. It is important to point out that IAM, IPyA and TAM pathways co-exist in *Fusarium* species (SZMC 26990, 27017 and 27037). EF were capable of synthetizing IAA through three pathways including IPyA – TAM *Didymella* species (SZMC 26648 and 26991) and IPyA – IAM *Fusarium* species (SZMC 26654 and 26979) and IAM – TAM *Fusarium* species (SZMC 26983) pathway pairs. This study also proves that IAA production of EF could occur through different pathways and in certain cases, two or more pathways could also coexist in EF.

7 CONCLUSIONS

Endophytic fungi (EF) in symbiotic partnership with their host plants are well known to boost plant growth, to protect them from pathogen attack and to diminish the adverse effects of both biotic and abiotic stresses. Hence, EF are excellent producers of compounds that can be exploited for agriculture and medicinal targets based on their biological activity. They produce plant growth regulating hormones like gibberellins and IAA, which raise crop yield and quality. Minerals such as phosphate solubilization and siderophore production produced by EF can regulate plant development and release plant growth in a stressful environment.

In the present study, 62 EF were isolated from *Astragalus melilotoides, Convolvulus arvensis, Halerpestes salsuginosa, Nitraria sibirica, Oxytropis glabra, Sophora flavescens, Sphaerophysa salsula* and *Thermopsis dahurica*. The EF belonged to 7 genera involving *Fusarium*, *Alternaria*, *Didymella*, *Penicillium*, *Phoma*, *Camarosporidiella* and *Xylogone*. *Alternaria* and *Fusarium* species were identified from most of the isolates.

The ferment broth extracts of the isolates showed higher inhibitory activity on bacteria than mycelial extracts in the antibacterial assays. Furthermore, *F. tricinctum* SZMC 27041 and *F. armeniacum* SZMC 26659 were the best candidates for further detailed work due to their highest antimicrobial activity of their EtOAc extracts. EtOAc extract of *D. glomerata* SZMC 26649 showed the highest inhibition zone during the antifungal tests. In total, 64 extracts of the isolates were found to show potential against *C. albicans* which isolates mostlybelonged to *Fusarium* and *Alternaria* genera.

All endophytic fungi were screened for their indirect plant promoting abilities including phosphate solubilization and siderophore production. It could be concluded that the phosphate solubilization and the siderophore production are common features of the isolated endophytes, because 34 and 31 strains proved to be active in the

phosphate solubilization and in the siderophore production assays, respectively. Furthermore, the IAA productions were generally improved due to the additional Trp in the ferment broth. The 6 selected isolates could increase the length of the primary root in *Arabidopsis thaliana*, while the biomasses of the plants were significantly lower than after the control IAA treatment. Significant alterations have also been detected in the photosynthetic pigment content after the fungal extract treatments.

EF were able to produce IAA at least through 3 different pathways such as IAM, IPyA and TAM.

8 SUMMARY

From time-to-time, medicinal plants have been used in therapies for numerous diseases linked to various medicinal activities. It is well-known that in many ancient cultures, such as in Mongolians and Chinese, the natural sources were used for the prevention of illnesses. Nowadays, mankind is facing an issue of extinction of many wild medicinal plant species due to the gaining urgent demand for plant metabolites. Therefore, it is vital to investigate alternative approaches to gather biologically active compounds from novel biological sources. In fact, medicinal plants offer a special source to boost bioactive endophytes that could be useful for numerous applications. Endophytic fungi (EF) that have the ability to produce minerals such as phosphate, siderophore as well as phytohormones like auxin could be potentially vital for agriculture. This study provided fundamental information to this field and it contributed to a better understanding of the plant-promoting phenomena of endophytic fungi (EF).

In this study, investigation of EF isolated from Mongolian medicinal plants (*Convolvulus arvensis, Sphaerophysa salsula, Nitraria sibirica, Astragalus melilotoides, Thermopsis dahurica, Halerpestes salsuginosa, Oxytropis glabra, S. flavescens*) was undertaken. Results of this study were divided into four parts: i) isolation and molecular identification of EF, ii) discovering the direct and indirect plant promoting abilities of EF, iii) screening of the bioactive potential of EF, and iv) determination of IAA biosynthetic pathways of the IAA producing EF.

Altogether, 62 strains were isolated from 8 plants. Certain isolates were identified at species level, while eighteen isolates at genus level. The isolates belong to 7 genera involving *Fusarium* (24), *Alternaria* (21), *Didymella* (9), *Penicillium* (3), *Phoma* (3), *Camarosporidiella* (1) and *Xylogone* (1). Based on the analysis of the ITS sequences of the strains, the members of *Alternaria* and *Fusarium* were found most frequently. This study found some EF for the first time from the host plants such as, *Alternaria*

species were found for the first time from the host plants *S. flavescens, O. glabra, S. salsula* and *H. salsuginosa* and *Didymella* species from *S. flavescens, N. sibirica, S, salsula* and *C. arvensis.* In case of *Fusarium* species, they were isolated for the first time from 3 different host plants which were *S. flavescens, S. salsula* and *H. salsuginosa.* For *Xylogone* and *Camarosporidiella* species, they were isolated from *S. flavescens* and *A. melilotoides. Phoma* species were isolated from *A. melilotoides* and *S. salsula* and *Penicillium* species were isolated from *A. melilotoides* and *C. arvensis.*

Three agar plate assay experiments were applied to observe the plant growth promoting abilities of EF. The indirect way is the mineral solubilization for plant growth promotion, in which the isolates were capable of producing both siderophores and to solubilize phosphate. In total, 34 endophytes were positive for their siderophores sequestering ability and binding iron from the medium. The largest zone appeared as 32 mm on the plate of the *F. tricinctum* SZMC 26658 (isolated from the stem part of *S. flavescens*) strain. 31 isolated were discovered as phosphate solubilizers. Interestingly, the highest phosphate solubilizator strain (SZMC 26657, 40 mm) was also the member of same species as the best siderophore producing strain (*F. tricinctum*) and both were isolated from the stem of *S. flavescens.*

Furthermore, each isolate was noted to produce IAA on either Trp supplemented medium or on simple PDA medium using IAA plate assay. From these, 25 EF showed positive results only in the presence of Trp, 6 EF only in the absence of Trp and 26 EF in both cultivation conditions. In the case of mycelial extracts, both *Alternaria* (SZMC 26993) and *Fusarium* (SZMC 26657) isolates accumulated remarkable amounts of IAA on PDB medium and 27 EF proved to be also outstanding producers on supplemented media. Furthermore, mycelial extracts of 9 isolates *F. tricinctum* (SZMC 26984), *F. proliferatum* (SZMC 26989), *Alternaria* sp. (SZMC 26993 and 26996), *X. sphaerospora* (SZMC 26661), *A. alternata* (SZMC 26651), *D. glomerata* (SZMC 26655) and *F. tricinctum* (SZMC 26657) on media without Trp source found to be higher IAA producers than their ferment broth extracts.

Based on the results, it could be concluded that the production of IAA in most of the isolates was improved due to additional Trp in both cases (the ferment broth and the mycelia). The production of IAA was higher in cultivation medium than in mycelial extracts. Therefore, six strains *D. glomerata* (SZMC 26648), *A. alternata* (SZMC 26652), *F. tricinctum* (SZMC 26658), *F.* sp. (SZMC 26660), *A. alternata* (SZMC 26651) and *A. alternata* (SZMC 26653), producing the highest amounts of IAA were applied to evaluate the effects of the endophytic IAA on plants. The length of the primary root of the model *A. thaliana* was increased in several cases. Significant alterations have also been detected in the photosynthetic pigment (chlorophyll-a, -b and carotenoids) content due to the fungal extract treatments.

A total of 372 extracts were tested for their bioactivity. The highest activity of the extracts was shown against *S. aureus* and *S. albus* in the ranges of 80 - 96% based on 287 and 269 extracts of the isolates, respectively. Most of these isolates belonged to *Fusarium* and *Alternaria* species. The extracts of *F. tricinctum* (SZMC 27041) and *F. armeniacum* (SZMC 26659) strains were the most promising candidates with the highest activities on all six bacteria. In general, the ferment broth extracts of the isolates presented higher inhibitory activity on the bacteria than the mycelial extracts. In case of antifungal activity, 64 extracts showed potential against *C. albicans*. Nearly the same number of extracts showed significant inhibition against *A. niger* and *R. solani* by involving 38 and 41 extracts, respectively. The least bioactive extracts of isolates were identified against *F. culmorum*. In addition, ethyle-acetate extract of *D. glomerata* (SZMC 26649) caused the biggest halo around the colony by significant inhibition activity of 23 mm.

This study recorded, that the isolates produced IAA through at least three different pathways including IAM, IPyA and TAM pathways as well as the presence of TSO pathways could also be possible based on the detected metabolite profiles. In general, most strains operated IPyA pathway in their IAA biosynthesis, which was followed by IAM pathway. Interestingly, most strains have conducted two or more biosynthetic pathways for producing IAA. Moreover, 8 EF have functioned only one pathway for their IAA production. Altogether, 26 IAA producing EF used IPyA pathway, 16 strains

produced IAA via IAM pathway and 8 isolates were able to produce IAA from TAM pathway.

This study showed that IAM, IPyA, TAM pathways co-exist in three *Fusarium* species. Besides, five EF were also capable of utilizing multiple pathways proving the co-existence of these pathways in both *Didymella* and *Fusarium* species. This study provides reliable data that the IAA production of EF could be occurred through different pathways and two or more pathways could exist in the same fungus.

In summary, present results confirm, that the isolated and deposited EF are outstanding reservoirs of bioactive secondary metabolites, however, further studies are needed to isolate the pure compounds from the screened strains. The presented results offer a proper framework to apply these isolates for medical purposes. Furthermore, the achieved results also highlight the potential of the EF in plant growth promotion, which could serve a good base for novel innovative agricultural solutions.

9 ÖSSZEFOGLALÁS

A gyógyhatású növényeket már évszázadok óta alkalmazzák különböző betegségek kezelésére és azok tüneteinek enyhítésére. Már az ősi mongol- és kínai tradicionális orvoslásban is számos gyógynövényt tartottak nyilván, melyek a bennük található szekunder metabolitoknak un. természetes vegyületeknek köszönhetik gyógyhatásukat. Az emberiség korai időszakában maguk a növények és a növényekből készített nyers kivonatok képezték a gyógyászati kezelések alapját. Mára már azonban legtöbbször a növényekből tisztított metabolitok találhatóak a készítményekben és a növényi metabolitok iránt jelentősen megnövekedett kereslet hatására számos vadon élő gyógynövényfaj a kipusztulás határára sodródott. Ennek elkerülése érdekében fontos olyan új lehetőségek feltárása, melyek új forrásokat biztosíthatnak a biológiailag aktív vegyületek kinyerése. Az endofiton mikroorganizmusok, főként a gyógynövények endofiton gombái ezen új lehetőségek felkutatása során kiemelt szerepet játszhatnak jelentős szekunder metabolit termelő képességüknek köszönhetően. Ezen túl az endofiton gombák képesek jótékonyan befolyásolni, serkenteni a gazdanövények fejlődését is. A növekedés serkentésének alapja általában az, hogy az ásványi anyagokat a növények számára hozzáférhetővé teszik, a talajban lévő foszfát mobilizálása vagy a sziderofórok termelése révén, valamint bizonyos törzsek képesek fitohormonok, például az auxin vagy giberellin termelésére is. Ezen tulajdonságaik alapján az endofiton gombák mezőgazdasági jelentősége megkérdőjelezhetetlen. Mezőgazdasági szempontból ugyanis fontos a termesztett növények egészségének és jó kondíciójának megőrzése a jobb termésátlagok elérése érdekében, lehetőleg vegyszermentesen, amelyhez nagy segítséget nyújthat az endofiton mikroorganizmusok alkalmazása a termelésben.

Munkánk során mongóliai gyógynövényekből (*Convolvulus arvensis*, *Sphaerophysa salsula*, *Nitraria sibirica*, *Astragalus melilotoides*, *Thermopsis dahurica*,

Halerpestes salsuginosa, *Oxytropis glabra*, *Sophora flavescens*) izolált endofiton gombákat vizsgáltunk a következő szempontok alapján: i) az izolált gombák taxonómiai helyzetének megállapítása, molekuláris azonosítása, ii) az endofiton gombák növényi növekedést serkentő képességeinek felmérése beleértve a közvetlen és közvetett hatásokat, iii) az endofiton gombák által termelt metabolitok bioaktivitásának vizsgálata és iv) az indolecetsav (IAA) termelő törzsek esetében a bioszintézis útvonalak feltérképezése.

A növényi minták begyűjtését követően összesen 62 törzset izoláltunk a 8 említett növényből. Az izolátumok egy részét faj szinten, tizennyolc izolátumot pedig nemzetség szinten azonosítottunk. A törzsek a *Fusarium* (24), *Alternaria* (21), *Didymella* (9), *Penicillium* (3), *Phoma* (3), *Camarosporidiella* (1) és *Xylogone* (1) nemzetségekhez tartoztak. A törzsek ITS szekvenciáinak elemzése alapján a gyógynövényekben a legnagyobb gyakorisággal az *Alternaria* és a *Fusarium* nemzetség képviselői fordultak elő.

Az endofiton gombák növényi növekedést serkentő hatásainak vizsgálatára agarlemezes kísérleteket alkalmaztunk. A talajban lévő ásványi anyagok szolubilizálása, talajoldatba vitele, a növényi növekedés serkentésének egy közvetett útja. Az általunk izolált endofiton törzsek képesek voltak mind a szideroforok termelésére, mind pedig a foszfát oldatba vitelére. A vizsgálatok eredményei szerint 34 endofiton törzs termelt sziderofórokat, és megközelítőleg ugyanennyi törzs volt képes a foszfát szolubilizálására is. A legnagyobb zónát a sziderofór tesztben a *F. tricinctum* SZMC 26658 (*S. flavescens* szárából izolált) törzse (32 mm) esetében regisztráltuk. A legnagyobb aktivitást (zónaátmérő 40 mm) a foszfát szolubilizációs tesztben szintén ezen faj képviselője okozta (SZMC 26657), amely szintén a *S. flavescens* szárából került izolálásra. Az agarlemezes vizsgálatok alapján minden izolátum képes volt az IAA termelésére Trp-vel kiegészített tápközegben vagy egyszerű burgonya-dextróz táptalajon. Huszonöt endofiton gomba csak Trp jelenlétében, 6 csak Trp hiányában

mutatott pozitív eredményt, 26 izolátum pedig mindkét tenyésztési körülmény között termelt IAA-t. A HPLC-MS vizsgálatok során az *Alternaria* sp. SZMC 26993 és a *Fusarium* sp. SZMC 26657 izolátumok micéliumában jelentős mennyiségű IAA halmozódott fel burgonya-dextróz tápoldatban tenyésztve, azonban az izolátumok jelentősebb része (27 törzs), a Trp-vel kiegészített tápoldatban mutatott kiemelkedő IAA termelést. Az eredmények rámutattak arra, hogy általában magasabb IAA szinteket lehetett mérni a micéliumokban és a fermentlevekben is, ha a tápoldat tartalmazott Trp-t is. A legjobb 6 IAA termelő törzs bevonásával megvizsgáltuk az IAA tartalmú fermentlékivonatok hatását *in vivo* növényi kísérletekben is. A vizsgálatok során azt tapasztaltuk, hogy modellként alkalmazott *Arabidopsis thaliana* primer gyökerei több esetben erőteljesebb növekedést mutattak a kezelés hatására. A növények fotoszintetikus pigment (klorofill -a, -b és karotinoidok) tartalmában is tapasztaltunk szignifikáns változásokat, de ezek az eltérések nem mutattak határozott trendeket.

Az izolátumok által termelt metabolitok bioaktivitásának vizsgálata során összesen 372 kivonatot teszteltünk, a legtöbb kivonat a *S. aureus* (287) és a *S. albus* (269) ellen mutatott jelentős, 80% feletti gátló hatást. Az aktív metabolitokat termelő izolátumok többsége a *Fusarium* és *Alternaria* nemzetségbe tartozott. A 62 vizsgált izolátum közül az *F. tricinctum* SZMC 27041 és a *F. armeniacum* SZMC 26659 törzsek kivonatai emelkedtek ki szélesspektrumú hatásukkal, mert mind a hat baktérium esetében magas gátlást tapasztaltunk a kivonataikkal szemben. Általában elmondható, hogy az izolátumok fermentlevének kivonatai nagyobb gátló hatást mutattak a baktériumokra, mint a micélium kivonatok. A gombaellenes hatások tesztelése esetén 64 kivonat mutatott jelentős aktivitást a *C. albicans* élesztővel szemben, valamint 38 kivonat gátolta az *A. niger*, 41 pedig a *R. solani* növekedését, míg a *F. culmorum* ellen gátló hatást mutató kivonatok száma volt a legkevesebb. A tesztek során a legnagyobb gátló aktivitást (23 mm-es gátlási zóna) a *D. glomerata* SZMC 26649 törzs etil-acetát kivonata mutatta a *C. albicans* törzzsel szemben. A bioaktivitási eredmények alapján

elmondható, hogy az izolált és deponált endofiton gombák nagy része termel antimikrobiális hatású bioaktív másodlagos metabolitot, azonban a tiszta vegyületek izolálásához és szerkezetének meghatározásához a már kiválasztott törzsek esetében további vizsgálatokra van szükség.

Az IAA bioszintézis útvonalak feltérképezését HPLC-MS mérésekkel végeztük. Az izolátumokban az indol-3-acetonitril (IAN) nem volt kimutatható, míg az indol-3-acetamidot (IAM), az indol-3-acetaldehidet (IAAld), az indol-3-tejsavat (ILA), az indol-3-piroszőlősavat (IPyA), a triptamint (TAM) és az indol-3-etanolt (TOL) azonosítani tudtuk. Ez alapján megállapítható, hogy az izolátumok legalább három különböző úton, köztük az IAM, az IPyA és a TAM útvonalon képesek az IAA bioszintézisére, valamint a TSO útvonal jelenléte sem zárható ki a detektált metabolitok alapján. Általában a legtöbb törzs IAA bioszintézise az IPyA útvonalat követi. Azt tapasztaltuk továbbá, hogy több törzs is két vagy több bioszintetikus úton is képes volt az IAA előállítására. Összeségében elmondható, hogy 26 törzs esetében azonosítottuk az IPyA útvonal, 16 törzsnél az IAM útvonal, 8 izolátumnál pedig a TAM útvonal működését. Kimutattuk, hogy az IAM, IPyA, TAM szintézis útvonalak három *Fusarium* fajban párhuzamosan is működnek, valamint 5 további izolátumban két útvonal egymás melletti működését is igazoltuk.

Munkánkkal új ismeretekkel járultunk hozzá az endofiton mikroorganizmusok mélyebb megismeréséhez, valamint eredményeink rávilágítottak az endofiton gombák növényi növekedést serkentő hatásainak hátterében lévő folyamatokra. A kutatásaink során nyert bioaktivitási adatok jó kiindulópontot adnak az izolátumok későbbi, esetleges gyógyszeripari célokra történő alkalmazására és új bioaktív metabolitok azonosítására. Az elért eredmények továbbá rávilágítanak a növényi növekedés serkentése terén az endofiton gombákban rejlő lehetőségekre, amelyek jó alapot biztosíthatnak új, innovatív mezőgazdasági megoldásokhoz.

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12 LIST OF PUBLICATIONS RELATED TO THIS THESIS

Adiyadolgor Turbat, David Rakk, Aruna Vigneshwari, Sándor Kocsubé, Huynh Thu, Ágnes Szepesi, László Bakacsy, Biljana D. Škrbić, Enkh-Amgalan Jigjiddorj, Csaba Vágvölgyi and András Szekeres, 2020. Characterization of the plant growth-promoting activities of endophytic fungi isolated from *Sophora flavescens*. *Microorganisms*, 8, 683: doi:10.3390/microorganisms8050683.

Gábor Endre, Zsófia Hegedüs, **Adiyadolgor Turbat**, Biljana Škrbic, Csaba Vágvölgyi and András Szekeres, 2019. Separation and purification of aflatoxins by centrifugal partition chromatography. *Toxins*, 11, 309: doi:10.3390/toxins11060309.

Adiyadolgor Turbat, Enkh-Amgalan Jigjiddorj, Csaba Vágvölgyi, András Szekeres, 2018. Antimicrobial activity of endophytic fungi isolated from *Sophora flavescens*. In 16th Wellmann International Scientific Conference.

Adiyadolgor Turbat, David Rakk, Enkh-Amgalan Jigjid, Csaba Vágvölgyi and András Szekeres, 2017. Determination of plant-growth promoting compound in endophytes, isolated from *Sophora flavescens*. ACTA MICROBIOLOGICA ET IMMUNOLOGICA HUNGARICA 64 (Supplement 1): 183-184.

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13 APPENDICES

Table S1. Antimicrobial effects of isolates extracted with chloroform from ferment broth

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
26975	91.19±2.47	81.61±1.51	66.59±2.56	84.67±1.62	94.96±2.11	85.13±3.99
26976	83.92±3.37	86.74±5.24	66.37±2.33	86.85±2.99	92.03±2.51	83.26±2.43
26977	83.56±3.40	91.73±2.77	48.73±1.32	81.79±3.14	93.02±3.80	91.51±2.02
26978	92.22±4.08	93.04±2.63	87.68±2.51	94.14±2.94	92.99±2.12	84.19±4.40
26979	93.32±2.74	91.99±1.71	93.86±4.41	83.80±1.49	92.58±2.11	91.25±2.94
26980	87.02±4.41	66.44±4.54	91.41±6.49	54.74±4.88	91.51±3.02	56.91±3.35
26981	61.60±14.8	76.47±3.27	94.19±3.78	33.19±7.26	70.21±3.24	58.91±3.70
26982	86.72±4.40	41.77±8.55	83.87±3.15	37.97±3.42	87.99±2.11	63.98±1.31
26983	87.17±5.24	60.81±8.75	92.27±4.06	47.27±4.54	86.65±4.49	57.13±1.45
26984	86.15±3.45	63.44±8.12	95.85±2.15	65.95±3.55	91.32±1.61	92.85±3.03
26985	84.14±1.22	49.31±2.51	88.37±2.24	67.48±2.35	87.54±2.16	84.91±6.78
26986	95.04±2.40	91.49±3.88	93.34±3.26	66.01±3.27	84.80±2.06	82.30±7.87
26987	91.86±2.81	87.96±5.95	96.46±3.29	67.50±6.46	87.65±2.15	76.99±2.21
26988	82.52±3.33	71.14±5.43	87.02±1.84	48.57±7.34	95.27±0.78	86.16±4.48
26989	85.71±4.24	55.62±4.08	95.96±2.59	58.51±2.64	94.64±1..96	90.83±4.38
26990	85.02±2.97	54.69±4.01	93.07±2.53	60.00±4.36	92.90±0.45	91.96±2.83
26991	82.98±3.09	62.75±7.21	92.37±2.59	42.86±4.63	92.23±2.43	92.52±2.88
26992	87.01±2.60	60.41±4.10	94.06±2.63	66.58±3.85	85.85±3.02	90.83±1.69
26993	93.32±1.65	55.94±5.87	91.04±3.27	67.02±3.78	93.36±2.96	91.78±4.35
27024	90.81±3.45	92.57±2.60	94.47±1.72	70.39±3.97	88.38±1.87	91.80±4.84
27025	82.71±3.45	38.34±6.35	87.02±1.06	33.09±8.46	92.50±2.21	62.56±10.5
26996	87.02±2.41	36.04±10.3	90.69±1.74	37.63±6.15	84.78±4.28	64.41±5.59
26997	85.08±4.17	40.95±13.7	89.90±1.46	58.85±2.77	93.97±4.12	61.18±4.20
26998	91.80±3.69	56.02±10.7	93.41±1.23	68.11±2.98	90.95±2.15	65.97±2.79
26999	92.67±1.56	90.73±2.26	91.11±2.82	70.12±6.77	83.64±2.63	62.22±3.12
27000	91.41±1.96	84.22±3.00	92.04±1.87	71.38±10.09	85.61±2.07	60.22±2.01
27001	85.18±4.28	75.08±7.83	93.17±03.65	62.72±4.47	93.46±3.13	61.26±1.78
27002	84.59±2.08	84.48±3.01	70.37±2.64	89.52±1.31	86.73±3.62	82.27±8.35
27003	93.15±2.97	85.62±4.35	81.37±4.21	64.89±5.63	91.76±1.31	57.26±1.50
27004	86.54±3.35	43.16±12.3	86.25±3.01	65.45±5.15	78.64±2.03	61.55±4.27
27005	85.72±3.40	65.64±3.77	79.82±3.16	63.37±4.32	86.63±3.11	60.42±2.94
27021	92.30±2.36	76.90±3.48	76.12±1.74	67.20±3.20	95.35±2.16	64.53±3.84
27033	93.46±1.76	92.46±2.22	74.12±3.51	64.51±4.77	91.21±1.60	58.91±3.17
27036	93.63±2.45	33.70±15.7	74.29±4.87	69.49±4.31	91.44±2.52	65.21±2.63
27034	86.63±3.97	77.58±3.68	75.90±2.71	73.63±6.17	91.57±1.96	63.83±1.01
27006	68.96±6.15	33.62±7.71	76.08±2.26	65.10±3.58	87.60±1.86	59.66±5.03
27012	87.93±1.94	65.47±5.90	74.36±2.77	62.68±2.36	89.31±0.99	56.45±3.52
27035	92.98±1.74	50.30±5.77	76.01±4.89	54.61±3.22	91.07±2.04	63.54±0.78
27007	92.41±2.21	92.04±1.93	75.15±3.43	89.68±3.84	88.96±1.57	92.34±2.82
27041	92.71±2.23	92.82±3.05	85.02±3.99	88.50±1.13	87.54±2.97	92.42±3.69
27037	89.64±0.77	93.40±1.88	74.64±3.79	84.99±3.34	84.20±3.18	92.54±2.47
27038	94.33±3.59	93.48±2.77	74.81±4.72	86.33±3.38	85.37±2.31	91.44±5.43
27039	91.38±3.07	93.05±1.79	77.22±3.93	85.36±2.55	93.39±3.72	85.96±1.05
27016	92.19±1.99	63.29±6.68	72.88±2.33	58.16±3.11	92.70±1.15	63.84±2.38
27017	85.79±2.96	69.32±7.91	71.15±2.02	59.33±4.07	86.23±1.85	62.28±2.62

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
27018	88.00±1.52	65.62±5.83	77.74±5.59	58.37±2.70	88.89±1.84	63.49±3.05
27019	93.68±2.85	66.20±9.09	75.54±3.45	59.69±3.85	90.99±1.85	44.17±4.41
26647	93.41±4.47	90.03±2.19	92.46±4.76	83.42±3.22	92.86±2.73	84.76±1.85
26648	92.46±5.02	76.26±3.16	89.73±2.97	76.26±4.28	85.78±4.44	87.31±2.02
26649	77.25±3.30	74.05±3.25	79.70±1.63	69.14±5.55	75.10±3.70	83.13±2.34
26661	78.59±0.56	90.80±1.17	90.69±2.62	78.90±4.30	83.89±2.22	81.57±4.17
26660	77.94±3.22	73.85±3.65	82.89±0.83	79.72±5.89	73.44±3.05	79.68±0.90
26650	91.95±2.02	88.72±3.26	77.83±1.33	82.01±6.43	78.29±2.39	83.63±2.53
26651	84.91±4.30	91.62±2.87	91.53±2.41	73.44±4.59	82.98±3.30	84.26±9.87
26652	74.94±2.37	76.37±7.26	78.92±1.14	66.45±2.88	85.45±2.74	84.93±1.60
26653	66.78±3.52	65.56±7.33	91.47±3.26	65.12±14.87	72.02±4.33	77.78 ±2.05
26654	83.65±2.76	74.67±2.50	91.11±1.17	66.39±6.60	80.69±5.60	84.29±3.45
26655	80.33±5.50	75.52±2.91	84.78±2.48	74.00±6.75	90.21±2.35	91.55±3.77
26656	84.07±5.03	76.27±2.78	76.45±1.46	72.10±2.16	85.41±0.73	85.57±1.49
26657	74.12±3.61	61.68±4.99	64.29±3.56	73.17 ±7.00	64.02±4.73	76.94±3.74
26658	83.98±2.84	80.96±4.77	84.34±3.81	74.37±5.17	91.14 ±10.9	89.70±4.38
26659	65.21±1.95	90.17±4.20	87.24±3.54	83.16±3.34	91.11±1.44	85.15±2.34

Table S2. Antimicrobial effects of isolates extracted with hexane from ferment broth

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
26975	75.04±2.91	44.24±7.03	72.82±3.47	55.60±4.89	75.83±2.35	65.30±3.38
26976	70.52±3.28	34.82±10.7	86.97±2.49	50.09±0.57	85.88±3.55	47.06±3.01
26977	76.87±4.07	35.42±7.09	86.12±1.16	26.03±13.5	75.91±1.77	55.81±5.56
26978	78.27±1.72	28.27±7.41	85.53±3.36	21.30±6.28	66.53±2.61	53.82±5.86
26979	71.84±2.39	39.06±3.10	86.35±3.12	30.08±1.49	86.70±0.66	61.47±6.91
26980	84.62±2.99	50.66±6.88	94.83±4.15	30.80±8.48	90.10±3.60	52.86±5.44
26981	84.09±1.32	45.22±4.11	87.32±2.20	50.90±8.11	84.36±1.75	63.17±7.16
26982	78.24±1.91	40.79±2.47	87.29±1.72	47.09±4.78	87.62±3.53	46.35±2.50
26983	74.05±1.80	48.14±4.07	87.66±1.32	27.80±4.75	84.71±2.68	29.25±3.81
26984	84.59±3.44	53.49±5.14	83.01±3.58	46.58±3.61	87.15±2.58	48.63±2.97
26985	82.58±2.70	54.62±4.18	85.94±0.43	42.89±7.37	84.03±2.02	53.64±3.73
26986	75.77±2.87	37.01±5.98	83.81±3.47	32.62±4.89	84.57±0.44	64.06±3.52
26987	79.82±0.75	45.33±5.98	82.43±2.47	41.51±7.43	86.80±3.93	56.39±3.82
26988	83.08±0.90	48.22±4.50	87.79±1.27	49.77±4.66	85.67±2.32	75.67±2.52
26989	85.09±2.94	33.71±3.28	87.04±2.51	42.85±4.78	86.30±1.14	48.35±2.32
26990	76.84±1.54	46.36±6.32	80.40±6.55	43.49±5.64	85.46±1.91	62.35±2.31
26991	78.50±1.33	33.67±5.16	86.62±1.70	33.68±8.47	87.68±2.47	56.76±2.88
26992	86.65±2.51	81.63±6.71	84.99±3.26	86.48±3.40	86.32±1.97	84.50±3.55
26993	64.80±4.31	36.65±2.27	85.10±4.74	42.79±2.85	85.31±0.69	75.93±3.13
27024	67.97±7.32	63.46±13.5	84.65±3.71	55.51±3.60	85.10±2.43	76.63±4.80
27025	65.07±4.06	56.13±3.90	86.64±3.04	46.61±3.39	82.21±1.72	75.37±2.11
26996	63.49±4.11	88.24±4.95	85.30±2.24	88.79±1.86	85.44±4.00	92.04±1.53
26997	61.20±1.02	82.97±2.86	86.62±1.73	87.27±2.14	85.41±1.72	82.98±4.82
26998	61.68±0.60	58.09±1.86	85.26±2.98	43.49±5.67	84.32±1.42	62.01±3.85
26999	65.36±0.48	53.54±3.47	82.64±3.03	35.90±3.48	86.13±1.56	55.54±4.27
27000	62.59±2.56	88.35±6.00	81.64±2.98	87.42±1.77	85.96±2.89	64.64±4.43

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
27001	58.30±6.99	50.27±2.59	85.40±3.03	48.13±2.31	82.90±3.03	74.54±1.95
27002	60.92±2.06	56.46±9.52	85.27±1.82	49.16±4.91	84.94±2.17	74.85±3.37
27003	50.27±5.01	42.42±4.99	81.79±2.70	46.29±3.26	84.94±2.77	66.03±3.09
27004	84.27±1.14	34.63±13.7	83.99±3.75	43.61±3.61	87.14±1.80	51.09±2.18
27005	86.34±2.63	50.54±17.6	84.13±4.74	46.64±2.78	86.35±2.33	56.15±3.93
27021	84.64±1.23	64.78±11.5	84.56±3.07	54.74±4.01	84.96±2.26	69.79±4.22
27033	86.26±2.03	43.75±7.52	84.56±3.98	37.39±7.33	83.87±4.84	40.79±1.42
27036	71.73±1.33	43.79±8.91	84.97±2.24	49.04±3.99	83.54±2.39	55.13±3.95
27034	77.35±1.95	45.69±13.4	86.81±2.48	40.77±4.47	84.02±2.89	55.58±5.01
27006	74.34±3.64	57.09±5.68	83.11±2.04	57.43±4.19	86.65±4.27	55.52±3.84
27012	76.75±2.68	35.28±4.73	87.36±2.48	39.93±2.54	84.62±3.37	48.96±5.34
27035	82.41±2.00	53.79±6.27	83.56±1.96	48.11±5.24	85.55±2.57	61.91±3.95
27007	80.27±4.17	56.45±4.50	88.07±2.20	45.79±6.07	82.88±3.02	68.36±3.62
27041	83.93±3.33	86.69±1.64	84.50±2.77	81.84±1.87	85.15±3.55	86.88±2.49
27037	83.45±2.58	56.53±3.35	75.14±2.74	37.82±3.57	84.37±1.77	51.72±3.03
27038	76.83±2.99	73.16±4.65	76.13±3.15	66.13±4.44	83.26±3.93	56.27±3.73
27039	76.71±1.14	63.77±8.02	72.67±1.84	35.93±4.78	75.08±1.27	60.91±2.64
27016	68.88±1.55	61.70±14.9	69.55±3.18	37.83±4.35	77.10±2.38	53.16±4.82
27017	79.46±1.36	44.40±7.73	62.00±3.02	37.37±6.40	78.96±2.20	45.69±4.95
27018	62.01±1.38	45.42±7.64	69.86±2.67	45.78±5.05	84.44±1.62	60.99±1.40
27019	61.44±2.02	49.67±7.35	76.92±4.89	50.35±7.63	83.25±2.37	53.35±5.18
26647	69.65±1.67	64.85±4.38	84.27±3.82	70.76± 2.22	84.42±1.44	56.07±7.09
26648	67.94±1.47	60.18± 8.44	86.11±3.20	73.45±4.30	90.78±3.44	54.95±6.74
26649	77.40±2.71	65.45±1.04	84.87±3.24	77.52±2.23	87.29± 2.52	55.39±3.45
26661	76.04±4.02	59.68± 3.37	86.41±3.83	73.15±1.88	87.79±2.47	65.38±3.37
26660	76.40±2.87	63.57±4.29	91.96±2.80	78.25±1.04	85.09±0.50	78.84±10.2
26650	79.44±1.86	59.99±8.67	83.58±1.69	70.56±1.27	87.20±5.26	56.84±2.27
26651	66.42±2.95	58.11±2.02	86.35±3.28	81.26±2.62	86.58±2.28	66.94±3.30
26652	71.32±0.33	62.77±3.66	88.22±2.05	63.23±3.00	86.66±2.04	68.43±5.01
26653	67.09±3.18	64.00±2.71	86.71±1.90	64.57±3.51	85.13±2.52	71.77±5.34
26654	74.03±1.40	65.49±8.28	80.64±2.48	70.70±8.97	86.47±5.25	67.13±2.66
26655	79.08±0.48	69.04±4.23	81.62±1.65	68.18±1.13	87.79±3.72	56.11±4.12
26656	79.11±1.51	61.92±2.57	82.63±0.83	71.63±1.65	84.50±3.74	62.20±8.24
26657	80.80±3.28	63.77±6.72	88.75±4.29	65.67±4.07	86.17±1.56	62.28±10.8
26658	70.05±1.21	57.36±2.67	82.92±4.01	73.00±3.79	89.15±2.94	61.68±9.10
26659	80.45±0.87	79.38±1.92	82.67±3.33	81.73±2.60	83.09±1.44	86.40±3.50

Table S3. Antimicrobial effects of isolates extracted with ethyle-acetate from ferment broth

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
26975	92.02±1.73	93.34±2.83	94.04±3.23	93.42±2.77	94.81±1.77	84.30±1.70
26976	95.95±2.18	91.98±1.65	93.80±2.17	92.36±1.90	93.83±2.13	83.59±2.13
26977	96.58±0.81	89.47±1.14	90.89±1.27	94.08±2.35	90.35±0.67	83.16±3.06
26978	94.95±2.94	89.78±0.41	90.92±1.91	84.12±2.18	94.29±0.98	80.42±1.00
26979	91.78±1.58	88.16±1.65	93.31±1.71	87.42±1.97	93.37±2.31	83.65±3.48
26980	85.97±3.26	92.55±1.64	92.70±2.01	88.58±1.13	93.91±0.99	75.85±3.85
26981	93.89±1.58	92.58±1.49	93.54±2.35	90.98±2.14	93.39±2.20	81.52±1.99

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
26982	96.98±1.54	86.88±1.98	85.89±2.82	87.47±2.22	92.17±2.07	81.87±1.43
26983	94.06±2.03	91.04±0.53	94.34±2.92	92.14±1.36	66.93±4.13	46.37±6.00
26984	91.97±1.88	83.50±3.97	90.90±1.58	85.88±1.17	86.79±2.00	86.03±3.14
26985	94.44±1.78	87.16±2.31	92.59±2.51	89.22±1.29	92.97±1.41	86.75±0.95
26986	94.19±1.99	84.87±3.86	93.96±0.85	92.81±2.41	93.38±2.75	90.38±1.14
26987	83.62±2.24	84.88±1.72	90.11±1.45	87.22±2.33	92.78±1.60	92.50±1.22
26988	92.96±3.54	93.00±2.55	82.08±2.44	91.64±2.75	88.83±0.77	79.63±2.38
26989	97.09±0.54	94.24±2.64	93.44±2.74	92.42±2.14	86.10±3.31	89.55±1.48
26990	93.53±3.08	86.02±2.63	94.14±3.32	91.41±1.77	94.00±2.56	91.35±1.10
26991	92.25±2.49	92.32±1.34	95.17±1.40	92.58±2.44	91.31±2.47	91.37±2.17
26992	96.14±0.86	92.89±2.13	92.59±2.50	88.95±7.42	92.99±1.58	88.48±0.99
26993	87.75±1.51	92.60±1.45	88.65±2.49	93.52±2.02	90.44±1.62	87.16±2.72
27024	89.70±0.68	87.43±2.71	92.41±2.47	92.36±1.36	92.64±2.97	86.79±3.59
27025	93.63±1.71	91.71±0.96	96.32±1.46	92.42±2.46	92.45±1.93	91.99±1.53
26996	96.23±3.50	92.63±2.29	95.27±4.21	92.98±2.29	93.14±1.72	87.70±3.02
26997	95.73±2.45	92.93±1.75	96.79±2.02	38.10±6/87	93.03±2.56	90.32±3.07
26998	96.54±0.86	74.98±3.70	90.85±1.39	92.42±1.75	89.72±1.68	89.03±1.41
26999	95.13±1.51	87.35±2.76	96.11±1.51	91.99±1.75	93.81±2.14	92.15±1.06
27000	92.21±0.68	92.12±2.48	96.66±1.93	90.42±4.25	93.25±2.09	90.27±2.65
27001	97.76±1.71	87.63±4.75	96.55±2.10	92.31±2.14	95.05±1.27	90.55±0.58
27002	91.49±3.50	92.16±2.32	87.32±2.01	89.64±3.08	92.07±1.71	92.72±1.46
27003	94.55±2.45	93.09±2.51	91.81±2.81	90.88±3.26	92.14±1.63	89.80±1.86
27004	96.69±0.86	88.04±5.52	96.14±2.69	90.25±2.64	94.78±1.84	85.77±1.46
27005	88.97±2.48	91.74±2.81	93.62±3.34	90.49±1.31	75.85±1.35	74.44±1.86
27021	94.09±2.24	87.77±5.31	97.58±1.15	90.46±2.31	92.87±1.19	88.14±5.59
27033	97.71±2.21	85.12±3.43	95.82±2.64	90.91±1.84	89.13±1.25	92.08±3.70
27036	96.01±2.76	91.68±1.53	96.70±1.60	92.54±1.34	93.10±2.13	92.21±4.48
27034	95.25±1.76	91.92±1.49	93.30±3.57	88.07±3.73	94.38±1.08	93.38±1.66
27006	63.76±1.35	91.72±1.96	96.12±2.17	93.27±2.67	92.18±2.16	93.00±0.80
27012	92.51±0.87	90.32±2.66	90.91±1.35	91.71±3.79	93.27±2.42	86.81±2.34
27035	91.47±1.80	81.72±2.04	95.98±1.54	85.90±3.38	96.35±1.50	84.48±0.99
27007	96.83±1.58	93.01±1.20	95.15±2.49	90.18±2.54	93.07±1.58	83.35±2.75
27041	92.82±3.44	87.80±2.04	97.05±1.46	91.39±1.47	94.34±2.53	86.26±2.56
27037	92.81±2.52	91.94±1.26	94.54±3.33	92.57±2.44	93.49±1.50	87.68±2.16
27038	92.45±1.96	93.13±1.57	90.24±1.29	85.39±3.57	95.52±1.79	86.73±2.81
27039	92.92±3.26	89.07±1.44	94.63±3.35	87.59±2.27	93.46±2.01	85.17±2.38
27016	89.39±3.07	88.57±2.85	91.31±2.38	86.09±2.83	92.17±5.76	90.53±1.44
27017	78.19±2.59	86.36±3.09	91.79±2.84	69.97±4.80	94.33±1.52	92.02±1.71
27018	93.86±2.43	93.21±2.72	92.21±3.26	90.67±1.61	89.23±3.45	85.67±3.05
27019	89.74±3.14	78.07±2.39	92.54±1.87	93.23±2.36	95.62±1.46	91.22±2.00
26647	93.61±2.09	90.96±4.10	82.10±2.37	92.51±3.12	92.03±3.18	84.55±4.85
26648	93.34±2.18	90.15±1.59	83.99±4.08	92.78±2.20	90.50±4.66	84.71±3.96
26649	92.24±2.01	90.62±2.46	84.66±4.17	91.16±2.51	88.25±5.56	83.29±4.48
26661	92.61±5.17	91.79±1.62	84.36±9.46	92.68±3.30	93.02±3.32	83.52±4.19
26660	93.08±3.05	91.48±2.98	89.56±0.99	80.61±2.67	90.56±0.74	89.17±3.39
26650	92.92±4.63	90.64±3.32	83.67±6.67	91.08±2.13	91.55±1.19	87.38±4.07
26651	91.51±4.84	91.18±1.58	85.69±3.69	91.16±1.10	90.53±2.71	82.24±2.59
26652	92.26±5.21	91.50±1.04	82.26±2.63	93.69±2.99	88.70±1.62	87.28±2.37
26653	93.63±2.93	89.23±5.05	92.41±2.57	92.75±2.36	92.21±1.99	86.13±3.94

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
26654	92.95±3.85	88.89±6.84	86.31±3.49	83.07±2.69	90.99±2.20	89.40±1.01
26655	84.69±4.26	80.66±1.75	82.41±4.39	82.75±3.35	85.51±3.24	87.56±3.14
26656	91.80±3.66	87.20±3.19	82.62±8.15	92.36±2.79	94.19±2.48	85.27±0.62
26657	93.57±3.75	91.56±3.29	86.42±6.24	80.71±2.27	90.47±1.83	86.01±3.77
26658	91.99±4.22	88.71± 3.70	76.95±3.12	93.09±1.82	91.84±2.48	87.78±2.04
26659	94.84±2.46	92.34±2.07	84.30±4.67	88.26±3.57	90.59±0.87	88.76±4.78

Table S4. Antimicrobial effects of isolates extracted with chloroform/MeOH from mycelia

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
26975	85.97±2.14	62.31±5.28	75.82±3.83	46.17±3.99	82.46±3.61	45.45±3.99
26976	87.73±2.73	61.51±6.29	91.10±2.31	73.18±2.12	84.92±2.91	50.81±4.87
26977	74.62±5.11	73.33±4.04	94.60±1.81	75.07±4.11	75.46±4.49	82.56±2.02
26978	89.54±2.31	67.24±11.5	88.38±1.36	76.01±5.82	87.15±2.59	81.94±4.40
26979	91.29±0.95	51.40±9.68	85.29±4.23	69.33±0.94	91.70±1.42	77.06±2.94
26980	84.93±2.61	72.69±6.07	71.05±3.06	64.03±2.96	90.45±1.99	77.58±3.35
26981	84.60±3.68	67.01±4.27	65.81±3.67	54.99±3.40	88.26±2.69	73.79±3.70
26982	79.71±1.49	32.58±17.1	86.49±3.10	61.85±1.31	70.65±7.81	83.50±1.31
26983	84.17±2.91	43.05±10.9	78.87±2.19	60.81±1.16	87.20±2.53	91.15±1.45
26984	86.54±2.76	54.19±3.83	84.53±2.91	58.15±2.50	91.39±1.65	88.18±3.02
26985	89.43±3.96	46.36±3.17	80.98±3.97	64.55±4.01	87.20±0.99	86.36±6.67
26986	91.52±3.06	50.50±0.69	56.54±5.59	65.40±2.74	92.06±1.24	84.67±7.77
26987	93.12±2.55	57.79±9.36	75.72±4.88	61.42±3.18	85.80±1.45	91.61±2.31
26988	75.93±3.54	60.64±6.69	66.56±3.12	60.03±3.31	75.89±3.07	90.25±4.48
26989	87.83±2.15	58.28±11.6	86.44±3.60	68.24±4.84	85.40±3.73	72.94±4.28
26990	63.19±11.2	48.58±6.69	90.15±1.06	63.70±4.84	61.95±5.75	91.52±2.83
26991	82.47±1.87	42.73±2.67	90.47±1.80	66.41±7.16	86.71±2.31	83.95±2.88
±26992	85.27±.4.30	62.64±7.95	92.27±2.66	69.50±4.75	83.97±1.43	83.19±1.69
26993	76.01±3.06	46.17±3.33	91.73±2.35	77.39±4.33	65.02±5.77	75.13±4.35
±27024	75.27±3.80	42.07±2.17	86.17±3.32	64.59±1.99	78.94±2.87	59.70±4.84
27025	60.88±22.9	65.91±2.51	90.97±1.55	65.79±3.47	84.40±4.95	48.20±10.7
26996	85.62±.4.32	27.55±4.91	87.13±2.34	65.17±3.07	87.40±2.06	46.57±5.59
26997	91.28±6.25	47.97±5.29	85.80±2.48	74.10±5.68	85.82±1.06	57.18±4.20
26998	86.09±4.18	66.08±3.46	67.38±4.63	64.81±2.39	85.30±1.97	50.37±2.79
26999	91.05±1.65	47.12±4.24	81.28±5.67	65.85±4.31	82.48±3.03	53.91±3.12
27000	90.99±1.64	60.50±4.49	85.59±3.60	59.31±0.97	86.22±2.96	55.01±2.01
27001	88.70±2.70	39.58±2.45	81.05±2.81	63.02±3.68	82.65±4.17	51.36±1.78
27002	66.81±5.80	46.04±2.65	85.47±1.32	81.32±2.63	76.99±2.52	57.19±8.35
27003	86.22±2.54	49.88±5.72	71.55±2.33	56.23±1.95	89.04±1.44	54.29±1.50
27004	88.25±1.62	40.72±9.65	76.75±8.26	66.77±2.91	86.25±1.52	55.96±4.27
27005	87.08±2.02	55.31±5.14	72.95±6.76	61.55±2.15	82.93±4.09	53.14±2.94
27021	91.36±1.15	59.69±7.67	65.73±5.26	77.17±3.13	87.70±1.71	54.66±3.84
27033	92.27±1.70	66.25±5.35	75.64±4.74	63.36±1.71	86.58±0.97	52.75±3.17
27036	87.44±2.47	58.67±8.86	76.06±4.40	75.28±4.18	87.30±2.22	54.89±2.62
27034	89.27±1.06	63.34±2.69	74.03±4.73	61.42±2.66	85.37±5.50	54.99±1.01
27006	83.85±1.35	36.23±4.99	70.54±0.64	64.28±4.10	83.36±4.45	63.24±5.03
27012	86.26±0.89	54.54±1.42	68.68±1.11	66.11±3.33	86.12±3.53	48.27±3.52

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
27035	85.65±3.04	45.38±3.19	68.81±4.32	56.48±2.88	84.70±3.04	53.60±0.78
27007	86.92±1.40	68.72±1.97	76.40±3.57	56.58±1.52	90.28±4.22	60.44±2.82
27041	95.91±3.12	85.45±3.53	83.37±3.36	81.37±2.97	93.29±0.88	85.73±3.69
27037	85.79±1.33	72.71±3.78	79.73±4.22	55.39±3.19	90.78±2.19	56.45±2.47
27038	89.95±1.67	59.28±5.16	73.90±1.03	74.28±3.00	85.51±2.61	54.81±5.34
27039	93.77±2.68	68.13±4.14	76.21±3.20	74.04±5.30	91.50±4.12	59.03±1.06
27016	90.84±3.00	55.12±5.19	69.68±1.48	64.47±4.22	82.17±2.91	52.95±2.38
27017	71.80±1.66	66.40±7.04	64.80±4.01	58.86±4.03	80.15±2.86	51.63±2.62
27018	88.02±1.31	54.70±5.44	66.14±4.67	64.36±1.95	85.80±2.62	59.60±3.05
27019	88.58±1.81	54.98±4.71	72.23±2.80	75.29±4.29	87.11±2.05	55.65±4.41
26647	86.14±1.02	88.56±6.06	93.39±2.93	84.81±4.44	92.02±1.91	91.98±1.85
26648	83.91±1.36	74.84±2.69	74.22±5.24	79.90±4.68	79.62±2.82	74.34±2.02
26649	90.65±1.82	72.95±5.06	91.98±2.97	76.26±3.74	89.06±2.54	74.06±2.34
26661	92.09±1.06	75.65±4.30	88.70±5.54	75.21±3.12	79.71±1.59	82.41±4.17
26660	88.16±1.53	69.89±3.56	89.21±2.64	77.77±3.63	71.92±3.88	81.58±0.90
26650	92.13± 1.31	77.95±3.58	91.85±1.79	78.28±4.48	74.72±2.82	82.61±2.53
26651	92.33±1.36	73.86±7.82	88.20±4.54	76.25±2.65	74.30±1.37	77.15±9.85
26652	88.69±1.82	68.51±4.56	75.68±3.56	80.22±3.65	82.08±2.82	83.81±1.60
26653	88.28±1.06	71.04±8.97	88.72±3.49	74.11±4.12	70.71±4.17	76.32±2.05
26654	93.15±1.53	74.60±4.40	77.86±2.10	77.69±4.89	83.08±2.39	84.60±3.45
26655	91.75±1.21	80.87±4.30	87.41±4.30	76.05±3.76	80.57±3.39	78.71±3.77
26656	87.63±2.27	73.19±5.45	78.02±1.74	72.81±4.83	79.84±3.29	83.98± 1.49
26657	90.69±1.77	72.13±6.90	73.20±3.28	74.48±3.02	80.14±3.05	80.57±3.74
26658	92.86±1.42	74.34±4.48	83.46±6.40	75.95±5.52	74.17±2.46	83.29± 4.38
26659	85.54±3.99	70.57±2.89	82.76±4.80	76.54±4.20	82.83±2.17	78.23±2.34

Table S5. Antimicrobial effects of isolates extracted with hexane from mycelia

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
26975	80.30±3.13	31.94±8.95	84.14±1.58	26.99±3.00	57.21±2.20	26.96±6.47
26976	77.51±9.10	26.25±9.02	85.15±1.95	46.87±2.82	70.28±1.98	49.24±3.25
26977	75.91±3.81	62.10±3.53	38.04±4.34	66.10±4.87	75.02±3.87	62.62±2.66
26978	74.05±1.25	31.77±7.10	45.90±6.62	28.16±3.21	49.26±1.96	32.41±4.76
26979	73.66±1.78	47.57±4.12	88.67±2.09	63.35±6.61	46.35±3.25	51.82±3.63
26980	78.86±1.67	33.51±19.2	74.26±4.76	26.77±2.43	82.84±2.42	26.36±15.5
26981	71.12±2.26	38.41±13.7	86.43±3.84	35.57±2.82	77.25±1.96	27.47±7.94
26982	63.36±9.28	51.55±7.15	43.95±4.25	36.41±1.76	66.99±4.04	31.77±6.85
26983	62.18±10.0	32.36±17.0	70.47±3.39	28.51±2.42	90.31±0.86	41.64±7.68
26984	71.74±4.09	48.79±4.83	64.46±11.5	45.61±5.92	87.70±2.29	25.64±5.99
26985	66.31±9.95	33.92±12.1	43.75±4.43	58.54±2.07	86.74±3.13	38.72±4.32
26986	74.67±4.86	35.42±4.73	57.24±6.21	59.88±3.82	86.45±2.66	51.86±2.62
26987	70.46±3.51	31.24±5.64	46.76±5.38	37.34±4.22	83.37±2.59	52.70±3.90
26988	75.54±4.03	37.43±2.26	47.54±4.32	44.45±6.24	86.75±2.51	53.94±10.6
26989	74.22±7.39	22.92±6.12	55.65±4.01	37.08±3.96	86.48±4.85	49.23±3.42
26990	68.80±3.33	34.93±4.18	56.84±4.49	59.32±4.15	83.37±3.03	52.60±2.74
26991	70.02±13.2	56.66±4.93	59.61±3.73	54.87±4.58	86.25±2.37	54.61±7.47
26992	73.26±2.87	39.42±6.54	93.89±13.8	57.41±2.35	85.13±3.73	52.54±2.61

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
26993	74.18±7.26	43.65±6.08	46.05±6.80	43.18±4.63	84.17±1.94	52.63±7.30
27024	64.53±5.69	31.25±13.6	47.56±4.31	50.50±2.77	86.56±1.25	51.88±2.37
27025	74.40±2.58	58.86±5.64	93.41±4.34	61.79±5.78	85.35±3.35	56.87±3.32
26996	70.33±3.31	48.87±4.86	68.57±3.92	87.10±2.17	90.84±1.40	89.09±1.35
26997	85.40±3.30	69.23±2.95	75.41±11.3	37.69±1.21	86.64±1.07	85.24±4.48
26998	80.82±3.10	36.42±7.74	48.12±8.30	43.35±2.59	84.29±3.30	30.69±11.5
26999	70.36±3.84	45.90±2.96	40.02±8.26	26.19±4.67	87.33±2.64	49.79±4.94
27000	75.42±5.51	73.23±5.99	58.73±6.05	74.43±3.83	84.41±2.85	50.26±5.20
27001	69.90±3.43	49.90±6.78	63.23±2.28	25.32±1.85	86.97±1.82	46.01±4.80
27002	64.69±9.09	34.87±4.51	91.98±5.66	37.64±0.74	83.33±1.11	39.68±3.56
27003	83.95±0.85	35.53±13.8	52.76±6.74	26.66±1.54	86.71±1.96	38.48±7.3
27004	74.12±3.44	29.91±9.99	56.13±3.84	48.96±3.01	83.72±3.34	39.17±5.29
27005	68.21±4.14	35.54±10.1	55.25±3.85	56.34±2.02	83.59±4.43	47.24±3.93
27021	74.20±1.01	31.62±8.57	39.96±6.19	33.02±3.92	74.77±2.78	49.74±4.47
27033	67.04±9.52	38.84±4.62	63.87±5.70	35.58±4.27	80.00±1.42	28.03±15.6
27036	76.01±2.71	70.77±5.66	46.84±3.04	57.87±1.18	89.01±2.43	60.39±3.49
27034	71.26±5.70	37.71±5.19	76.85±4.11	44.90±2.37	83.90±1.89	43.87±5.68
27006	75.14±6.99	27.88±4.32	53.54±3.10	35.48±3.57	87.36±1.78	36.18±3.70
27012	68.00±4.64	34.86±4.39	76.39±1.44	44.80±4.26	87.06±0.88	23.50±6.13
27035	66.20±3.36	52.03±3.22	57.22±2.34	36.35±1.80	80.37±2.50	29.35±4.49
27007	85.96±3.12	43.47±5.11	87.49±1.28	66.72±3.89	85.85±2.4	52.93±2.07
27041	88.21±1.08	86.65±3.17	86.37±1.28	84.80±3.88	87.31±1.59	82.03±2.37
27037	72.07±2.66	46.77±2.62	88.54±2.71	43.56±3.87	70.47±4.80	37.30±5.45
27038	74.34±4.32	61.89±5.69	86.98±1.08	34.47±3.79	57.66±4.51	26.44±13.7
27039	75.61±3.40	40.54±5.17	87.99±2.53	45.07±3.86	69.11±4.54	33.85±7.51
27016	76.79±4.18	44.75±3.35	79.31±5.45	42.36±6.75	66.05±4.11	46.76±3.58
27017	66.66±6.00	35.37±2.62	85.47±2.00	33.20±6.87	68.85±5.75	30.15±6.90
27018	72.82±2.69	34.80±5.28	85.74±2.81	61.66±6.08	71.35±5.58	32.61±3.66
27019	69.86±3.94	33.05±6.56	87.67±1.85	41.26±1.40	69.57±9.00	34.67±7.24
26647	63.33±2.23	48.13±0.50	83.09±2.71	64.23±2.97	92.08±2.46	60.61±4.90
26648	65.77±4.07	48.05±3.20	85.02±3.42	67.32±2.69	87.03±3.23	57.06± 5.34
26649	71.26±0.77	51.76±3.49	82.84±2.33	68.62±0.34	84.38±3.38	63.94± 6.36
26661	68.00±1.50	53.29±4.04	75.32±4.30	65.24±1.80	84.46±1.72	60.57± 5.34
26660	70.01±3.14	52.44±4.36	81.75±2.76	68.37±2.84	83.21±3.08	69.35± 2.59
26650	71.79±1.14	52.55±1.72	85.16±3.23	73.25±6.04	81.26±4.58	56.81± 2.67
26651	71.33±1.72	49.49±3.82	85.25±2.49	66.43±2.65	85.26±2.25	62.58±6.68
26652	70.78±2.53	49.37±4.34	82.28±7.10	69.38±2.56	83.6±2.85	69.63±4.07
26653	77.82±3.39	80.64±4.55	81.94±3.03	82.90±3.60	83.19±2.16	80.46±2.60
26654	82.16±1.70	79.17±8.34	80.80±3.70	74.38±3.59	85.54±2.25	87.21±7.27
26655	74.75±4.18	57.32±1.87	80.81±2.70	64.28±12.6	84.29±3.42	60.64±2.61
26656	70.78±8.07	62.31±4.50	84.15±3.76	67.81±10.7	84.15±2.76	64.49±7.17
26657	67.96±1.33	57.55±4.66	77.78±6.23	68.14±3.18	85.92±4.03	56.74±5.48
26658	80.13±7.04	59.06±10.9	85.01±1.95	68.07±1.10	91.48±2.58	55.35±4.89
26659	70.13±2.11	62.05± 8.44	80.10±3.21	75.01±4.04	83.91±2.94	63.47±5.85

Table S6. Antimicrobial effects of isolates extracted with ethyle-acetate from mycelia

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
26975	76.03±2.72	34.00±7.24	96.18±2.50	76.46±1.42	75.72±3.68	64.89±3.50
26976	76.91±1.90	44.90±2.83	84.99±2.71	77.62±1.94	79.22±0.98	71.62±1.63
26977	61.75±3.73	42.35±4.73	84.20±3.29	75.34±6.22	87.50±1.95	31.37±5.99
26978	73.46±1.75	90.46±2.85	94.82±3.00	77.91±5.57	81.53±2.72	41.55±3.22
26979	75.85±2.91	91.11±1.92	85.34±2.76	74.86±4.10	86.78±3.02	50.83±4.07
26980	69.64±1.94	68.46±4.39	71.42±7.60	78.14±3.25	79.19±5.26	55.59±5.82
26981	72.36±1.95	91.58±2.57	87.11±2.88	51.09±4.32	82.24±3.71	52.61±6.25
26982	82.13±1.93	91.27±1.05	96.48±2.73	77.35±1.80	78.75±2.99	71.42±3.45
26983	77.37±1.82	93.20±1.64	94.24±4.17	88.33±1.20	89.85±3.33	73.76±7.23
26984	72.63±2.18	92.43±2.37	83.87±5.19	74.63±4.18	77.73±5.99	72.01±6.97
26985	95.26±2.67	91.74±1.75	86.02±3.43	78.28±0.92	79.65±4.40	53.58±5.21
26986	92.57±2.15	93.35±4.63	92.95±3.76	69.07±1.85	83.89±1.45	92.03±2.15
26987	67.82±7.02	44.08±3.23	81.22±2.93	74.64±3.55	75.34±2.11	73.18±6.81
26988	95.46±2.34	69.97±5.81	63.37±6.05	73.54±2.31	84.94±2.74	90.92±2.05
26989	85.73±0.98	35.21±1.97	85.26±4.81	78.31±1.37	74.91±4.54	74.75±5.66
26990	92.66±3.02	91.52±0.89	94.90±4.51	70.92±2.10	67.20±2.53	41.85±2.94
26991	95.97±1.75	92.14±3.89	96.19±2.32	86.27±3.54	73.58±2.28	89.13±2.71
26992	85.54±3.11	84.35±1.47	84.43±4.92	85.43±2.75	92.48±2.06	91.06±1.73
26993	94.52±1.74	91.83±4.07	94.81±4.51	76.51±2.35	87.46±2.01	91.79±1.29
27024	85.56±3.67	64.72±2.54	93.74±4.28	75.79±4.76	76.99±2.30	64.29±4.95
27025	86.82±2.83	92.56±6.67	86.07±2.49	78.67±1.47	86.00±3.48	89.71±3.59
26996	82.74±2.45	53.73±6.03	89.97±1.70	76.08±3.01	76.10±3.39	75.46±2.65
26997	82.99±2.54	84.22±0.90	48.62±3.41	76.91±2.90	76.22±3.03	76.26±3.85
26998	92.13±1.87	91.34±8.27	86.14±2.21	61.69±3.48	77.66±3.55	50.45±8.51
26999	63.47±6.37	73.25±5.44	90.77±4.88	72.23±3.86	51.88±3.06	59.68±5.29
27000	85.23±1.62	79.01±13.8	91.05±4.99	73.55±1.19	75.27±3.42	71.41±2.83
27001	81.84±1.66	33.28±8.97	93.58±4.57	78.58±1.93	75.69±5.39	74.45±2.56
27002	85.40±4.25	36.23±1.70	63.90±4.13	81.01±3.53	52.32±3.99	74.39±2.74
27003	93.71±3.02	92.24±3.08	94.45±4.27	81.69±3.29	75.65±3.79	47.03±4.65
27004	93.03±3.73	90.51±2.53	94.63±4.16	88.21±2.83	85.82±3.49	89.71±2.73
27005	84.24±4.88	73.99±4.00	93.89±1.87	84.73±5.57	56.13±2.49	55.78±5.16
27021	81.79±1.63	73.37±3.62	94.92±6.34	84.21±5.77	76.37±1.15	70.67±2.48
27033	93.44±2.69	83.39±2.59	57.92±3.50	93.16±2.95	94.10±3.06	87.82±4.93
27036	84.92±4.24	83.03±0.97	68.19±6.96	78.20±11.3	65.01±3.29	86.66±3.19
27034	85.29±3.90	90.68±3.86	79.05±4.21	80.28±5.64	75.88±3.09	84.26±3.69
27006	78.37±2.88	74.10±1.85	83.14±3.35	80.49±3.31	66.04±2.87	83.98±4.10
27012	92.48±4.79	92.44±7.61	75.09±3.27	82.65±3.76	77.80±3.89	72.33±4.25
27035	82.85±1.23	62.97±3.26	74.78±0.60	83.15±4.91	76.29±3.42	63.62±6.79
27007	68.03±5.73	83.97±3.23	89.01±2.43	72.32±4.50	86.20±2.3	54.35±4.62
27041	84.26±4.43	83.77±5.02	88.79±0.60	83.60±6.32	84.41±4.66	86.11±1.75
27037	81.47±2.59	81.11±2.16	88.18±2.49	78.58±6.08	80.92±3.23	55.62±4.98
27038	84.92±3.30	67.75±5.29	84.66±4.25	78.19±2.90	83.76±5.46	60.49±3.92
27039	84.69±4.61	63.91±4.69	83.29±2.74	80.42±2.99	80.16±5.48	73.45±4.82
27016	86.41±2.56	68.37±3.74	89.56±0.63	77.53±5.06	80.59±5.78	76.77±2.19
27017	92.76±4.45	92.86±3.00	83.70±4.54	75.27±3.20	75.56±3.43	88.98±3.37
27018	95.03±3.43	91.50±3.01	86.27±4.84	76.87±4.17	64.08±5.43	53.77±6.77
27019	83.96±1.29	65.67±4.04	87.33±2.62	79.88±4.58	93.77±2.09	66.35±2.86

SZMC number	Inhibition rate (%)					
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. albus*	*M. luteus*
26647	65.91±4.01	70.10±3.38	82.65±1.85	74.61±3.57	87.20±4.12	70.57±2.28
26648	78.56±1.06	88.83±2.13	82.72±3.15	71.89±1.05	88.10±3.22	78.01±1.29
26649	89.36±0.97	92.56±2.14	85.30±2.19	74.95±3.55	86.68±4.54	85.06±6.08
26661	73.22±6.34	84.64±3.88	85.64±3.54	68.93±4.28	84.02±1.95	71.29±4.74
26660	93.40±5.46	73.5±1.35	92.05±3.37	72.69±2.00	86.14±3.56	68.84±4.43
26650	92.66±5.13	88.28±6.37	84.29±2.45	75.65±4.85	84.39±2.64	85.64±2.14
26651	93.46±4.57	64.75±9.40	66.39±5.60	74.80±4.06	70.49±2.72	68.92±2.46
26652	91.10±0.91	76.40±3.23	80.65±0.38	66.61±3.89	86.61±3.40	84.79±3.97
26653	86.51±3.57	66.31±11.9	84.43±3.40	73.42±5.69	86.24±4.56	68.67±2.99
26654	94.21±4.40	84.96±2.31	75.84±3.50	65.67±4.04	81.00±3.04	66.27±2.22
26655	83.32±9.35	90.92±1.60	85.08±3.70	64.10±8.96	92.02±1.33	81.37±0.70
26656	80.82±1.21	84.98±4.49	81.31±3.57	79.22±2.13	84.23±3.52	64.69± 3.26
26657	85.02±3.93	70.59±7.43	85.43±4.21	73.93±4.50	84.50±4.33	62.74±10.4
26658	74.11±8.26	88.39±3.52	85.10±3.14	74.53±4.51	85.25±2.74	84.44±2.01
26659	66.90±4.43	83.58±3.60	84.18±4.66	74.44±4.61	85.90±3.87	84.69±1.82

Table S7. Results of anti-fungal assay of the fungal extracts against *Candida albicans* and *Aspergillus niger* (results shown only where inhibition was detected).

SZMC number	*Candida albicans* (mm)						*Aspergillus niger* (mm)					
	HB	HM	EB	EM	CB	CM	HB	HM	EB	EM	CB	CM
26654								6	12	9	3	
26655					9	4						
26656	11				14		4					
26657					11			7			9	
26659				14								
26653					9	8						
26649				23		22		8		18		
26661			17									
26660			15						17			
26650		7						5			13	
26651					11						14	9
26647									18			
26648		9	14	15				5	11			
26975	10	11										
26976	9				9							
26977	8	9	14	12					18			
26978		5	6			4						
26979		3	3		6	7					9	
26980			5	8								
26982					7						7	
26983					5							
26984					8							
26985				7						9		
26986				11						5		
26987												3
27024			6									2
27025				5	2					5	7	
26998											13	
27001				8	7						8	
27002				4							5	
27003											7	
27021						5						
27035							6					
27038			3									

27016					8							
27017											6	

Table S8. Results of anti-fungal assay of the fungal extracts against *Rhizoctonia solani* and *Fusarium culmorum* (results shown only where inhibition was detected).

SZMC number	***Rhizoctonia solani* (mm)**						***Fusarium culmorum* (mm)**					
	HB	**HM**	**EB**	**EM**	**CB**	**CM**	**HB**	**HM**	**EB**	**EM**	**CB**	**CM**
26654						4						
26656			2	5	8							
26657											5	
26658										3		
26652					11	9						
26653					14	11						
26649					6	15				6		
26661					9	9						
26660				13	13							
26650				11		9		4				
26651				5	7							
26647				3	6							
26648				5	3	14				9		3
26976					19	11						
26977		3			17	8					7	
26978					9							
26979						11	14				3	
26980											9	
26981				16								
26983				18								
26984				13	11	9						6
26987										9		
26988										17		
26993				14								
27025					3					6		
26996										3	9	
27003										13		

Table S9. Siderophore detection, phosphate solubilization and IAA detection of the fungal endophytes by agar plate assay.

SZMC number	Plant growth-promoting plate assays			
	Siderophore (mm)	Phosphate (mm)	IAA	
			Trp+	Trp-
26654			+	
26655	18.83 ± 1.26		+	+
26656	26.00 ± 3.61		+	+
26657		40.33 ± 1.53	+	
26658	32.00 ± 3.62		+	+
26659	21.33 ± 2.08	38.83 ± 1.89	+	+
26652	24.33 ± 2.52		+	
26653			+	+
26649	25.17 ± 1.76		+	+
26661	19.33 ± 1.53	31.00 ± 2.00	+	
26660	21.33 ± 1.53		+	
26650	17.33 ± 1.53		+	
26651	27.00 ± 2.00	38.67 ± 1.53	+	+
26647	14.83 ± 2.25		+	+
26648	25.00 ± 2.65	34.67 ± 2.52	+	
26975	13.67 ± 2.08	22.33 ± 1.15		+
26976	20.33 ± 2.08	32.67 ± 1.53	+	+
26977	13.67 ± 2.08	9.33 ± 1.53	+	
26978		10.67 ± 0.58		+
26979	9.33 ± 1.53	4.00 ± 1.00	+	
26980			+	+
26981	14.67 ± 1.53	6.67 ± 0.58	+	
26982		6.33 ± 2.31	+	+
26983	24.67 ± 2.52	8.33 ± 0.58	+	
26984	29.33 ± 1.53	13.00 ± 1.00	+	
26985			+	+
26986		15.33 ± 0.58	+	
26987		14.67 ± 2.08	+	
26988			+	
26989	14.67 ± 2.08		+	+
26990			+	
26991	29.33 ± 1.53	19.67 ± 1.53	+	
26992		13.67 ± 0.58	+	+
26993	12.00 ± 1.00	17.00 ± 1.73	+	+
27024	20.00 ± 1.00	13.33 ± 1.53	+	
27025		22.67 ± 1.15	+	
26996	24.00 ± 1.00		+	+
26997		23.67 ± 2.52	+	
26998	18.00 ± 1.00			+
26999			+	
27000			+	
27001			+	
27002	24.00 ± 2.00	32.33 ± 2.08	+	+

SZMC number	Plant growth-promoting plate assays			
	Siderophore (mm)	Phosphate (mm)	IAA	
			Trp+	Trp-
27003		28.33 ± 0.58	+	
27004	12.33 ± 1.53		+	
27005			+	
27021	10.00 ± 1.00		+	
27033			+	+
27036		31.67 ± 0.58		+
27034	24.00 ± 2.00	36.67 ± 1.53	+	+
27006	20.33 ± 1.15	33.00 ± 2.00	+	+
27012		13.00 ± 2.00	+	+
27035		7.67 ± 1.53		+
27007		4.67 ± 1.53	+	+
27041		28.00 ± 1.00	+	+
27037	20.67 ± 0.58	24.33 ± 1.15	+	+
27038	16.00 ± 1.00	7.67 ± 1.53	+	+
27039		34.33 ± 3.06	+	
27016			+	
27017	24.33 ± 3.06	8.00 ± 1.73	+	
27018		12.67 ± 2.09	+	
27019	30.00 ± 1.00		+	+