IDENTIFICATION AND ISOLATION OF ENDOPHYTIC FUNGI PRODUCING L-ASPARAGINASE IN REPRESENTATIVES OF THE ASTERATCEA FAMILY

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https://doi.org/10.5281/zenodo.7643932

Abstract. *L-asparaginase is an important anticancer enzyme used in the first-line treatment of acute lymphoblastic leukemia. This study was conducted to isolate L asparaginase-producing endophytic fungi from medicinal plants of the Asteraceae family. Seven healthy medicinal plants from the Asteraceae family were selected for the isolation of endophytic fungi using standard surface sterilization methods. A total of 837 isolates belonging to 84 species consisted of stem (55.6%), leaf (31.1%), root (10.6%) and flower (2.7%). Initial screening of L-asparaginase-producing endophytes was performed by qualitative plate assay on modified Czapex dox agar medium. L-asparaginase-producing endophytes were identified as Plectosphaerella, Fusarium, Stemphylium, Septoria, Alternaria, Didymella, Phoma, Chaetosphaeronema, Sarocladium, Nemania, Epicoccum, Ulocladium and Cladosporium species. This study showed that endophytic fungi from members of Asteraceae have high L-asparaginase production potential and can be used as an alternative source for anticancer production.*

Keywords: *Plectosphaerella, Fusarium, Stemphylium, Septoria, Alternaria, Didymella, Phoma, Chaetosphaeronema, Sarocladium, Nemania, Epicoccum, Ulocladium va Cladosporium, BLAST, NCBI*

Introduction: Endophytes are microorganisms that live in plant tissues without causing any symptoms or obvious negative effects on other plants. Endophytic fungi from medicinal plants are a rich source of new natural products for medical and commercial use. The close symbiotic relationship between endophytic fungi and host plants gives endophytes the ability to produce new bioactive compounds, the production of which is supported by basic plant carbohydrates. These bioactive compounds increase plant resistance to pathogens and herbivores, increase competitiveness, and improve growth. Endophytic fungal bioactive metabolites may be useful as new drugs due to their diversity of biological activities. In recent years, endophytic fungi have been considered as a source of secondary metabolites, including anticancer, anti-inflammatory, antibiotic and antioxidant substances. Enzymes produced by microorganisms are used for medical and industrial purposes. L-asparaginase is one of the enzymes that hydrolyze asparagine into aspartic acid and ammonia. In the food industry, L-asparaginase enzymes are used as additives to reduce the acrylamide produced at high temperatures in starchy foods and reduce the risk of cancer. This enzyme is one of the most important biochemical therapeutic enzymes used in the treatment of various leukemias such as acute lymphoblastic leukemia in children. In cancer treatment, L-asparaginase removes L-

asparagine from the blood serum and deprives tumor cells of large amounts of asparagine needed for growth. Currently, Lasparaginase from Escherichia coli is the main source of L-asparaginase. However, side effects of this bacteria-derived enzyme include chills, fever, abdominal cramps, and fatal hyperthermia. Isolation of fungal endophytes based on seven medicinal plants, including: *Matricaria chamomilla, Matricaria parthenium, Athemis triumfetii, Anthemis altissima, Achillea millefolium, Achillea filipendulina, and Cichorium intybus,* in which L-asparaginase from eukaryotes can cause relatively less toxicity and decrease immunity. and were selected for screening for L-asparaginase activity.

MATERIALS AND METHODS

Isolation and identification of fungal endophytes

Identification was carried out using morphological and molecular methods. Molecular identification of morphological endophytic fungi Endophytic fungal isolates were grown in 200 ml potato dextrose broth (PDB) for 7 days at 28 ÿC. The mycelia were washed with distilled water and ground with liquid nitrogen. Nucleic acid was extracted using the cetyl trimethyl ammonium bromide (CTAB) method (Dayle et al., 2001). The strains were sequenced with four: molecular markers, including ITS (internal transcribed spacer), LSU (partial large subunit nrDNA), TEF1. was carried out without

BLAST analysis was performed on the NCBI database. All sequences have been deposited in NCBI's GenBank database.

Screening of L-asparaginase-producing endophytes The isolated endophytic fungi were screened for their ability to produce asparaginase.

RESULTS AND DISCUSSION

Endophytes were obtained from a total of 837 isolates from 200 leaf, stem and root segments from all seven medicinal plant species. Endophytes were mainly *A. altissima (241 isolates), followed by A. millefolium (163 isolates), A. triumphetii (121 isolates), C. intybus (132 isolates), A. filipendulina (90 isolates), M. chamomilla found. (59 isolates) and M. parthenium (31 isolates).* Due to the abundance of fungal endophytes, the isolates were divided into 84 morphotypes based on different morphological and cultural characteristics. Using morphological and molecular methods, 84 species of endophytic fungi belonging to *Ascomycota and Basidiomycota* were identified. Several endophytic fungi such as *Acremonium sclerotigenum, Alternaria burnsii, Bjerkandera adusta, Colletotrichum tanaceti, Epicoccum nigrum, Fusarium acuminatum, Paraphoma chrysanthemicola, Plectosphaerella cucumerina and Stemphylium* and abundant amaranths from common plants were present.

Most of the isolates belonged to the genera *Alternaria, Fusarium, Phoma, Chaetosphaeronema and Plectosphaerella, which colonized several plant parts. Fusarium* isolates from stems, leaves, flowers and roots, Phoma spp. obtained from stem and leaf sample. Tissue specificity was also observed for some endophytes. This was evident only in Septoria species found in stem tissue. Basidiomycetous endophytes such as *Trametes versicolor, Bjerkandera adusta, Trichaptum biforme and Schizophyllum commune* were isolated from stem tissues. *Fusarium spp. Stained* dominant endophytes with 140 isolates were found, followed by Alternaria spp. (105 isolates). The results showed that the species composition and frequency of endophyte species depended on tissue and plant type.

L-asparaginase activity was not observed in endophytes of *M. parthenium.* This may be due to the low diversity of endophytes obtained from this medicinal plant.

Although *S. tormentillae* showed pink zones in the agar assay, enzymatic activity was low based on subsequent quantitative assays. The reason for the absence of enzyme activity in the quantitative evaluation may be related to the differences in the ability of fungi to produce the enzyme in solid and liquid state. According to the available literature, this is the first record of *L-asparaginase* production by endophytic fungi of the host plants considered in this study.

CONCLUSIONS

Studies conducted here showed that the diversity of some endophytic fungal communities was influenced by host plants and tissues. We isolated many fungal endophytes from seven healthy medicinal plants. Endophytes capable of L-asparaginase production belonged to the genera *Plectosphaerella, Fusarium, Stemphylium, Septoria, Alternaria, Didymella, Phoma, Chaetosphaeronema, Sarocladium, Nemania, Epicoccum, and Ulocladium.*
Cladosporium. Fusarium proliferatum isolate was found to have the highest L-asparaginase enzyme activity. Our findings are consistent with the hypothesis that endophytes associated with medicinal plants have potential medicinal properties.

We found that the production of L-asparaginase by endophytic fungi could be an alternative source for this enzyme.

Further studies involving enzyme isolation are needed to prove the utility of *Lasparaginases* from fungal endophytes.

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