ISOLATION AND ECOPHYSIOLOGY OF ENDOPHYTIC FUNGI FROM *AMBROSIA ARTEMISIIFOLIA*

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Summary

Ambrosia artemisiifolia causes health and environmental problems. It can live without negative symptoms with endophytic fungi, which belong to plant pathogen genera. Fungi were isolated from symptomless *Ambrosia*'s roots and measured their extracellular enzymes. Our goals were to isolate and identify endophytic fungi from roots of common ragweed deriving from typical sandy habitat types of Southern Great Hungarian Plain: grassland, abandoned field and field. In addition we characterized the fungal isolates, measured their secreted enzymes: cellobiohydrolase, xylanase, β-1,3-glucanase, laccase, β-glucosidase, trypsin, chymotrypsin, cellulase, exochitinase, lipase and pectinase. Endophytic fungi were isolated from symptomless *Ambrosia* roots from different habitats in Szolnok and near Maros in 2010, Kiskundorozsma and Ásotthalom in 2011. Our data may contribute to the development of biocontrol techniques aiming the environmental friendly reduction of *Ambrosia artemisiifolia*.

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Introduction

Ambrosia artemisiifolia (Common ragweed) is a highly allergenic and invasive plant in Europe causing serious problems in human health. In our work, we report on the isolation of endophytic fungi from *Ambrosia artemisiifolia* and their extracellular enzyme secreting capabilities.

Sampling method and sampling places

Samples were collected randomly from different areas in Great Hungarian Plain of Hungary.

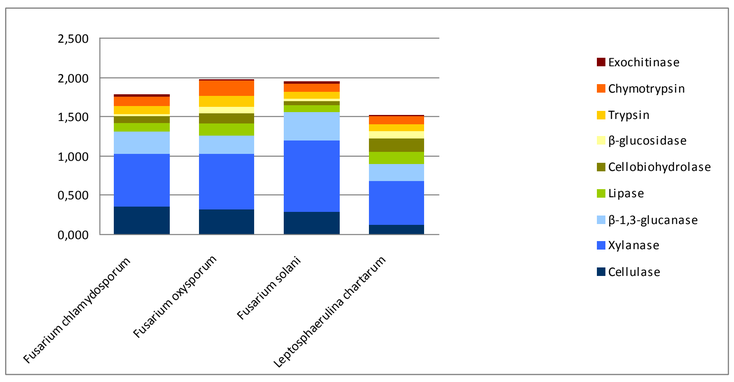
Roots were collected from three different habitats such as abandoned fields, lawns and fields, five replicates from each area.

In 2010 September from next to Maros near of Szeged from a natural habitat and at Szolnok from a degraded yard, and in 2011 May at Kiskundorozsma and at Ásotthalom.

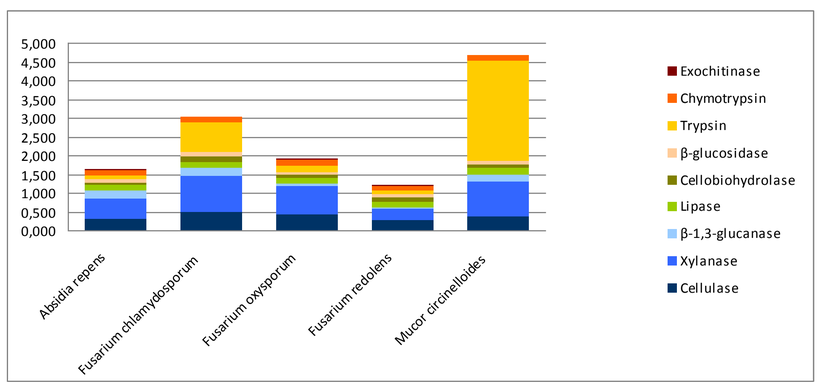
Plant roots were washed in running tap water and cut into 2-3 cm pieces. The pieces were surface-sterilized with 70% ethanol for 2 minute and rinsing in sterile distilled water and drying on sterile paper. Then they were put onto yeast extract - glucose agar (YEG) medium (1% D-glucose hydrate, 0.5% yeast extract, 0.5% potassium dihydrogen phosphate and 2% agar) in Petri dishes. Fungi were grown for 5 days at 25 °C. 105 colonies were isolated belonging first of all into four different genera, Alternaria, Fusarium, Mucor and Penicillium, based on their macro- and micromorphology.

Total DNA were extracted from the young mycelia using the Aqua Genomic Solution kit (MultiTarget Pharmaceuticals) according the recommendations of the manufacturer. PCR amplification of fungal DNA was performed with the primers ITS1 and ITS4 or ITS4 and ITS5, . Amplicons were sequenced commercially by LGC Genomics Ltd. from both directions with the same primers.

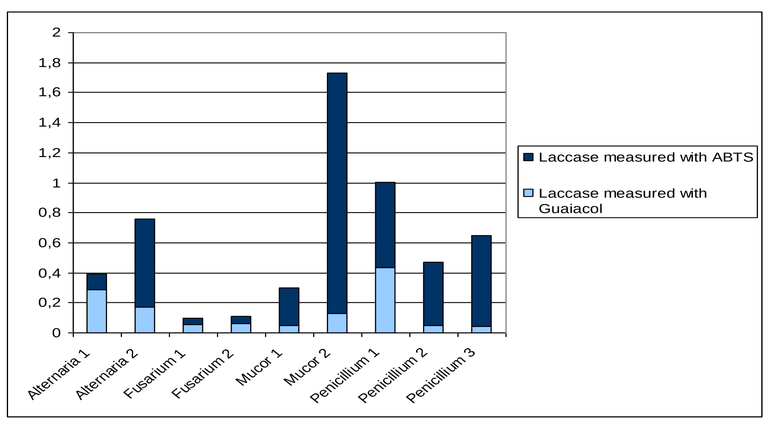
Results



Enzyme activities measured in the ferment broth of some endophytes isolated from roots of *Ambrosia artemisiifolia* collected at river Maros



Enzyme activities measured in the ferment broth of some endophytes isolated from roots of *Ambrosia artemisiifolia,* collected at Szolnok



Laccase activities measured in the ferment broth of some endophyte strains

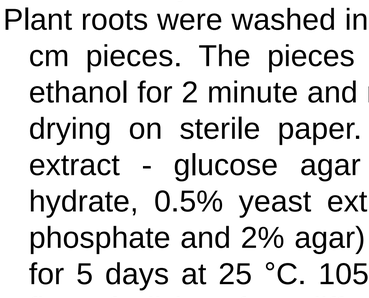
From the enzyme activity data, we can obtain information about the most highly secreted enzymes and we can suggest which enzymes are important for the fungus to live together with the host plant.

Isolated endophytes can secrete many types of enzymes. Generally, cellulase and xylanase enzymes showed the highest activities in the samples of the isolates from roots collected at Szolnok and the river Maros. These enzymes can degrade xylan and cellulose, which are the major components of the plant cell wall. A high amount of trypsin secretion was detected from *Mucor* species. Because of the impact on the living conditions of the plant, endophytic fungal enzyme production may be an important factor.

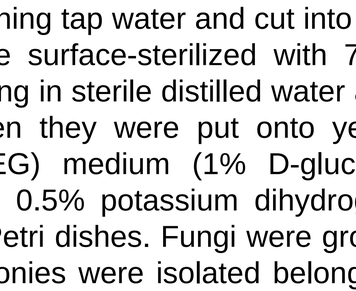
The most highly secreted enzymes were xylanase, trypsin, cellulase and β-1,3-glucanase.

The production of laccase secretion of an endophytic fungus could be advantageous for both the fungus and the host plant, as with this enzyme the endophyte could detoxify its environment.

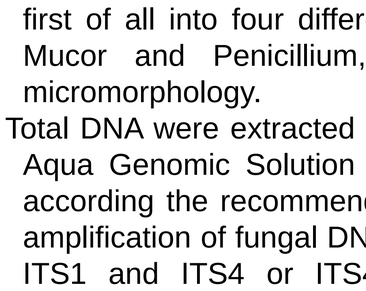
Colonies of isolated endophytic fungal strains



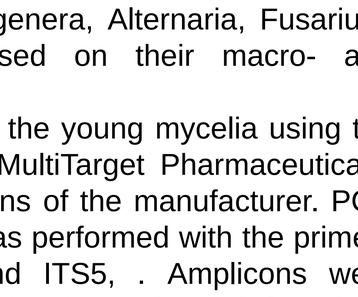
Penicillium sp.



Alternaria sp.



Fusarium sp.



Mucor sp.

Frequently isolated species, identified by sequencing:
Absidia repens,
Fusarium chlamydosporum,
Fusarium oxysporum,
Fusarium redolens,
Fusarium solani,
Leptosphaerulina chartarum,
Mucor circinelloides,
Penicillium aurantiogriseum.

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Conclusion

Our investigations expanded the list of endophytic fungi living in the roots of *Ambrosia. artemisiifolia*. We found more *Fusarium* species in the roots from the natural habitats than in those from the disturbed area. Furthermore, it was found that at least four genera (*Fusarium, Mucor, Alternaria* and *Penicillium*) are persistently present in the roots of the plant. Isolated endophytes can secrete many types of enzymes. Generally, cellulase and xylanase enzymes showed the highest activities. These enzymes can degrade xylan and cellulose, which are the major components of the plant cell wall. Intensive trypsin secretion was detected from *Mucor* isolates. Because of the impact on the living conditions of the plant, endophytic fungal enzyme production may be an important factor in the ballanced mutualistic symbiosis of the fungus and host plant..

Our data may contribute to the development of biocontrol techniques aiming the environmental friendly reduction of *Ambrosia artemisiifolia*.

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