

DISSERTATION SUMMARY

Echophysiological and molecular investigation of *Trichoderma* strains isolated from winter wheat rhizosphere

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There is a worldwide need to adopt the practice of sustainable agriculture, using strategies that are environment-friendly, less dependent on agricultural chemicals and less damaging to soil and water resources. One of the key elements of such sustainable agriculture is the application of biocontrol agents for plant protection. Species in the filamentous fungal genus *Trichoderma* are of great economic importance as sources of enzymes and antibiotics; plant growth promoters; degraders of xenobiotics, and most importantly, as commercial biofungicides (Howell 2003), thus they are potential candidates for biocontrol applications.

One hundred and twenty *Trichoderma* strains were isolated from roots of winter wheat grown in agricultural fields of southern Hungary from different defined test holes. The identity and diversity of species was examined based on morphological, biochemical and molecular characters. The morphological data were collected by measuring structure and shape of conidiophores, phialides and conidia. Differential utilization of a total of 100 carbon and 45 nitrogen sources were applied as biochemical markers. For the investigation of molecular diversity, sequence analysis of the internal transcribed spacer (ITS) region and cellulose-acetate electrophoresis (CAE) mediated isoenzyme analysis were applied. In the case of CAE, after initial testing of 13 enzymes for activity and resolution of bands, 7 of them (glucose-6-phosphate dehydrogenase, glucose-6-phosphate isomerase, 6-phosphogluconate dehydrogenase, peptidase A, B, D and phosphoglucomutase) proved to be appropriate for the analysis of the full sample set. Comparing the different electrophoretic types, each of the enzymes applied could be used as molecular marker in the identification of *Trichoderma* spp (Szekeres et al. 2004a). The differential utilization of carbon and nitrogen sources, the sequenced ITS regions and the isozyme data of the isolates were used for the construction of a phylogenetic dendrogram.

The isolated strains were investigated for the production of β -xylosidase, α -glucosidase, β -glucosidase, β -galactosidase, cellobiohydrolase, trypsin-, chymotrypsin- and chymoelastase-like proteases and *N*-acetyl- β -glucosaminidase,

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which are extracellular enzymes important for the biocontrol activity. The secretion of enzymes was revealed by means of specific chromogenic *p*-nitroanilide and *p*-nitrophenyl substrates. Some of the examined enzymes were secreted constitutively and their amounts showed high variability within the isolates.

Biocontrol properties were tested *in vitro* against *Fusarium culmorum* NRRL 29371, a significant pathogen of wheat. Direct confrontation assay was applied for recording the inhibition effect, which was expressed as the value of biocontrol index (BCI) calculated from the image analysis of ratio of the area occupied by *Trichoderma* and the plant pathogen. A modified plant pathogen aggressiveness test was carried out on wheat seeds for the investigation of reduction in diseases severity in the presence of *Trichoderma* strains.

After collecting the taxonomical and ecophysiological data, the relationships and correlations between the biocontrol efficiencies, the productions of several extracellular enzymes and location of isolation were examined by statistical analysis. To improve the antagonistic capacity of a selected fungal strain, a mutagenetic program was undertaken for the construction of derivatives overproducing extracellular proteases. The mutant strains were obtained by means of UV-irradiation and were selected for *p*-fluorophenyl-alanine resistance or altered colony morphology. Both trypsin-like and chymotrypsin-like protease secretion was elevated in most of the mutant strains. The profiles of protease isoenzymes were different between the mutants and the wild-type strain, when examined by gel filtration chromatography. Certain mutants proved to be better antagonists against plant pathogens in *in vitro* antagonism experiments (Szekeres et al. 2004b).

References

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