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DISSERTATION SUMMARY

Investigation of the applicability of the *Agrobacterium*-mediated transformation in Zygomycetes

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Fungi belonging to the Zygomycetes can cause serious post-harvest losses in various agricultural products; they are well known as opportunistic human and animal pathogens. Among them, *Mucor circinelloides* is frequently studied, e.g. due to its dimorphic nature, the ability of fungus to produce extracellular enzymes and the unique sex pheromones of its mating system. Transformation approaches, based on the complementation of auxotroph markers, have been worked out for *Mucor*. However, in these experiments foreign DNA was maintained exclusively in an autonomously replicating form in the cells and the transformation required the construction of auxotroph strains. *Agrobacterium tumefaciens* is a Gram-negative plant pathogenic bacterium, which is able to transfer a part of its tumour-inducing plasmid (T-DNA) into the genome of the infected cell. *A. tumefaciens*-mediated transformation (ATMT) proved to be efficient tool of gene transfer to a wide variety of plants. Recently, this approach was successfully applied also to transform some fungal species (de Groot et al. 1998).

The aims of the present study were: (i) to adapt the *Agrobacterium*-mediated transformation method to *Mucor* and other zygomycetous fungi and to develop a protocol which can be applied routinely to obtain integrative transformants, and (ii) to elaborate a direct selection method for the transformants which does not require the usage of auxotroph markers.

We have worked out the conditions of ATMT for *M. circinelloides*. The usage of antibiotics, such as hygromycin B, which inhibits fungal growth, would make possible the direct transformation of wild-type isolates, so the need for mutagenesis to obtain auxotroph strains could be avoided. *Agrobacterium* transfers T-DNA to the host cell, which integrates into the nuclear genome at a random position. This method can integrate heterologous sequences (as the *hph* gene in this study) into the *Mucor* genome, without the need for flanking homologous regions in the transforming vector to direct the homologous recombination.

Experiments revealed that media supplemented with the combination of dichloran and rose bengal not only reduces the colony size of transformants, but also increases the sensitivity of these fungi to hygromycin B. Transformation was performed with different plasmids containing the hygromycin B resistance gene controlled by the *Aspergillus nidulans trpC* promoter (Mullins et al. 2001) or *M. circinelloides gpd* promoter (Ács et al. 2002). The transformation event, the presence of the *hph* gene in the genomic DNA of transformed isolates was verified by PCR reaction with *hph* specific primer pair (Ács et al. 2003; Nyilasi et al. 2003). Other experiments (e.g. the hybridization of transformant DNA with *hph* specific probe) also verified the successful transformation event.

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