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DISSERTATION SUMMARY

Cloning and sequence analysis of *Mucor circinelloides* glyceraldehyde-3-phosphate dehydrogenase gene and development of new vector systems for transformation of zygomycetes

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Mucor circinelloides is a filamentous fungi belonging to the Zygomycetes. Its special biochemical, morphological and physiological features established a longstanding interest of both applied and theoretical research. Until now, the transformation systems developed in *Mucor circinelloides* are based on complementation of (*e.g.* amino acid) auxotrophy. In spite of the efficiency, such transformation systems have a serious drawback: a definite stable mutant has to be isolated from each strain before the transformation. Therefore, it seems desirable to establish a transformation system based on a strong native promoter allowing efficient expression of heterologous resistance gene as selection marker.

In the present study a genomic library of *Mucor circinelloides* ATCC 1216b has been constructed in Lambda Fix II vector. The library has an average insert site of 10 kb and covers the genome 12 times. The *M. circinelloides* gene encoding glyceraldehyde-3-phosphate-dehydrogenase (*gpd)* was isolated from this library by hybridization of the recombinant phage clones with a *gpd*-specific gene probe generated by PCR reaction. The complete nucleotide sequence encodes a putative polypeptide chain of 339 amino acids interrupted by 3 introns. The predicted amino acid sequence of this gene shows a high degree of sequence similarity to the GPD proteins from other filamentous fungi. The promoter region containing a consensus TATA and CAAT box and a 298 nucleotid long termination region were also determined (Ács et al. 2002). The predicted amino acid sequence of this gene shows a high degree of sequence similarity to the glyceraldehyde-3-phosphate dehydrogenase proteins from yeast and filamentous fungi (Papp et al. 2003; Vastag et al. 2003).

New transformation systems based on selective drugs have been tested for use in zygomycetes. Transformation vectors have been constructed which contain hygromicin B resistance gene under the control of the promoter of the glyceraldehide-3-phosphate-dehidrogenase (*gpd*) gene from *M. circinelloides*. In contrast to other transformation systems which rely on nutritional auxotrophic markers for the selection of transformants, the combination of a *gpd* promoter sequence and a dominant selectable marker allows the transformation of wild type strains (Nyilasi et al. 2003). Optimal conditions for transformation which increase the sensitivity of these fungi for hygromicin have been worked out.

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