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DISSERTATION SUMMARY

Isolation and characterization of the gene encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) of *Rhizomucor miehei*

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Rhizomucor miehei is a practically and theoretically important member of the genus *Rhizomucor* (order *Mucorales*). Certain strains of this genus are used in the food industry, and some isolates are agents of opportunistic infections in man and animals. The genus *Rhizomucor* involves two ubiquitous species: *R. pusillus* and *R. miehei*. The purpose of our study was to clone and characterise the coding and regulatory region of the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) gene of *R. miehei,* a homothallic species of the genus. HMG-CoA reductase (EC 1.1.1.34) catalyses the reduction of HMG-CoA to mevalonate, which is the first step of the acetate/mevalonate pathway, leading among others to the synthesis of the characteristic mating pheromone (trisporic acid) of zygomycetes.

Degenerated primers designed to the most conserved region of known *hmgr* genes were used to amplify a short conserved region of the gene by polymerase chain reaction. The resulting 314 bp length DNA segment of the *hmg* R gene was labelled with non-radioactive dioxigenine and was used as a homologous probe to select positive clones from a genomic library prepared by Vastag et al. (2004) from the strain *Rhizomucor miehei* (NRRL 5901) in λ FixII phage. One λ phage clone was selected and, after purification steps, it was revealed that this clone contains an insert of about 8000 bp. This insert was cloned into pBluescript plasmid with *Xho*I restriction endonuclease and was used for further subcloning and sequencing experiments. As a result of these experiments, the complete nucleotide sequence was determined and analysed. The putative protein sequence proved to be 1059 amino acids in length. Five introns have been identified in the HMG-CoA reductase gene, dispersed in the whole coding region. Two types of method were carried out in order to determine the *hmgr* gene copy number of *R. miehei*: quantitative PCR and Southern blot experiments revealed that both *R. miehei* and *R. pusillus* have two *hmgr* genes. In order to prove that the cloned *hmgr* gene of *R.miehei* is expressed, total RNA was isolated and reverse transcription was carried out with specific primers.

The sensitivity to lovastatin, a competitive inhibitor of HMG-CoA reductase, was determined in the two *Rhizomucor* species, in order to investigate if the *hmgr* gene could be utilised in transformational experiments by providing lovastatin resistance to the transformants (Vágvölgyi et al. 2004). It was observed that *R. pusillus* isolates are more susceptible to lovastatin than *R. miehei,* despite both having two copies of the *hmgr* gene. The difference in the susceptibilities of *R. pusillus* and *R. miehei* is so marked that besides the isoenzyme (Vastag et al. 1998) and random amplified polymorphic DNA (RAPD) patterns (Vastag et al. 2000), it could be used for differentiation of the two *Rhizomucor* species. Accordingly, a simple and reliable method for species-level differentiation of these species (Lukács et al. 2004) has been devised.

In order to broaden the available expression data on the *R. miehei hmgr* gene, further experiments are in progress.

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