Analysis of the formation of submersspores and molecular characterization of different isolates of the entomopathogenic fungi *Metarhizium anisopliae*

Stefan Laut, Andreas Leclerque, Dietrich Stephan
Julius Kühn-Institut, Institute for Biological Control
stefanlaut@web.de

The genus *Metarhizium* is one of the best characterized entomopathogenic fungi and the pathogenicity is known against 200 different insect species. To increase the fields of application and reduce the production costs of these fungi, it is essential to maximize the rate of spores obtained during liquid fermentation. Under this aspect, the work concentrates on testing of fermentation parameters in shake-flask cultures, like media composition, incubation-temperature and agitation of the flasks. During these tests, a media composition was found where the fungi produced spores, whereas in another setup no sporeformation could be detected. The observation of this different growth led to another approach in which the differential gene expression was analyzed for *M. anisopliae* strain 43 cultivated in two different liquid media. Therefore, RNA was isolated from the cultures and reverse transcribed into cDNA. Subsequently, the method of suppression subtractive hybridization PCR (SSH-PCR) was used to amplify differentially expressed genes. Due to optimization of the SSH-PCR, a necessary combination between amplification and suppression of the PCR was achieved. After verifying that these genes are indeed differentially expressed, the results should enable the identification of sporulation related genes.

Parallel to this work DNA was extracted from some isolates of *Metarhizium* to amplify a partial sequence of the β-Tubulin gene (*tubB*) and the ITS-region of the ribosomal RNA operon. With these results, phylograms were created with the methods of Neighbor-Joining (NJ), Minimum Evolution (ME) and Maximum-Likelihood (ML) to determine the phylogenetic relationships of the isolates.