several Zygomycetes fungi was tested in solid-state fermentation assays. Some *R. miehei* strains showed intensive extracellular enzyme activity. The aim of our present study is the identification and molecular and biochemical characterization of a beta-glucosidase enzyme and its coding gene (*bgl*) from *R. miehei*.

Degenerated beta-glucosidase-specific primer pairs were designed to conserved regions of fungal glycoside hydrolase family 3 genes and a 493 bps long fragment was amplified by PCR from the genomic DNA of the *R. miehei*. The sequence of the amplicon was determined; it showed high homology with the C-terminal domains of the beta-glucosidases belonging to the family 3. Based on this sequence, specific primers were designed for inverse PCR. The original fragment has been lengthened to a 4063 bps long sequence which contained the 2826 bps long beta-glucosidase gene encoding a protein with a length of 743 amino acids. *Rhizomucor bgl* showed the highest homology with the beta-glucosidases of *Phanerochaete chrysosporium*, *Trichoderma reesei* and *Piromyces* sp. strain E2.

For gene expression studies two transformation vectors were constructed: the plasmid pTM1 contained *bgl* under the control of the regulator sequences of the related *Mucor circinelloides gpd1* gene, while the plasmid pTM4 harboured the promoter region of the *bgl* fused with a green fluorescent protein gene. In the lack of an efficient transformation system in *R. miehei*, genetic transformations were started in a heterologous system: PEG-mediated protoplast transformations were performed in an uracile auxotrophic *M. circinelloides* strain. Induction of the *bgl* promoter by different substrates was studied in the *M. circinelloides* transformants harbouring the pTM4 plasmid. Strong fluorescence was observed only in the transformants growing on cellobiose containing medium. Analysis of the transformants containing pTM1 is in progress.

For production of the extracellular beta-glucosidase enzyme in high amount, *R. miehei* was grown on wheat bran medium for six days at 40°C. The enzyme was purified from the crude extract to homogeneity by ammonium sulphate fractionation and two-step chromatographic separation through Sephadex G100 and G200 columns was performed. The molecular mass of the purified enzyme was determined by SDS-polyacrylamide gel electrophoresis. The optimum temperature and pH for the action of the enzyme were at 60°C and 4.0 to 5.0, respectively; the beta-glucosidase proved to be highly stable at temperatures up to 50°C but it almost lost its activity at temperatures above 70°C. The enzyme was fairly stable at pH 4.0 to 6.0 and 20% of the activity remained after incubation at pH 3.0.

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Map based cloning of leaf developmental abnormality in *Medicago sativa* and comparison of the rDNA (NOR) regions in *Medicago truncatula* and *Medicago sativa*

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A *Medicago sativa* mutation called sticky leaf (*stl*), which appears in the nature occasionally was reported previously (Stanford 1959) and used as a morphological marker in cross-fertilizations. The *stl* mutant is characterized by the adhesion of the adaxial sides of adjacent leaflets in the same leaf, as well as adhesion of opposite halves of the same leaflet. The inheritance of the mutant in tetraploid populations suggested that the *stl* character is determined by a single recessive gene (Stanford 1965). This was confirmed later in a diploid F2 segregating population originating from a cross between *M. sativa* ssp. *quasifalcata* and *M. sativa* ssp. *coerulea* (Endre G. PhD thesis 1997). Genetic mapping of this trait in this population placed the *Stl* gene on Linkage Group six (LG6) in the close vicinity of the rRNA coding region (NOR). In the closely related model legume *M. truncatula* that shows very high overall macrosynteny with *M. sativa* the position of NOR region is different, it is located on LG5.

Our aim is to identify the mutant gene which is responsible for the *stl* phenotype in *M. sativa* with the help of the available genomic information of the model *M. truncatula*. For this the localization of the ortholog *Stl* gene in *M. truncatula* is needed, therefore we compare the NOR and its flanking regions in these *Medicago* species. To explore this syntenic relationship we used three approaches. One of these was to map the molecular markers linked to the *M. sativa stl* trait on the *M. truncatula* linkage map. The second direction was to search for and use structural genomic information of *M. truncatula* sequenced BAC clones carrying rDNA sequences. We have identified the repeat unit sequences and looked for discrete sequences for mapping on *M. sativa*. As a third approach we have screened a *M. truncatula Tnt1* insertional mutant plant collection for leaf phenotypes similar to *stl*.

Molecular markers Q5C and P16C closely linked to *stl* phenotype on the *M. sativa* map were used to identify *M. truncatula* BAC clones. Some have already been mapped in *M. truncatula*, but their position was neither on LG5 near NOR region nor on LG6 in syntenic position of *stl* in *M. sativa*. We have subcloned and sequenced other BACs with unknown location but only intergenic repetitive sequences were identified not suitable for syntenic mapping purposes.

Following the second approach with the help of BAC sequences we determined the rDNA units (the 18S-5.8S-25S rRNA coding + IGS sequences) in *M. truncatula*. The thorough analyses of these BACs identified only one putative other coding sequence but so far mapping efforts failed in *M. sativa*.

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In the meantime a publication reported about positioning *M. truncatula* FUT2 (α-1.3-fucosyltransferase) genes by FISH method on five chromosomes (LG1,4,5,7,8), one of them inserted in the NOR region. Based on this information we checked if FUT2 gene was present also in *M. sativa* NOR region or not. Southern blot experiments suggested that FUT2 gene has lower copy number in the *M. sativa* genome and no position inside the rDNA region was detected. This further suggests a low synteny between these regions in the two *Medicago* species.

Insertional mutagenesis technology is important tool for isolation of new genes by phenotypes (forward genetics) or study their function (reverse genetics). We have found a number of plants with leaf phenotype similar to *stl* among *M. truncatula* insertional mutants carrying tobacco *Tnt1* retrotransposons. Sequence of *Tnt1* flanking regions of these mutant lines were determined by AFLP-PCR method. These sequences have been analyzed by their potential coding function as well as by their map position. Possible candidates were identified based on location (Mt LG5, LG6 or unknown) and are subject for further studies.

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Typing of bacterial symbionts of entomopathogenic nematodes, and their potenital use as biocontrol agents

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The extensively used biocontrol organisms, entomopathogenic nematodes belong to the *Heterorhabditis* and *Steinernema* genus are symbiotically associated with *Photorhabdus* and *Xenorhabdus* bacteria. The bacterial partners have an outstanding role in the life-cycle of their nematode hosts: they produce wide range of toxins, hydrolitic exoenzymes and antibacterial compounds that are responsible for the death and bioconversion of the infected insect larvae and prevent other soil organisms from degradating the insect cadavers. The bacterial partners highly determine the effectiveness of the symbiotic complex against different insects, therefore bacteria have an interests from the viewpoint of biocontrol practice. The aim of this study was to survey the diversity of the Hungarian *Photorhabdus* isolates, and to obtain comprehensive view about their potential use as industrial entomotoxin and antimicrobial compound producers.

Photorhabdus strains from entomopathogenic nematodes isolated from Hungarian soils (Tóth 2006) were characterized by morphological, physiological and genetic properties to survey the diversity of bacterial symbionts of *Heterorhabditis* species of commercial importance. Entomopathogenic bacteria (EPB) were isolated from 245 entomopathogenic nematode strains originated from different part of Hungary. There were 156 *Photorhabdus* and 77 *Xenorhabdus* from the successfully cultured 233 EPB isolates. 65 *Photorhabdus* isolates representing the whole collection from the point of view of geographical and nematode host distribution were analysed. First stage bacteria cells selected on NBTA indicator plates were used to determine the morphological traits and to perform physiological tests using Biolog GN microplates and API20E strips. Cytotoxic and antibacterial properties of cell-free culture broth were measured against *Drosophila melanogaster* S2 and *Spodoptera frugiperda* Sf9 cell lines or *Stpahylococcus aureus* and *Bacillus subtilis* bacteria, respectively. Morphologically and physiologically homogenous groups of *Photorhabdus* isolates were characterized by partial sequencing of 16S rRNA and *gyr*B subunit gene.

High physiological and morphological diversity were proved among the *Photorhabdus* isolates, and all of physiological and morphological bacteria types could be isolated both from *Heterorhabditis megidis* and *H. downesi*. A number of bacteria isolates were shown only moderate 16S rRNA gene sequence similarities with type strains of all described *Photorhabdus* species/subspecies. Using *gyrB* sequences to the phylogenetic analysis, these isolates were proved to be part of the species *Photorhabdus temperata*, with clear separation from both palearctic and American strains (phylogenetic distances are 93.1% and 92.1%, respectively). The physiological and carbon source utilization characters supported the phylogenetic position of these strains, therefore a new subspecies, *Photorhabdus temperata* subsp. *cinerea* (Tóth and Lakatos 2008).

The 39% and 13% of all studied isolates were uneffective against *S. aureus* and *B. subtilis*, respectively, while 26% and 7% were much more effective, than 100 ppm streptomycine, which was the control. About 10% of the studied isolates do not produce effective ingredients against *S. aureus* and *B. subtilis* bacteria, while 10% of them were highly effective against both bacteria.

59% and 8% of isolates had no cytotoxic effect on S2 and Sf9 cells, while 3% and 21% were highly toxic to dipteran and lepidopteran cells. There was not any *Photorhabdus* isolates, of which fermentation liquid was toxic to both cell types.

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