Abstract

Anaerobic fungi inhabiting the digestive tract of large herbivores secrete a variety of hydrolytic enzymes, including cellulases, xylanases, mannases, esterases, glucosidases, and glucanases, which effectively hydrolyze plant biomass consisting mainly of cellulose and hemicellulose, the most abundant polysaccharides in the biosphere. The immense progress has been achieved in investigation of *Neocallimastix*, *Piromyces*, however other genera are less studied. This work therefore concentrated the effort to analyze the hydrolytic system of genera *Anaeromyces, Orpinomyces, Caecomyces* and fungus KF9 described as new type of fungus belonging to the genus *Piromyces*. The studied anaerobic fungi grown in batch culture on M10 medium with rumen fluid and microcrystalline cellulose as carbon source produced a broad range of enzymes requisite for degradation of plant structural and storage carbohydrates including cellulase, endoglucanase, xylanase, α-xylosidase, β-xylosidase, α-glucosidase, β-glucosidase, β-galactosidase, mannosidase, cellobiohydrolase, amylase, laminarinase, pectinase and pectate lyase. These enzymes were detected in both the intra- and extra-cellular fractions, but production into the medium was prevalent with exception for intracellular β-xylosidase, chitinases, N-acetylglucosaminidase, and lipase. Xylanase activity was predominant among the polysaccharide hydrolases. Xylanolytic enzymes were therefore studied in detail.

Activity of endoxylanase and xylosidase of studied rumen fungi *Anaeromyces, Orpinomyces, Caecomyces* and new fungus KF9 were the highest in 4-7 days after inoculation and were produced on all studied carbon sources (glucose, cellobiose, phospho-cellulose, mc-cellulose, xylose, hemicellulose, cellulose, cm-cellulose, cm-xylan, starch, lactose, arabinose and xylan). Activities of endoxylanase and β-xylosidase were detected in 6 different cell fractions including medium (extracellular), cell wall, hydrogenosome, lysozome, microsomal and cytosolic fraction. In general, endoxylanase was secreted into the culture medium while β-xylosidase was associated with fungal cells and cytosolic fraction. Endoxylanases of studied rumen fungi exhibited several isoformes of xylanases with approximate molecular weights ranging from 22 to 146 kDa. Xylosidases exhibited 2 isoforms with molecular weight 39 and 89 kDa. Endoxylanase and xylosidase of rumen fungus KF8 of genera *Anaeromyces* was purified using SEPHADEX G-100. While xylosidase was found to produce only one expressive peak, the endoxylanase produced several similar peaks. Xylanases of strains of *Anaeromyces* and *Orpinomyces* have been subjected to phylogenetic analysis. Randomized Axcelerated Maximum Likelihood phylogenetic analysis resulted in tree, where xylanase

sequences of *Anaeromyces* and *Orpinomyces* grouped well together with GenBank sequences derived from *Neocallimastix* and *Piromyces* sp. creating monophyletic group of xylanases of anaerobic fungi. On the other hand, inside this group, *Orpinomyces* and *Anaeromyces* xylanase sequences grouped separately and both formed sister clade of mixed *Neocallimastix* and *Piromyces* xylanase clade. Surprisingly xylanases of anaerobic fungi are more similar to bacterial xylanases (clade of clostridia and bacilly) then fungal xylanases (clade of dikarya), which indicates the possible horizontal gene transfer between rumen bacteria and rumen anaerobic fungi.

The increase attention was further paid to chitinolytic system of anaerobic polycentric rumen fungi of genera *Orpinomyces* and *Anaeromyces*. Activities of four chitin splitting enzymes including endochitinase, exochitinase, N-acetyglucosaminidase and deacetylase were studied in three crude enzyme fractions- extracellular, cytosolic and cell wall fraction. Endochitinase was found as a dominant enzyme with highest activity in cytosolic fraction. Endochitinases of both genera were stable from pH 4.5 to 7 with optimal pH 6.5. *Orpinomyces* endochitinase was stable up to 50 ^{0}C with optimal temperature 50 ^{0}C, while *Anaeromyces* endochitinase was stable up to 40 ^{0}C with optimal temperature also 40 ^{0}C. The most suitable substrate for both endochitinases was fungal cell wall chitin. Enzyme activities were inhibited by Hg^{2+} ions and activated by Mg^{2+} ions. Both endochitinases were inhibited by 10 mM SDS and activated by iodacetamide.

The last part of this work is devoted to description of new fungus KF9. This fungus with monocentric thallus was isolated from cattle rumen fluid. Strain KF9 was willing to produce sporangia of very different shapes: globular (average 25 až 70 μm), bifurcated or elongated (size 20-25 x 50-100 μm). On some sporanga the separation from the flexuous sporangiophore by septum was visible. The nuclei of thalli were indicated in sporangia and sporangiophores. The whole mycelium except sporangia is composed of branched narrow hyphae with many stolons. Uninucleate and polyflagellate (3-12 flagella, 30-40 μm, posteriorly orientated) zoospores is elipsoidal or globular (average 7-12 μm). The main end products of the strain KF9 was found to be acetate. Sequence analysis of ITS1 fragment of rDNA indicated that this strain belong to genus Piromyces, however represents a new type. Probably new species, which has to be described.