Abstract

The anaerobic rumen fungus, *Orpinomyces joyonii* KF2, was isolated from cow's rumen fluid. Its chitinolytic system was studied in 3 fractions, in medium, in cytosol and in cell wall. The fungus grew in medium M10 [27] with 20 % cell-free rumen fluid and 3 g/l glucose as energy source. Activities of exochitinase, N-acetylglucosaminidase and deacetylase were very low. The most important chinolytic enzyme is endochitinase. The greatest endochitinase activity was found in cytosolic and cell wall fraction. Endochitinase produced extracellulary did not achieved activity comparable with enzyme activities detected in two intracellular fractions. Concentration of medium by amonium sulfate precipitation and ultrafiltration through polyethersulfon membranes (10- 300 kDa) did not bring increasing of endochitinase activity. Ultrafiltration of enzymes of cytosolic fraction was not succesful as well, only the fraction containing the bigest proteins was active.

Endochitinase of three cell fractions of fungus KF2 was furher characterized. The optimal pH of extracellular endochitinase was 5, while cytosolic and cell wall fraction had optimal pH 6. The optimal temperature of extracellular endochitinase was 30°C , cytosolic endochitinase was 50°C and cell wall endochitinase was 40°C. The endochitinase was stable from pH 4 to 7 and lost the function at 50°C in medium and at 60°C in others cell fractions. The intracellular chitinases hydrolyzed fungal chitin and CM-chitin more rapidly then other tested substrates. The enzyme activity was inhibited by Mn^{2+}, thimerosal, SDS and EDTA. Mg^{2+}, on the other hand iodoacetamid and iodoacetic acid stimulated enzyme activity. Zymograms demonstrated that endochitinase exist in several isoenzyme forms; their molecular weights range from 43 to 134 kDa.