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Purification and Characterization of Alkaline Carboxymethyl Cellulases and Xylanases from Alkalophilic *Cephalosporium* sp. RYM-202

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An alkalophilic fungus, *Cephalosporium* sp. RYM-202, capable of producing extracellularly cellulase and xylanase was isolated from soil. This organism grew best at an initial pH 9.5 and produced cellulase and xylanase maximal at the initial pH 9.5-10.0. Two carboxymethyl cellulases (CMCases, C-I and C-II) and three xylanases (X-I, X-II and X-III) from the culture supernatant of this strain were purified to homogeneity by a series of chromatography separations. The molecular weights of C-I and C-II were estimated to be 56 and 22 kDa, respectively, by SDS-polyacylamide gel electrophoresis (SDS-PAGE). The hydrolysis patterns revealed that the CMCases were endoenzymes. The K_m values of C-I and C-II on carboxymethyl cellulose (CMC) were estimated to be 14.29 and 10.52 mg/ml, respectively. The optimum pH for C-I was 8.0 and that for C-II was 7.5-9.5. Both enzymes were stable over a wide range of pH and retained more than 80 % of their activities after exposure to pH 11 for 24 h. The CMCases were also stable in the presence of various laundry detergent components, indicating their possible application as an effective additive for laundry detergents. The molecular weights of X-I, X-II and X-III were 35, 23 and 24 kDa by SDS-PAGE and the isoelectric point values 6.3, 4.4 and 4.3, respectively. Three xylanases had similar temperature (50 °C) and pH (7.5-8.0) optima. These enzymes were considerably stable at the pH 4.5-11.5. The K_m values of X-I, X-II and X-III on birchwood xylan were estimated to be 3.18, 2.20 and 2.33 mg/ml, respectively. Three xylanases were free from cellulase activity. Among three xylanases, X-I contained relatively higher levels of alanine, leucine and lysine than X-II and X-III. On the other hand, amino acid composition of X-II and X-III showed great homology, having relatively higher contents of histidine and tyrosine than X-I has. The analyses of predominant products from xylan and xylo-oligosaccharides indicate that these xylanases are endoenzymes having trans-xylosidase activity. Chemical modification with N-bromosuccinimide (NBS) revealed that the number of tryptophan residue, essential in the catalysis, is at least one for X-I and two for X-II and X-III, respectively.