Gene Cloning of Hyper-thermo-stable Cellulase and its Characterization

M.M. Rahman
UNK-Kirin Fellow from Bangladesh
Enzyme Laboratory, National Food Research Institute

A cellulase gene (TM1751, Swissprot Q9X273, GenBank AAD36816) of *Thermotoga maritima* was cloned and expressed in *E. coli.* The recombinant protein was purified by a combination of Ni-NTA and Q-Sepharose FF column chromatography. SDS-PAGE analysis of Q-Sepharose active fractions showed a homogeneous protein band with expected molecular mass of 38 kDa. The specific activity of the enzyme increased about 16-fold while the recovery was 20 % after purification. The optimal temperature of the enzyme was found to be 90℃ while that of pH was 6.6. The enzyme was active over a wide pH range (4-9.5) and stable up to 85℃. Kinetic studies showed that the PNP-b-D-cellotetraoside and PNP-b-D-cellopentaoside had similar *Km* values of 0.25 and 0.24 mM respectively. However, maximum catalytic efficiency (*kcat/Km*) was observed with PNP- β -D-cellotetraoside. Further analysis of *kcat* and *Km* revealed that the addition of even one more glucopyranose unit to the non reducing end of PNP-b-D-cellotetraoside had little or no effect on binding or catalysis, suggesting that the carbohydrate binding cleft of the enzyme may be composed of four subsites for glucopyranose units. Hydrolysis of PNP-oligosaccharides (DP up to 5) yielded a range of products with cellobiose and cellotriose being the major products. CMC hydrolysis also predominantly produced cellobiose and cellotriose. The enzyme hydrolyzed mixed linked β -1,3/1,4 soluble substrates such as barley glucan and lichenan more strongly than CMC. Strong transglycosylation activity was also found to be taking place with smaller soluble cellooligosaccharides.