Purification and enzyme properties of an extracellular Endo- -1, 4- glucanase from *Paenibacillus amylolyticus MTCC 8084* isolated from sugarcane fields

P. ANURADHA, D. JHANSI RANI, S. ABID PASHA AND K.SRAVANI

Asian Journal of Environmental Science, Vol. 3 No. 2 : 183-188 (Dec., 2008 to May, 2009)

See end of the article for authors' affiliations

Correspondence to :
P. ANURADHA
Criya Biolabs,
TIRUPATI, (A.P.)
INDIA

SUMMARY

Endo- -1, 4 - glucanase is an extracellular key enzyme used by bacteria to decompose cellulose of sugarcane crop residue. It has been purified from *Paenibacillus amylolyticus MTCC 8084* isolated from sugarcane fields. The dialysed crude enzyme preparation was loaded on to a DEAE-Sepharose anion–exchange column. Active fractions were collected and loaded on to a Sephadex G-75 column for further purification. The purification fold was 11.7 with a recovery yield of 29.4 %. Specific activity of enzyme was 4.16 U/mg. The purified endo- -1, 4–glucanase gave a single protein band on polyacrylamide gel electrophoresis and molecular weight is approximately 93 kDa. The optimal temperature and pH were 50^{0}C and 5.0, respectively. Apparent kinetic parameters K_m and V_{max} were determined 8.2 mg/ml and 167 U/min/mg, respectively. Enzyme activity was stimulated by Cu^{+2} and inhibited by Hg^{+}, Ag^{+2}, Al^{+3} and EDTA.

Key words : Endo-β-1, 4–glucanase, *Paenibacillus* sp., Purification, Cellulose.

Bacterial degradation of cellulosic biomass of agricultural waste plays a vital role in carbon recycling. For the same reason, treatment of cellulose by cellulolytic enzymes for practical purposes has attracted the continuing interest of biotechnologists. Interest in transformation of biomass is both fundamental and applied (Clarke, 1997). In order to enhance the rate of saccharification, it has become necessary to search for highly efficient cellulolytic organisms with secretion of copious amount of cellulose.

Paenibacillus spp. produce several celluloses. Most of the enzyme is extracellular and a small amount is cell bound. Celluloses are characterised by a multiplicity of enzyme components whose exact number varies from one organism to another. Cellulose is the key enzyme produced by cellulolytic microorganisms for the degradation of cellulose. It is a complex enzyme comprising three major components, *viz*. Endoglucanase (E.C 3.2.1.4), Exoglucanase (E.C 3.2.1.91) and Cellobiase (E.C 3.2.1.21) which act synergistically and completely solubilize crystalline cellulose to glucose. Endoglucanases randomly hydrolyze internal glycosidic linkages, which results in rapid decrease in polymer length and a gradual increase in the reducing sugar concentration (Beguin and Aubert, 1994; Wood and Bhat, 1988). Exoglucanases hydrolyze cellulose chains by removing cellobiose either from the reducing ends or non reducing ends (Teeri, 1997). Glucose is produced primarly by the action of glucosidases on cellobiose.

Frequently, cellulolytic organisms also produce other polysaccharases, including xylanases, mannanases, galactosidases, which hydrolyze associated plant polysaccharides and thus facilitate cellulose access to the substrate. Besides the degradation of organic matter, celluloses are used in various industries like food, brewery, wine, textile, leather, paper, pulp and printing ink (Hamlyn, 2000) etc. This potential has stimulated the search for new microorganisms with better cellulolytic capabilities. The commercial possibility of using cellulose preparations to produce glucose, alcohol is under intensive study. A number of biomass conversion methods have been proposed and employed ranging from direct chemical methods like acid hydrolysis and pyrolysis to biological methods such as application of cellulose enzymes (Cooney *et al*., 1978).

Enzymatic hydrolysis of cellulosic wastes may give a relatively pure product with the consumption of less energy during the process (Fennington *et al*., 1982). Substantial efforts have been made by enzyme suppliers and industrial users to improve existing enzymes (Brennan, 1996). In this report, the purification and partial characterization of extracellular endo-β-1, 4 - glucanase from *Paenibacillus*

Accepted :* *November, 2008