The biotechnological perspective of Beta-Glucosidases

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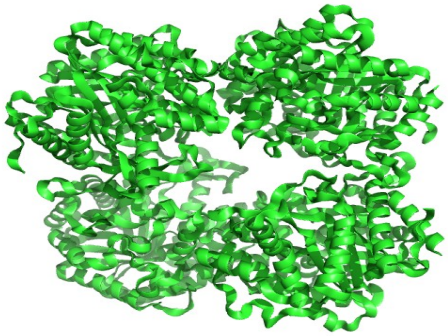
Abstract

Biotechnology is the art and science of making and technology to process biological materials for the welfare of human being. This discussion includes the beta glucosidase classification, its structure, application, and recent research interests in relation to its significance and comparison with eukaryotic Beta glucosidases including plant, animal and yeast as well. The study also comprise of the various stress factors and modifications thereby the organism to cope it. The focus of the study is on the various kinetics principles and their full potential exploitation to increase the activity of the enzyme. As such making the enzyme a potent vehicle for various industrial, pharmaceutical and research purposes. It also include the various glycosylhydrolases of families 1 and 3 that include beta-glucosidases.



A ribbon depiction of beta-glucosidase A from bacterium Clostridium cellulovorans (pdb 3AHX; Jeng et al. J.Struc.Biol., 2010)

Introduction

Beta-glucosidases are able to cleave the beta-glucosidic linkages in di- and oligo glucosaccharides and several other glycoconjugates. These enzymes are widely distributed and have important roles in many biological processes. In cellulytic microorganisms beta-glucosidase is involved in cellulose induction and hydrolysis (Bisara and Mishra, 1989; Tomme *et al*.,1995). In plants the enzyme is involved in beta-glucan synthesis during cell wall development, pigment metabolism, fruit ripening and defence mechanisms. (Brozobohaty *et al*., 1993; Easen,1993). In humans and other mammals the enzyme is involved in the hydrolysis of glucosyl ceramides (Barton,1990). The deficiency of the enzyme leads to Gauchers's disease. Like many hydrolases, the enzymes can be used for synthesizing a variety of glycoconjugates such as alkyl glucosides,

aminoglycosides and special disaccharide fragements of phytoalexin-elecitor oligosaccharides which are involved in plant and other microbial defence mechanisms (Bhatia, 2002).

Beta-glucosidase is a glucosidase enzyme that acts upon 1 - >4 bonds linking two glucose or glucose-substituted molecules (i.e., the disaccharide cellobiose). An exocellulase with specificity for a variety of beta-D-glycoside substrates. It catalyzes the hydrolysis of terminal non-reducing residues in beta-D-glucosides with release of glucose.[2]Cellulose is largely composed of polymers of beta-bond linked glucose molecules, and beta-glucosidases are required by organisms (some fungi, bacteria, termites) that can consume it.Lysozyme, an enzyme secreted in tears to prevent bacterial infection of the eye, is also a beta-glucosidase that cleaves 1 - >4 bonds between N-acetylglucosamine and N-acetylmuramic acid sugars within the peptidoglycan cell walls of gram-negative bacteria.

Closteridium thermocellum is the best known member of the anaerobic, thermophilic, cellulytic, ethanol producing bacteria, that have great potential in conversion of cellulose and Hemicellulose into liquid fuel. It’s cellulose system is exploited for saccharification of complex cellulosic substrates both crystalline and

amorphous cellulose (Johnson *et al*., 1982). A thermostable beta-glucosidase from Closteridium thermocellum, expressed in E.coli. the **restriction map** cloned in plasmid pALD7 was detrmined.

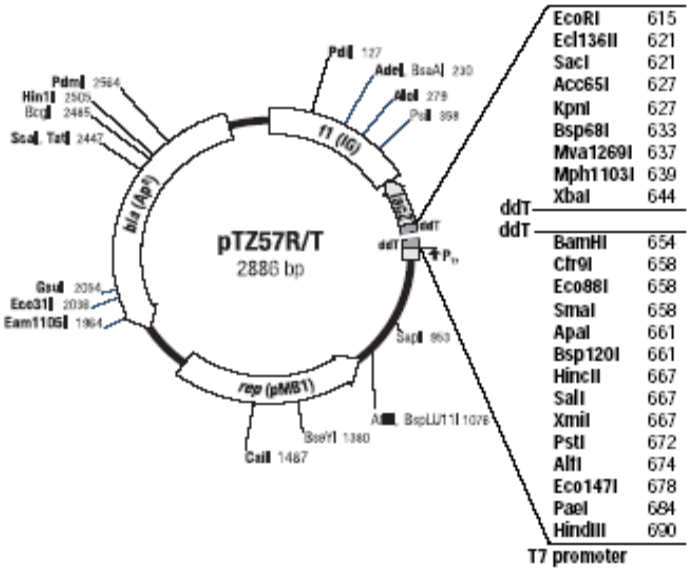


Fig.1. Restriction map of vector pTZ57R/T.

(InsTAclone™ PCR Cloning Kit)

Addition of E.coli cell extract having beta glucosidase, increased the conversion of crystalline cellulose (Avicel) to glucose. (Kadam and Demain., 1989). A genomic library of Closteridium thermocellum DSM 1237, was constructed in a bacteriophage lambda vector and recombinant clones expressing four beta-glucanses and two distinct beta glucosidases were obtained. The beta-glucosidases were identified as cellobiases hydrolyzing both aryl-beta-glucosides and cellobiose (Schwarz *et al*., 1985).

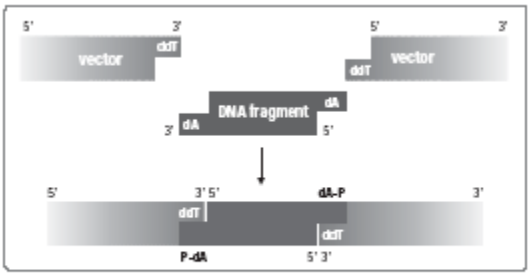
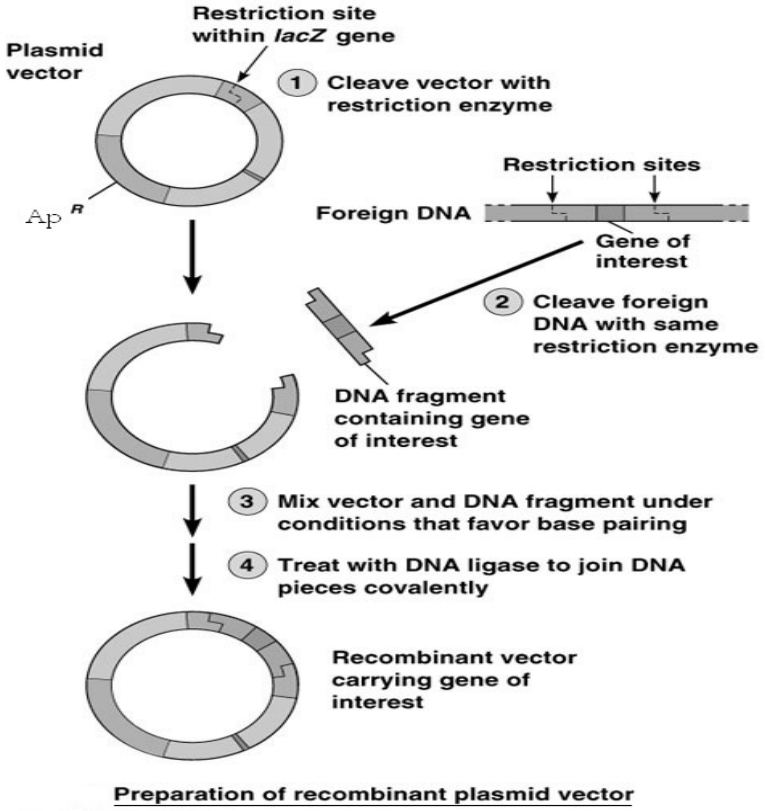


Fig. 2. Ligation of a PCR fragment into the pTZ57R/T vector.

Beta glucosidases, a major group among **glycosyl hydrolase enzymes**. Out of the 82 families classified under glycosylhydrolase category, these belong to family 1 and family 3 and catalyze selective cleavage of glucosidic bonds. It is important in degradation of structural and storage polysaccharides, cellular signaling, oncogenesis, host pathogen interactions, as well as in a number of biotechnological applications. Recent efforts have been directed towards molecular cloning, sequencing, mutagenesis and crystallography of the enzymes. The sources and properties of recombinant beta glucosidases, their classification schemes based on similarity at the structural and molecular levels, elucidation of structure function relationships, directed evolution of existing enzymes toward enhanced thermostability, substrate range, biosynthetic properties and applications (Bhatia *et al*., 2002)



An unusual **cold active beta-glucosidase** of family 3, glucoside hydrolase from psychrophilic isolate Paenbacillus sp. Strain C7 was characterized, some transformed E.coli isolates hydrolyzed X-gal a galactopyranoside below 30 ^{o}C. Sequencing of the cloned gene showed an ORF of 756 aminoacids protein, that rather than belonging to a family typically known for Beta-galactosidase activity, belonged to glycoside hydrolase family 3, a family of beta-glucosidase. (Shipkowski and Brenchley., 2005).

Key words: Beta-glucosidase, cloning, expression, biotechnology

Literature cited:

1) Bhatia Y., S. Mishra, V.S. Bisaria, Microbial b-glucosidases: cloning, properties and applications. CRC Crit. Rev. Biotechnol. **22** 375–407. **(2002)**.

2) Bisaria,V.S and S. Mishra, Cellulase biosynthesis and regulation, CRCCrit. Rev. Biotechnol. **5** 61–103. (1989)

3) Barton N.W., F.S. Furbish, G.T. Murray, M. Garfield, R.O. Brady. Therapeutic response to intravenous infusions of glucocerebrosidase in patients with Gaucher_s disease, Proc. Natl. Acad. Sci. USA. **87**. 1913–1916. **(1990)**.

4) Easen,A., b-Glucosidases. Biochemistry and Molecular Biology, American Chemical Society, Washington, DC, **(1993)**.

5) Johnson, E. A., M. Sakajoh, G. Halliwell, A. Madia, and A. L. Demain. Saccharification of complex cellulosic substrates by the cellulase system

from Clostridium thermocellum. Appl. Environ. Microbiol. **43**:1125-1132. **(1982)**.

6) Kadam SK, Demain AL..Addition of cloned beta-glucosidase enhances the degradation of crystalline cellulose by the Clostridium thermocellum cellulose complex. Biochem Biophys Res Commun. **15**;161(2):706-11. **(1989)**.

7) Tomme, P. R.,A.J. Warren, N.R. Gilkes.. Cellulose hydrolysis by bacteria and fungi, Adv. Microbiol. Physiol. **37**. 1–81. **(1995)**.

8) Shipkowski, S and J. E Brenchley..Characterization of an unusual cold-active beta- glucosidase belonging to family 3 of the glycoside hydrolases from the psychrophilic isolate Paenibacillus sp. strain C7. Appl Environ Microbiol.;**71**:4225-32 16085807. **(2005)**.

9) http://tools.neb.com/NEBcuter2/index.php

10) http://www.basic.northwestern.edu/biotools/oligocalc.html