

Novel Bi-functional Enzymes based on Endoglucanase and β -glucosidase

Endoglucanase and β -glucosidase are two key components of the enzyme cocktail used for the hydrolysis of biomass for bioethanol production. Design and expression of bi-functional cellulolytic enzyme will reduce the overall production cost. We have considered an Endoglucanase (Endo5A) belonging to a GH5 superfamily and a β -glucosidase (Gluc1C) belonging to a GH1 superfamily from *Paenibacillus* MTCC 5639 for construction of bifunctional chimeric proteins. The Endo5A of ~40 kDa hydrolyzed the amorphous cellulose efficiently and released cellobiose as the major product. The Gluc1C of 51 kDa hydrolyzed cellodextrin up to five chain length, with highest efficiency towards cellobiose.

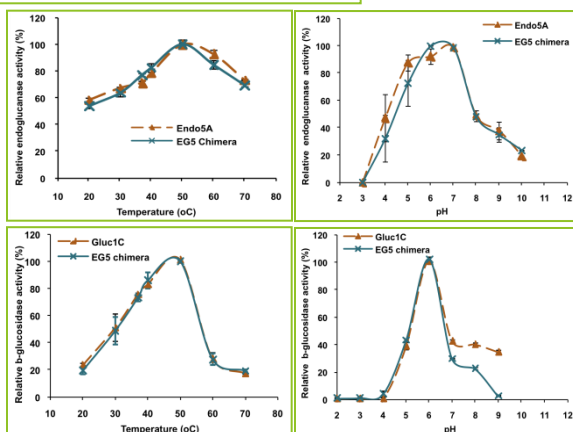
Six bifunctional chimeric proteins based on Endo5A and Gluc1C varying in the orientation and the size of linkers were constructed. One of the constructs, EG5, consisting of Endo5A-(G₄S)₃-Gluc1C, demonstrated 3.2- and 2-fold higher molar specific activity for β -glucosidase and endoglucanase, respectively. In addition, β -glucosidase activity of EG5 showed 4-fold higher half life at 55 °C as compared to Gluc1C alone. Hydrolysis experiments done on biomass and CMC indicated two fold higher product released by EG5 as compared to that by the physical mixture of Endo5A and Gluc1C, providing rational for channeling of intermediates.

Graphical Description

Activity and recombinant protein	Sp. act (U/mg)	Molar sp act (U μ mol ⁻¹)	Fold change in activity
β-glucosidase			
Gluc1C	5.8	307	1
EG5 (Endo5A-(G ₄ S) ₃ -Gluc1C)	10.5	997	3.2
Endoglucanase			
Endo5A	24.2	1016	1
EG5 (Endo5A-(G ₄ S) ₃ -Gluc1C)	22.6	2147	2.1

Table 1: Specific activity of recombinant enzymes

Figure 2: Comparative temperature and pH optima of EG5 with individual enzymes



Technology Exploitation

The lab scale recombinant enzyme technology is now ready to be upscaled industrially to meet the energy needs of human beings.

Reference for the Invention

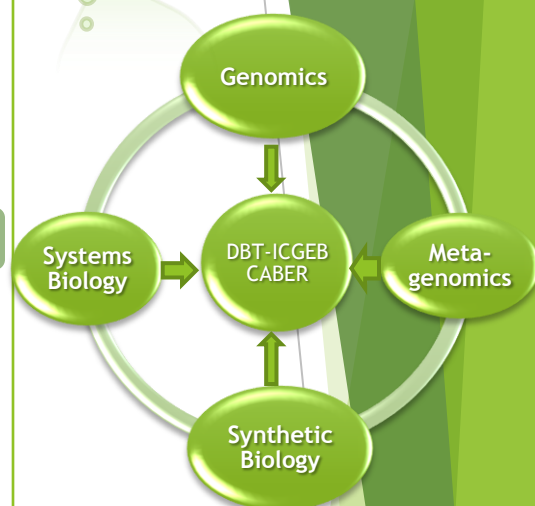
Syed Shams Yazdani And Nidhi Adlakha. 2012. Novel Cellulolytic Enzymes and their Chimera. Indian Patent Application No. 2695/DEL/2012.

Adlakha, N., Sawant, S., Anil, A., Lali, A., Yazdani, S.S. 2012. Specific fusion of β -1,4- endoglucanase and β -1,4- glucosidase enhances the cellulolytic activity and helps in channeling of the intermediates. Applied and Environmental Microbiology 78:7447-54.



DBT-ICGEB

CENTRE FOR ADVANCED BIOENERGY RESEARCH (CABER)



Contact Person:

Dr. Brajesh Barse

Email: barsebrajesh@icgeb.res.in

Dr. Syed Shams Yazdani

Group Leader, Synthetic Biology and Biofuels
Coordinator, DBT-ICGEB Centre for

Advanced Bioenergy Research

International Centre for Genetic Engineering
and Biotechnology (ICGEB)

Aruna Asaf Ali Marg, New Delhi 110067

Phone: +91-11-2674-1358 (Ext. 460)

Email: shams@icgeb.res.in