## Focal Point & the Purpose of Innovation

# Bifunctional enzyme based on Endo5A & Xyl11D

The saccharification of cellulosic biomass accounts for 30% of the overall cost of cellulosic biofuel production. One way to reduce saccharification costs is by producing enzymes economically. Because saccharification of lignocellulosic biomass requires the coordinated action of multiple enzymes, the production cost should be reduced if a single polypeptide has more than one catalytic function. The most striking feature of the isolated Endo5A and Xyl11D enzymes was that they exhibited optimal activity at the same temperature and pH and were, therefore, potential candidates for constructing a bifunctional polypeptide; however, constructing a chimeric protein often reduces the activity of one or more of the catalytic domains . One way to address this issue is to express chimeric proteins with various linkers to separate the two domains.

Therefore, we constructed four bifunctional chimeric proteins based on Endo5A and Xyl11D varying in the orientation and the size of linkers were constructed. One of the constructs, Model3, consisting of Endo5A-$(G_4S)_2$-Xyl11D, demonstrated 2.3- and 1.6-fold higher molar specific activity for β-xylanase and endoglucanase, respectively, as compared to Xyl11D and Endo5A alone.

## Graphical Description

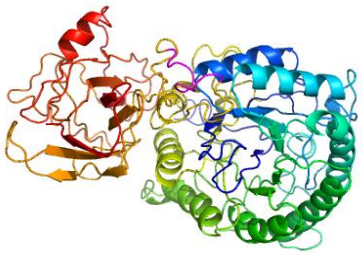

**Figure 1**: Three dimensional structure of Model3 as predicted by I-TASSER

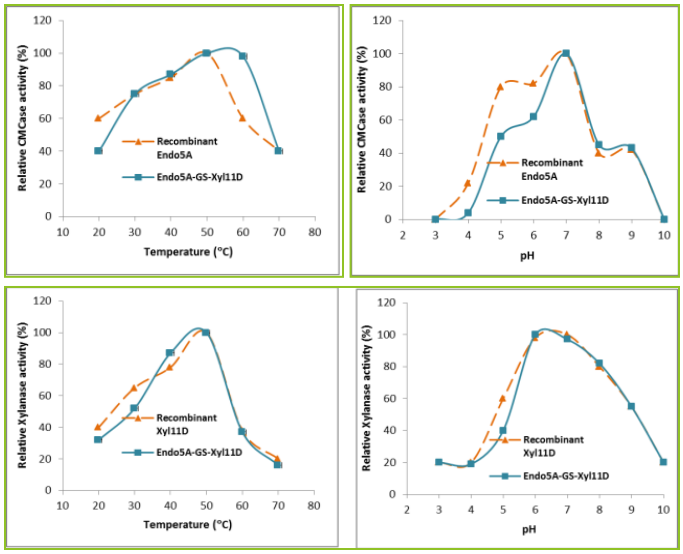


**Figure 2**: Comparative temperature and pH optima of Model3 with individual enzymes

## 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


Adlakha, N., Rajagopal, R., Kumar, S., Reddy, V.S., Yazdani, S.S. 2011. Synthesis and characterization of chimeric proteins based on cellulase and xylanase from an insect gut bacterium. Applied and Environmental Microbiology 77(14):4859-66.


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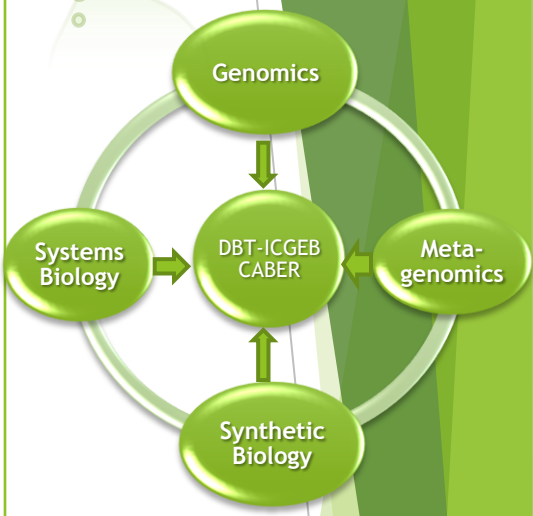



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