Thesis abstract

Protein engineering of β-1,4-endoglucanase and Chimera construction with β-glucosidase from *Clostridium thermocellum* for improving ligno-cellulosic biomass saccharification

Plant cell wall were composed of polymers for example cellulose, various hemicellulose and lignin and can be converted into alcohol that can be served as renewable source of energy. Saccharification of cellulose (major component of plant cell wall) requires three major enzymes i.e. endoglucanases and exoglucanases (cellobiohydolases and β-glucosidase) for its complete hydrolysis. But the use of these enzymes are limited because of their low catalytic efficiency and individual production of each enzyme increases the total cost of bioethanol production. So, it is important to engineer enzymes to i) improve catalytic efficiency and ii) production of chimera having multifunctional activity to reduce the number of enzymes required for complete degradation of lignocellulosic biomass. Site-directed mutagenesis of β-1,4- endoglucanase from family 5 glycoside hydrolase (*Ct*GH5) from *Clostridium thermocellum* was performed to develop a mutant *Ct*GH5-F194A that gave 40 U/mg specific activity against carboxymethyl cellulose, resulting 2-fold higher activity than wild-type *Ct*GH5. *Ct*GH5-F194A was fused with a β-1,4-glucosidase, *Ct*GH1 from *Clostridium thermocellum* to develop a chimeric enzyme. The chimera (*Ct*GH1-L1-*Ct*GH5-F194A) expressed as a soluble protein using *E. coli* BL-21cells displaying 3- to 5-fold higher catalytic efficiency for endoglucanase and β-glucosidase activities. TLC analysis of hydrolysed product of CMC by chimera 1 revealed glucose as final product confirming both β-1,4-endoglucanase and β-1,4-glucosidase activities, while the products of *Ct*GH5-F194A were cellobiose and cellooligosaccharides. Protein melting studies of *Ct*GH5-F194A showed melting temperature (Tm), 68ºC and of *Ct*GH1, 79ºC, whereas, chimera showed 78ºC. The action of Chimera (*Ct*GH1-L1-*Ct*GH5-F194A) was analysed on pretreared Sorghum stalk biomass. The TLC analysis showed Chimera displayed higher accumulation of glucose after 48h than the mixture of *Ct*GH1 and *Ct*GH5-F194A. Therefore, Chimera acts on the pretreated Sorghum stalk biomass and efficiently releases the glucose as final product. The *insilico* molecular modelling was performed to determine the molecular structure of Chimera. The modelled structure of the Chimera showed a stable modular conformation with 99.9% of residues in allowed region analysed by Ramachandran plot. The molecular dynamics simulation showed stability of the two modules in the chimeric form. Moreover, SAXS analysis of Chimera displayed elongated structure with two modules in fully folded form in the solution and showed an overall shape similar to a bean-bag contour. The enzymatic saccharifcation using cocktail of cellulases comprising of Chimera and cellobiohydrolases (*Ct*CBH5A) on dual alkali pretreated followed by organosolv of sugarcane bagasse gave maximum TRS yield at 30°C for 96h was 230 mg/g and glucose yield of 137 mg/g of pretreated biomass. The efficacy of the recombinant enzymes was obtained on the dual pretreated sugarcane bagasse. The effectiveness of the pretreatment processes was further confirmed using FESEM and FT-IR analysis.

List of publications
From Thesis:

1. **Nath, P.,** Dhillon, A., Kumar, K., Sharma, K., Jamaldheen, S. B., Moholkar, V. S., & Goyal, A. (2019). Development of bi-functional chimeric enzyme (*Ct*GH1-L1-*Ct*GH5-F194A) from endoglucanase (*Ct*GH5) mutant F194A and β-1, 4-glucosidase (*Ct*GH1) from *Clostridium thermocellum* with enhanced activity and structural integrity. *Bioresource Technology*, *282*, 494-501.
2. **Nath, P.,** K., Sharma, & Goyal A. (2019). Combined SAXS and computational approaches for structure determination and binding characteristics of Chimera (*Ct*GH1-L1-*Ct*GH5-F194A) generated by assembling β-glucosidase (*Ct*GH1) and a mutant endoglucanase (*Ct*GH5-F194A) from *Clostridium thermocellum*. *International Journal of Biological Macromolecules*, *148*, 364-377.
3. Nath[¥], P., Maibam[¥], P. D., Singh, S., Rajulapati, V., & Goyal, A. (2020). Comparative pretreatment using mild alkali and organosolv method for improving enzymatic digestibility of sugarcane bagasse by recombinant Chimera and Cellobiohydrolase for bioethanol production (submitted).
4. **Other Publications:**

1. Kumar, K., **Nath, P**., and Goyal, A. (2018). Structural characterization of an endo β-1, 3-glucanase of family 81 glycoside hydrolase (CtLam81A) from *Clostridium thermocellum*. *Journal of Proteins & Proteomics*, *9*(3).
2. Mohanapriya.N., Singh, S., Jamaldheen, S. B., **Nath, P.,** Moholkar, V. S., & Goyal, A. (2020)**.** Assessment of combination of pretreatment of *Sorghum durra* stalk and production of chimeric enzyme (β-glucosidase and endo β-1,4 glucanase, *Ct*GH1-L1-*Ct*GH5-F194A) and cellobiohydrolase (*Ct*CBH5A) for saccharification to produce bioethanol. Preparative biochemistry & biotech (in press)

Conferences

1. Presented a poster entitled "Construction and characterization of chimeric enzyme developed by fusing β-glucosidase (*Ct*GH1) and endoglucanase (*Ct*GH5-F194A) both from *Clostridium thermocellum* for enhanced catalytic efficiency and thermostability" in "International Conference on Biotechnological Research and Innovation for Sustainable Development" 15th BRSI convention. CSIR- Indian Institute of Chemical Technology (CSIR-IICT), Nov. 22-25, 2018, Hyderabad, India.
2. Presented a poster entitled "Protein engineering of endo β-1-4 glucanase (*Ct*GH5) from *Clostridium thermocellum* by site-directed mutagenesis for development of mutant with enhanced activity" in "Bioprocessing India, Recent Trends in Bioprocessing for Healthcare, Energy and Environment" Dec 9-11, 2017, IIT Guwahati, Assam India.
3. Presented a poster entitled "Identification of promising functional residues capable of introducing endo-xylanase activity into an exo-acting arabinofuranosidase (*Ct*43Ara*f*) with enhanced activity: An *in silico* approach" in "56th International Annual Conference of Association of Microbiologists of India (AMI)", December 7-10, 2015, Jawaher Lal Nehru University, New Delhi. Best Poster Award)

Awards

1. Best poster award on the work entitled "Identification of promising functional residues capable of introducing endo-xylanase activity into an exo-acting arabinofuranosidase (*Ct*43Ara*f*) with enhanced activity: An *in silico* approach*"* in "56th International Annual Conference of Association of Microbiologists of India (AMI)" organized by Jawaher Lal Nehru University, New Delhi during December 7-10, 2015.