Thermophilic GH5_7 project

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1 Introduction

Glycoside Hydrolases can be used for production of novel glyconjugates usng transglycosylation reactions. This is of interest in utilizing waste streams in bioconversion of wood material, such as lignocellulose, which is underutilized in current industrial settings. Instead this material stream can be used to produce novel materials and platform chemicals only accessible through petrochemical sources in the past. With this in mind, I've set out to assess the potential of some Thermophilic GH:s for use in these reactions, as the thermostability is potentially helpful in industrial application. I'm also investiated the possibility of the structure of these enzymes is conducive to increased stability in organic solvents at lower temperature, besides being more stable at higher temperatures. The enzymes I will be utlizing are TpMan5A, from *Thermotoga petrophila* and BpMan5A, from *Bacillus pumilus*. These are described previously in literature, and will be discussed briefly in the sections Below:

1.1 TpMan5A

T. petrophila is a hyperthermophillic, Gram-negative bacterium from the Kubiki oil resevoir in Niigata (Japan), and its enzymes show good promise for biotech application. Some work has already been done on TpMan5A, described below:

- Santos et al [1] describes how the crystal structure (PDB: 1QNR) was generated. There are two crystals, each from two separate crystallization procedure (is this the reason for the rotamers in 1QNR?).
- dos Santos et. al [2] describes how the stability is affected in different deletion mutants, thus elucidating stabilizing elements in the structure. They also carried out a central composite experimental design in order to find optimal pH and temperature values, results of which is included in table 1. In summary, they determined that the ΔCBM mutant showed no significant change in substrate specificity or catalytic effiency, but showed much worse thermal stability ($\Delta T_{melt} = 100 - 88$).
- da Silva et al. [3] attempts to characterize TpMan5A through structural analysis and some DLS studies of thermostability. They found out that the second immunoglobulin-like domain acts as a thermostabilizing domain and allows the enzyme to act even under hyperthermal conditions.

1.2 BpMan5A

BpMan5A is a GH5 endo-1,4-β-mannosidase from *B. pumilus*. *B. pumilus* is a Gram-positive, aerobic, spore-forming bacterium commonly found in the *rhizosphere*, the area close to plant roots directly under the influence of plant secretions etc. *B. pumilus* is non-pathogenic an highly resistant to environmental stress. The specific strain collected in Zang et al [4] is collected from Tibet and has shown good ability in decomposing lignocellulosic plant mass.

- Zang et al. [4] identified BpMan5A as a mannanase, expressed, purified, and finally characterized it. pH optimum, pH activity, temperature optimum, and temperature stability (figure: 1). Its products are mainly M2, M3, M5 when utlizing Locust Bean Gum (LBG) as substrate.

2 Activity Measurement / Basic Characterization

The goal for me was to map out how the activity and stability of TpMan5A and BpMan5A were affected by temperature, pH, and the presence of various acceptor solutions in order to determine proper reaction parameters for maximizing glyconjugate production yield. Some of these values were available in literature, and will be presented in table 1:

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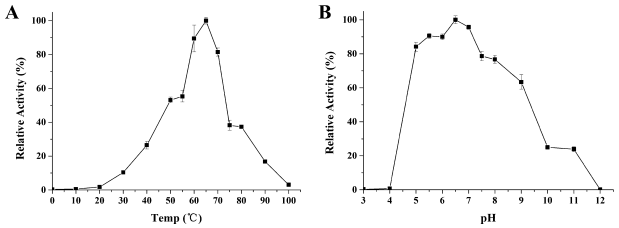

Fig. 3. Temperature (A) and pH (B) optima of the recombinant β-mannanase from *B. pumilus* GBSW19.
The optimal temperature was determined using 0.5% LBG in 0.1 M Tris–HCl buffer at 7.0, whereas the optimal pH was determined at 60 °C using 0.5% LBG.

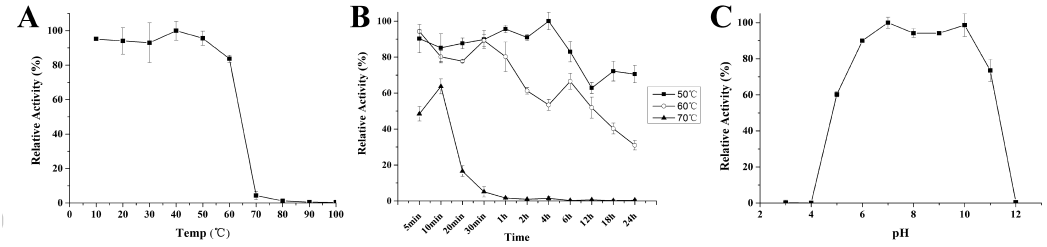

Figure 1: Shows some parameters characterized in Zang et al [4]

parameter:	TpMan5A	BpMan5A	source:
T_{opt}	65°C	65°C C	
T_{stab}	$90°C for 24h$	30min 70°C C	
pH_{opt}	6.5	6.5	
pH_{stab}	6→10	inactive at pH 3	

Table 1: Shows some optimal temp and pH for TpMan5A and BpMan5A, gathered on my own or supplied from literature.

3 Acceptor Screening

4 Future Project Plans

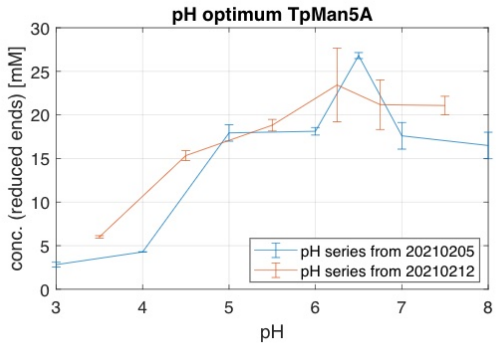

Figure 2: shows results of pH-resolved DNS assay of TpMan5A performed 20210212

References

[1] Camila Ramos Santos et al. "Cloning, expression, purification, crystallization and preliminary X-ray diffraction studies of the catalytic domain of a hyperthermostable endo-1,4-β-d-mannanase from Thermotoga petrophila RKU-1". In: *Acta Crystallographica Section F: Structural Biology and Crystallization Communications* 66.9 (2010), pp. 1078–1081. ISSN: 17443091. DOI: 10.1107/S1744309110029131. URL: www.pdb.org.

[2] Camila Ramos dos Santos et al. "Molecular insights into substrate specificity and thermal stability of a bacterial GH5-CBM27 endo-1,4-β-d-mannanase". In: *Journal of Structural Biology* 177.2 (Feb. 2012), pp. 469–476. ISSN: 10478477. DOI: 10.1016/j.jsb.2011.11.021.

[3] Viviam M. da Silva et al. "High-resolution structure of a modular hyperthermostable endo-β-1,4-mannanase from Thermotoga petrophila: The ancillary immunoglobulin-like module is a thermostabilizing domain". In: *Biochimica et Biophysica Acta - Proteins and Proteomics* 1868.8 (Aug. 2020), p. 140437. ISSN: 18781454. DOI: 10.1016/j.bbapap.2020.140437.

[4] Haoyu Zang et al. "A novel thermostable GH5$_7$$\beta$-mannanase from Bacillus pumilus GBSW19 and its application in manno-oligosaccharides (MOS) production". In: *Enzyme and Microbial Technology* 78 (Oct. 2015), pp. 1–9. ISSN: 18790909. DOI: 10.1016/j.enzmictec.2015.06.007.