Protein engineering of cellulase for shifting the optimum pH

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ABSTRACT

Cel12A from *Thermotoga maritima* is an endoglucanase that catalyzes the hydrolysis of β-1,4 linkages in β-glucans. Biodegredation of structural biopolymer of plant cell wall is a promising approach in industrial applications. Therefore, there are ongoing efforts to manipulate the structural and functional properties of cellulases. In this study, we aimed to manipulate the pH optimum of Cel12A. We employed directed evolution approach and used error-prone PCR to create a random library of mutants. The library was transformed into *E. coli* by electroporation and the Cel12A-encoding single colonies were screened on carboxymethyl cellulose agar plates under the manipulated pH conditions. Colonies that were capable of producing clear zones under acidic conditions were selected. The selected colonies were cultured and the recombinant enzymes were purified by Ni-chelate affinity chromatography. Catalytic activity of the purified enzymes were assayed by using carboxymethyl cellulose as the substrate. pH optimum, pH stability, optimum temperature, thermal stability and detergent stability parameters of the mutant enzymes were characterized and compared with the wild-type enzyme. A triple mutant of Cel12A showed a pH optimum shift from 5.5 to 5.0. Further work is ongoing in order to determine the effect of single point mutations on the pH shift.

PURPOSE

- **The purpose of this study is to manipulate pH optimum of TM Cel 12A by using the method called ‘’directed evolution’’.**

INTRODUCTION

- Cellulases are one of the most widely used industrial enzymes. Paper, detergent, food and beverage industry and bioethanol production are some areas that cellulases can be used[1].
- Manipulation of pH optimum of cellulases is of great importance for the application areas of cellulases[2].

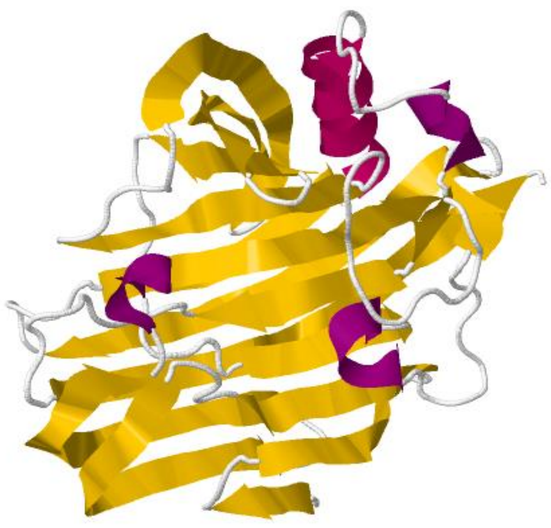

Figure 1 :Overall structure of the TM Cel12A

Rational Design and Directed Evolution

- Rational design is a technique that is based on the knowledge of structure-function relationship of protein [3].
- Directed Evolution is a powerful technique for the generation of novel enzymes with superior properties. Iterative cycles of mutagenesis and screening or selection are applied to modify protein properties, enhance catalytic activities, or develop compeletely new protein catalysts for non-natural chemical transformation[4].
- . In this study, we used directed evolution for the selection of enzyme variants that are active in more acidic conditions.

METHOD

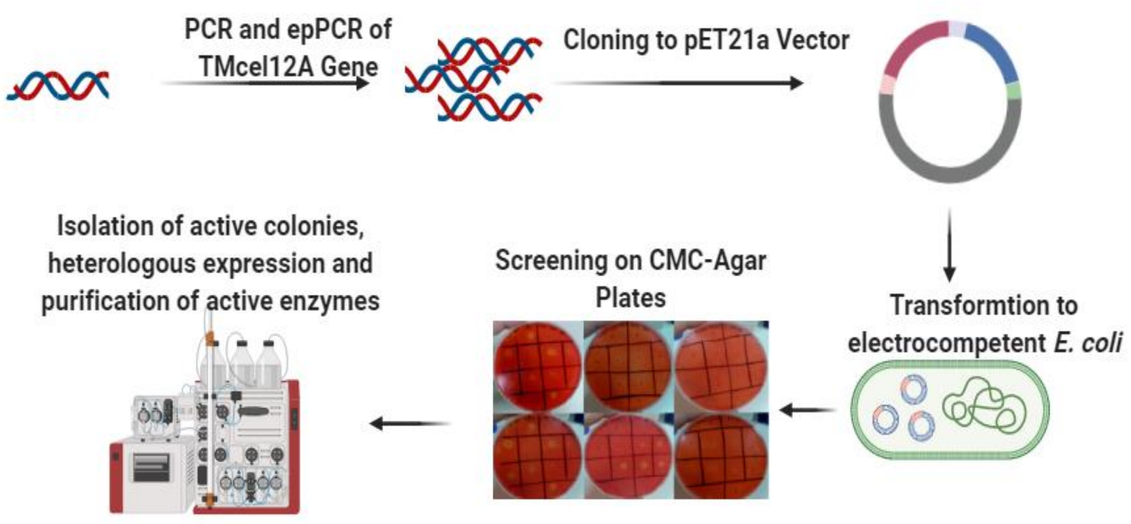

Figure 2 : Overall work flow

- In this study, directed evolution is the technique that was used for the screening of TM Cel12A variants according to their pH optimun profile.
- Random mutations introduced to the gene encoding TM Cel12A, cloned to pET21a vector and the library was transformed to electrocompetent *E.coli* cells.
- Single colonies screened on CMC-Agar plate and active colonies picked and used for heterologous protein expression. Purified enzyme variants were characterized according to their pH optimum,pH stability, optimum temperature, temperature stability and activity in the presence of detergents

RESULTS

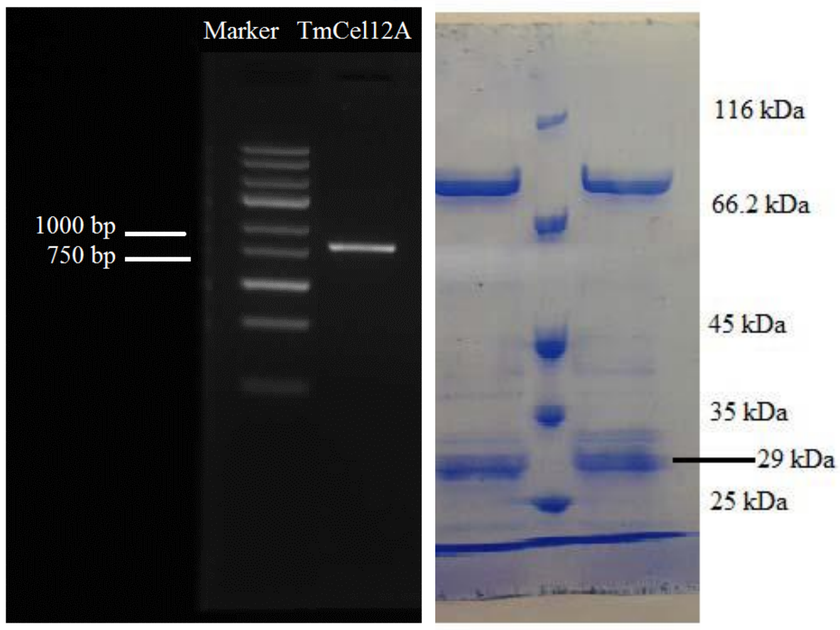

Figure 3: Agarose gel electrophoresis of TM Cel12A encoding gene and SDS-PAGE analysis of purified recombinant TM Cel12A. Agarose gel electrophoresis shows a single PCR product at 750 bp. SDS-PAGE analysis shows a 29 KDa TM Cel12A enzyme.

pH Optimum

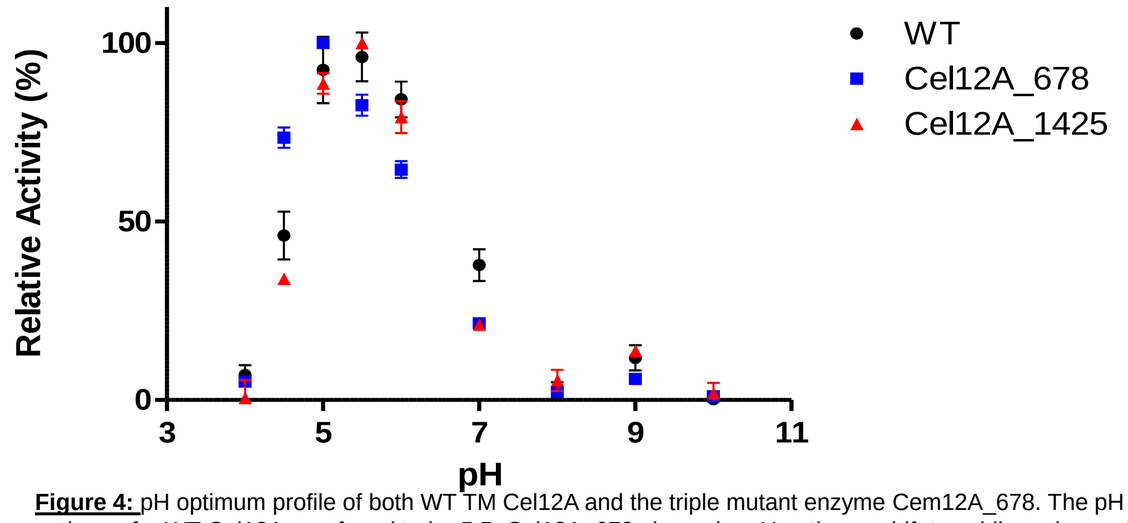

Figure 4: pH optimum profile of both WT TM Cel12A and the triple mutant enzyme Cem12A_678. The pH optimum for WT Cel12A was found to be 5.5. Cel12A_678 showed a pH optimum shift to acidic region and it was determined to be 5.0.

pH Stability

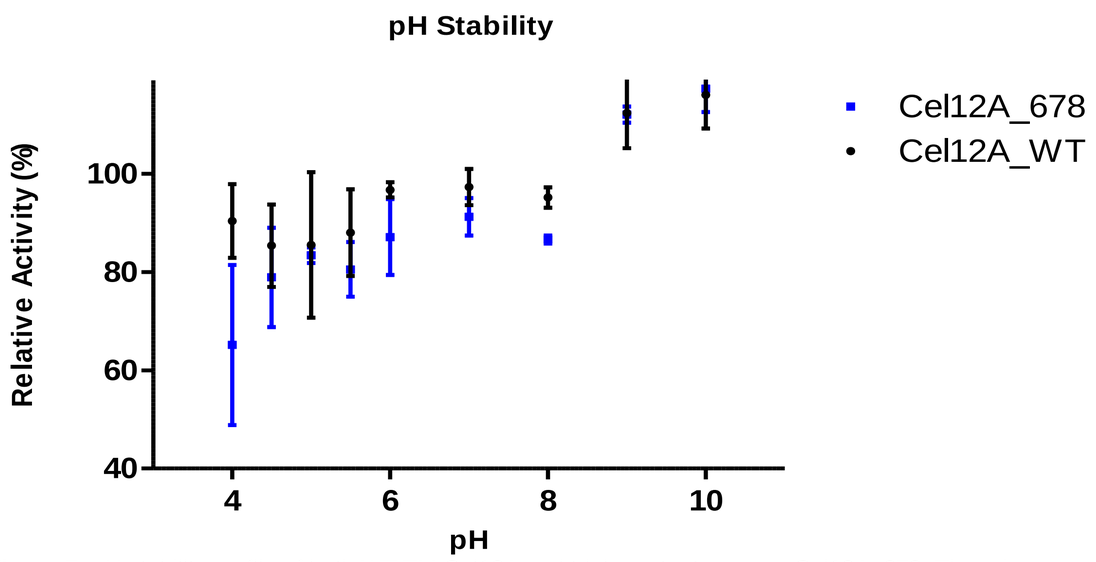

Figure 5: pH stability profile of both WT TM Cel12A and triple mutant enzyme Cel12A_678. There is no significant change in the pH stability profile of both WT and triple mutant enzyme.

ONGOING WORK

- ➢ Cloning, heterologous expression and purification of reverse mutated variants.
- ➢ Enzymatic characterization of reverse mutated TM Cel12A variants in order to understand point mutations that are responsible for pH optimum shift.

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