Effects of different domains on the activity of a GH78 α-L-rhamnosidase

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Key words: α-L-rhamnosidases; domain; enzyme activity; truncated mutants (TM).
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α-L-Rhamnosidases (EC 3.2.1.40) are a widely spread and industrially important group of glycoside hydrolases, which are responsible for cleavage of terminal α-L-rhamnose residues from the nonreducing end of carbohydrates and their derivatives (Yadav et al., 2010). α-L-Rhamnosidase has been widely found in nature and has been reported from animals, plants, yeasts, fungi, and bacteria (Naumoff and Dedysh, 2012). The majority of characterized bacterial α-L-rhamnosidases to date originate from Gram-positive bacteria, i.e. *Bacillus*, *Lactobacillus*, *Staphylococcus*, *Clostridium*, *Streptomyces* and *Thermomicrobium* species (Lise et al., 2016). Most known bacterial-L-*rham*nosidases are classified into the GH 78 family (GH78) because of possessing the glycoside hydrolase (GH) motif. According to the CAZy database, GH 78 family contains 470 proteins, including 13 proteins from the Archaea, 394 proteins from the Bacteria, and 59 proteins from members of the Eukaryota (Naumoff and Dedysh, 2012). Eighteen of these proteins are characterized biochemically and display the same type of enzymatic activity. For instance, up to now, two α-L-rhamnosidases were identified by activity assay in a comprehensive functional study on glycoside hydrolases (GHs) from cow rumen on the functional level (Ferrer et al., 2012). Two crystal structures of α-L-rhamnosidases have been determined: α-L-rhamnosidase B (BsRhaB) from *Bacillus* sp. GL1 and the putative α-L-rhamnosidase BT1001 from *Bacteroides thetaiotaomicron* VPI-5482, which are both from GH78 family (Ferrer et al., 2012). The α-L-rhamnosidases are of interest for chemical industry, especially pharmaceutical and food industries (Manzanares et al., 2007). The commercial enzyme preparations include naringinase and hesperidinase comprising α-L-rhamnosidase next to β-D-glucosidase activity, which are applied for debittering and clarifying of fruit juices (Li et al., 2018; Ribeiro, 2011). The aroma optimization of wine is a further potential field of application of α-L-rhamnosidases (Michlmayr et al., 2011). In addition, the derhamnosylating function of α-L-rhamnosidases can enhance the pharmacological properties of drugs like antibiotics as chloropolysporin, steroids as ruscin or gypenosides (Yu et al., 2004). Therefore, the α-L-rhamnosidases are of considerable interest given their suitability in various applications within the food and pharmaceutical industries, which are worth looking insight into their structure and regulatory mechanism for further applications.

Proteins are composed of evolutionary units called domains, the majority of proteins consist of at least two domains. As these domains and nature of their interactions determine the function of the protein, it is important to understand the principles of domain combinations and interactions (Christine et al., 2004). A combined sequence and function-based analysis of a metagenomic library DNA derived from elephant feces was performed and a novel bacterial α-L-rhamnosidase belonging to GH78 was identified in a published assay. Five different ORFs rhaB, rhaB1, rhaB2, and rhaB3 were amplfied and overexpressed, and the most active open reading frame (ORF) to be of 3081 bp designating rhaB1 was identified. RhaB presented an unexpected and novel enzyme domain architecture, comprising typical bacterial α-L-rhamnosidase domains co-organized with lipolytic GDSL-like lipase and acetyl-xylan esterase motifs. However, the detailed catalytic mechanism of rhaB1 is unclear, and the role of the surrounding domains are unknown. Therefore, we aimed to explore how domains function by studying the catalytic activities present in the wild-type polypeptide and its various mutants.

Fig 1. Modeled structures of rhaB1 wild type and its truncated mutants.

In the present study, in order to determin the catalytic machanism of rhaB1, we expressed the wild type gene and its truncated mutants to analyze their polypeptides for enzymatic activities. Based on the catalytic domain, five truncated mutants were designed (Fig 1). The physicochemical parameters were determined with *p*NP-rham as a substrate. Fig. 2A show that protein constructs were successfully expressed as soluble proteins. The predicted sizes of the truncated mutant proteins TM1, TM2 and TM3 were 76kDa, 56kDa and 46kDa, respectively, which were in accordance with obtained SDS-PAGE results. As shown in Fig. 2B, specific activities were determined for the wild-type protein and each of the mutants with *p*NP-rham. The enzyme activity of TM1 was 190.55 U/mL, which showed a slight increase compared to the WT enzyme with 169.95 U/mL. The explanation for this was probably that N-terminal module with 335 amino acids of rhaB1 wide type showed little significance on the catalytic module, leading to the increase in enayme activity in TM1. In contrast, no *α*-L-rhamnosidase activity was detected in *p*NP-rham assays of crude cell extracts of truncated mutant TM2, TM3, TM4, TM5. It was because the functions of the other domains contributed to the catalytic module, resulting in the decrease in the enzyme activity once they were cut off.

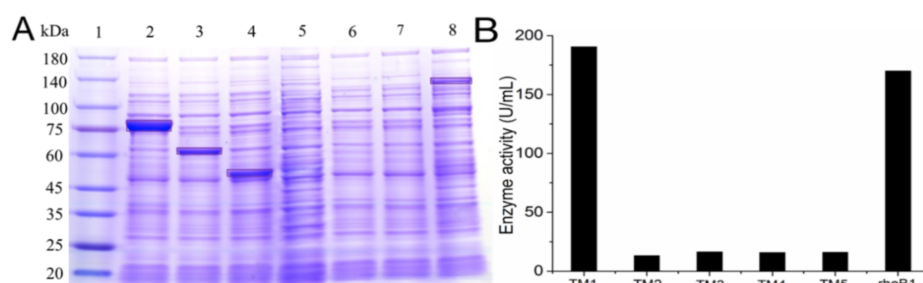

Fig 2. (A) SDS-PAGE of the rhaB1 wild type and its truncation mutants. Lane 1, protein molecular mass marker; lane 2, rhaB1TM1; lane 3, rhaB1TM2; lane 4, rhaB1TM3; lane 5, rhaB1TM4; lane 6, rhaB1TM5; lane 7, PET28a; lane 8, rhaB1 wide type. (B) Activity profiles of rhaB1 wild type and its truncation mutants.

In conclusion, effects of different domains on the activity of a novel GH78 *α*-L-rhamnosidases from elephant feces were analysed, TM1 was the most active truncated mutant of rhaB1 compared to the other mutants, which indicated the different functions of N-terminal and C-terminal module to the enzyme activity. Our fndings might discover a promising alternative biocatalyst for biotransformation and provide an opportunity for better understanding of the catalytic mechanism of GH78 *α*-L-rhamnosidases.

Acknowledgements: This work was supported by the Natural Science Foundation of China (21676130), the Key Project of University Science Research of Jiangsu Province (16KJA530002).

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