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Characterization of a novel hyperthermophilic glycosyltransferase GT-2 from the Archaea Pyrococus horikoshii

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PURPOSE OF THE ABSTRACT

Glycosyltransferases (GTs) are enzymes involved in the synthesis of glycoconjugates and polysaccharides, molecules with a wide range of biological activities, and as such are very attractive for biotechnological applications. GTs catalyze the formation of glycosidic bonds through the transfer of activated sugar donors to a diversity of acceptor molecules such as proteins, lipids or carbohydrate residues, with a very tight control either on the nature of both donor and acceptor substrates and on the stereochemistry of the glycosidic bond itself [1]. GTs are classified in more than 116 families according to the Carbohydrate-Active enzyme (CAZY) database [2].. Enzymes from the GT-2 family are involved in the synthesis of several linear polysaccharides such as cellulose, chitin or hyaluronic acid. The latter is of particular interest due to its numerous clinical and cosmetic applications. A genome-wide survey analysis of GT-2 encoding sequences in the three domains of life was performed and revealed an uncharacterized separate clade, closely related to a clade including hyaluronan synthases [3](Khaled et al., Unpublished; Amin et al., EPO under revision). Members of this family are typically processive enzymes, displaying a GT-A fold with two Rossman fold-like domains. A topology prediction revealed that proteins of this family are transmembrane proteins and show a similar folding as the hyaluronan synthase (HAS), either human or bacterial HAS, and hence were named HAS-like. Sequences corresponding to this new clade were particularly found in hyperthermophilic Archaea genomes. Enzymes from hyperthermophilic microorganisms are of high biotechnological interest as they generally display high robustness and thermostability allowing their use in various industrial processes requiring elevated temperatures [4]. The objective of our study was therefore to biochemically characterize a representative of this clade from the hyperthermophilic archaea Pyrococcus horikoshii, thereafter named Ph-has-like. The recombinant protein Ph-has-like was expressed in E.coli using different expression systems. The full-length transmembrane protein (1-314 aa) was first produced but was difficult to overexpress and purify. Domain analysis of the protein showed that the catalytic domain was located mainly in the N-terminal soluble cytoplasmic region. Site-directed mutagenesis was then performed to express a truncated form (1-232 aa) of Ph-Has-like displaying the catalytic domains but not the transmembrane domain. Several constructions were performed to express the soluble form of the protein with and without tags. After several optimizations using different E.coli strains and induction conditions, the protein was successfully produced as a soluble form from the BL21 (DE3) pLysS E. coli strain and purified by affinity chromatography. The biochemical activity of the protein is currently under investigation, particularly, the activity of the hyperthermophilic enzyme on a wide range of substrates used by processive GT-2 of the same family. For that purpose we have developed an HPLC-based method for the evaluation of such GT activity. Our work represents one of the few characterized GT-2 enzymes from Archaea so far. It will contribute to the elucidation of the enzymatic mechanism of polysaccharide synthesis and enlarge our understanding on the behavior of these enzymes at high temperature.

FIGURES

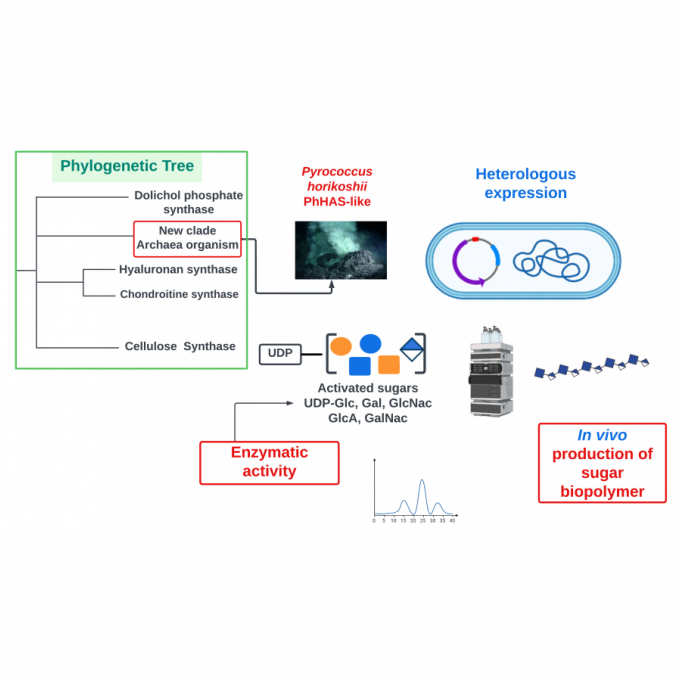

FIGURE 1

Overview of stategy developed to characterize a novel GT-2 from Pyrococcus Horikoshii

Enzymatic activity of recombinant PhHas-like purified (left). In vivo production of the new polysaccharide (right).

FIGURE 2

KEYWORDS

Glycosyltransferase GT-2 | Hyperthermophilic Archaea | Truncated protein | Glycosaminoglycan

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