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Phylogenetic Assessment of Cyclodextrin Glucanotransferase Producing Microorganisms and Structural Study

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ABSTRACT

Cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) is an extracellular enzyme found in various microorganisms which catalyzes three types of transglycosylation reactions like cyclization, coupling, disproportionation as well as hydrolysis action on various substrates. By a cyclization reaction, starch is converted into cyclodextrins that is widely used in various industries for its immense structural properties. To improve the activity of CGTases, screening of CGTase from novel organisms having higher yield of specific products, thermal stability, substrate specificity is required. This paper gives the path to identify the novel organism producing CGTase. The property of that enzyme with specific amino acid sequence will lead to the more specificity for the enzyme engineering with downstream processing.

Keywords: Cyclodextrin glucanotransferase, phylogeny, structural study, conserved domains

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INTRODUCTION

A unique member of the glycoside hydrolase 13 family is cyclodextrin glucanotransferase (EC 2.4.1.19). It catalyze the transglycosylation reactions like cyclization, disproportionation and coupling. Its distinctive characteristic is the capacity to convert starch into cyclodextrins via an intramolecular cyclization process. CGTase produce major α, β, γ- cyclodextrins via cyclization, where as coupling is used for synthesis and modification of alkyl glycosides[1]. The coupling reaction of CGTase opens the cyclodextrin ring and transfers glycosyl groups to acceptors, while the disproportionation reaction by CGTase shifts the linear oligosaccharides to another oligosaccharides (Figure 1) [2]. This transition of the glycosyl group improves water solubility, bioavailability, absorption, and bioactivity of acceptors [3]. Moreover, cyclodextrins can be used in foods, pharmaceuticals and cosmetics, and the cyclization reaction has, therefore, been extensively studied. CGTase belongs to the amylase family with five domain structures (A1, B, A2, C, D, E). Amylases have three conserved domain while CGTase have five domains. Domain A and B have the catalytic sites while Domain C and E have carbohydrate binding sites, which involve in raw starch binding [5].

In the present study, we summarize CGTase producing organisms and its relation between each other. Sequences of CGTase from various sources reveal the site specificity of CGTase for α-CD, β- CD, and γ-CD production, catalytic sites conserved throughout the CGTases in all organisms.

MATERIAL AND METHODS

Sequence Retrieval

For the evaluation of relationship between different types CGTase producing organisms, gene sequence demonstrated activity of all CGTase were retrieved from NCBI (available till November 2022). The structure of crystallographic protein was retrieved from PDB.

Phylogenetic analysis of CGTase producing organisms

Multiple sequence alignment of CGTase sequences were performed using MEGA software. Protein sequences were aligned with MUSCLE by using default settings and constructed phylogenetic tree using maximum likelihood method [6].

Amino acid sequence comparison of various CGTases

Manually annotated protein sequences were selected in the UniProt database for comparative analysis of CGTase sequences using Clustal W [7]. Sequence similarity and information of secondary structure was assessed by ESPript 3.0. Structure of various CGTase was compared for the further approach of thermostability and product specificity.

RESULT AND DISCUSSION

Phylogenetic analysis

Most of the CGTase enzymes are produced by various bacteria. *Bacillus*, *Geobacillus*, *Paenibacillus*, *Klebsiella*, *Pyrococcus*, *Brevibacterium*, , *Thermococcus*, *Haloferax*, *Thermoanaerobacter*, *Microbacterium* and *Anaerobranca* are some of the bacteria that produce the CGTase extracellularly [8]. Some of the archaea and fungi can also produce CGTase. The archaea that are studied for production of CGTase are *Haloferax* , *Thermococcus*, *Carboxydocella* and *Pyrococcus* [9]. Fungi that are producing CGTase are *Aspergillus* and *Trihoderma viride*. However, CGTase is most commonly produced by different strains of *Bacillus*, such as *B. agaradharens*, *B. circulans*, *B. stearothermophilus*, *B. macerans*, *B. firmus*, *B. cereus*, and *B. pseudalcaliphilus*. Other known CGTase producing bacteria include *Paenibacillus graminis*, *Klebsiella pneumoniae*, *Amphibacillus* sp. The CGTase producing microbes are mainly found in soil, lake water, hot springs, wastewater from flour industry and rotten potatoes. Phylogenetic relation of various CGTase producing organisms is shown in Figure 2.

Structural study and Amino acid sequence evaluation

GH13 family having four to seven conserved sequence regions (CSRs) [10]. Among all five domains of CGTase (Figure 3), the B domain pierces the folded TIM barrel-like structure of the A domain. The A1 domain is the section of the protein that starts at the N-terminus, while the A2 domain is the next segment after the B domain. The A1-B-A2 domains are folded into an architecture in which a groove or cleft serves as an active site for each of the four CGTase processes [11]. The C domain, which is involved in starch binding, has an anti-parallel beta-sandwich shape. The D domain is exclusively found in CGTase, but the function is unknown. Domain E is referred as the raw starch binding domain. Domain E is closely related to the cyclization activity of CGTase [12]. All CGTase The four structurally conserved regions present in the domain A that is covered by the strands β3, β4 , β5 and β7, other 3 conserved regions are present at the C-terminal of B domain that is covered by the strands β2, β8 [13]. The literature shows that the three catalytic residues in all CGTases are Asp222, Glu250, and Asp321 which are located at the conserved regions II, III, and IV, respectively shown in Figure 4, which have different catalytic roles in CGTase [14]. Three conserved histidine residues, His133, His226, and His320 are present in all CGTase sequence regions (CSR-I, CSR- II, and CSR-IV) and they are involved in the stabilization of the transition state and the substrate recognition of active sites [15]. An Asp residue present in CSR-V involved in the binding of the Ca^{2+} [16]. CGTases and α-amylases can be recognized by CSR-VI. While CSR-VI of α-amylases contains highly conserved glycine and proline residues, CSR-VI of CGTase has highly conserved tryptophan and glutamine residues [17]. The three active site residues in CGTase are Glu257, Asp229, and Asp328, as determined by the examination of several crystal structures for this enzyme. Asp229 creates a covalent intermediate with the cleaved substrate prior to CD formation, Glu257 functions as both a proton donor and acceptor, and Asp328 stabilizes the reaction intermediates [18]. A sequence alignment of selected CGTases was performed, revealing seven conserved amino acid residues, or conserved sequence regions. When compared to α-CGTases and β-CGTases, the area between residues 145 and 152 (based on the *B. circulans* 251 CGTase) that makes up six amino acids at subsite 7 is entirely absent in γ-CGTase. The residue 47 in subsite -3, which can be Arg and Lys (in β-CGTases), Lys (in α-CGTases), or Thr (in γ-CGTases), determines product specificity [19]. With only two mutations (Asn/Asp29 and Asp/His199), the two Ca^{2+} binding sites (CBSI and CBSII) were substantially conserved in each CGTase sequence, pointing to their potential importance in determining CD selectivity [20]. A sequence alignment of selected CGTases was shown in Figure 5.

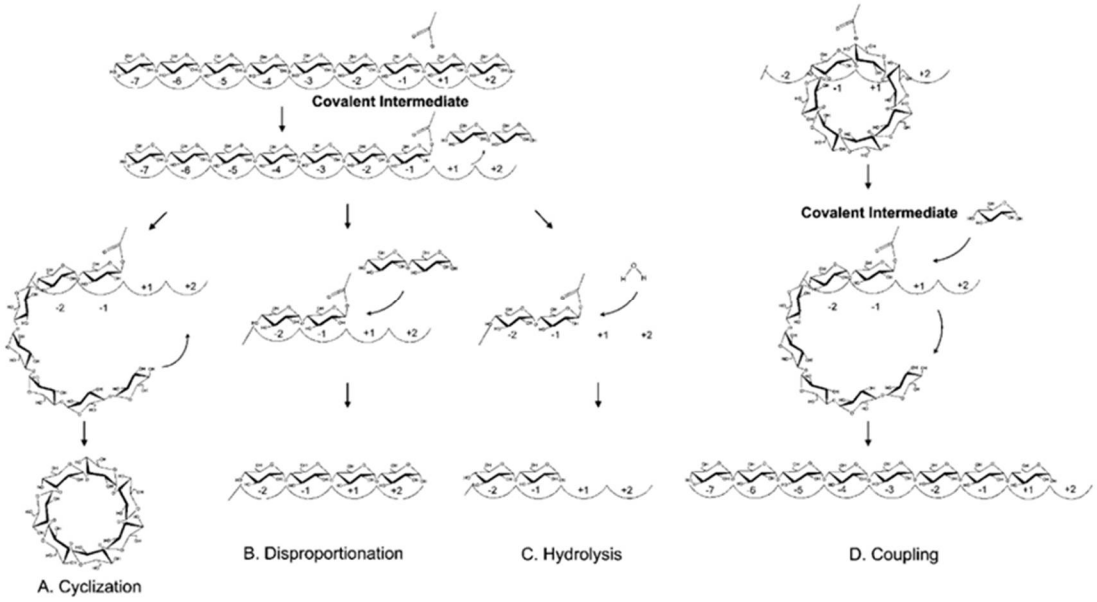

Figure 1: Schematic representation of various reactions catalyzed by Cyclodextrin glucanotransferase enzyme. This figure has been adapted from [4].

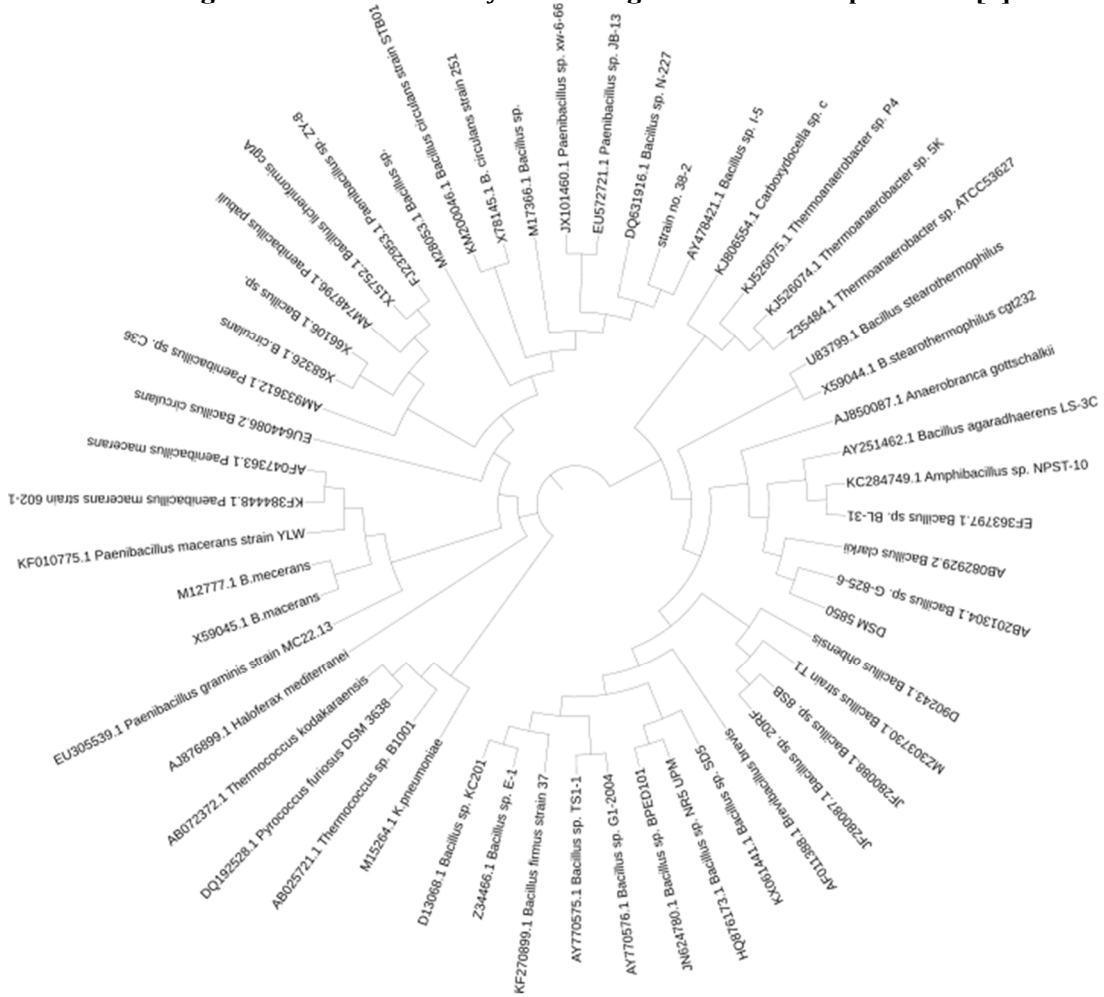

Figure 2: Phylogenetic relation between various CGTase gene sequence from source organisms using maximum likelihood method.

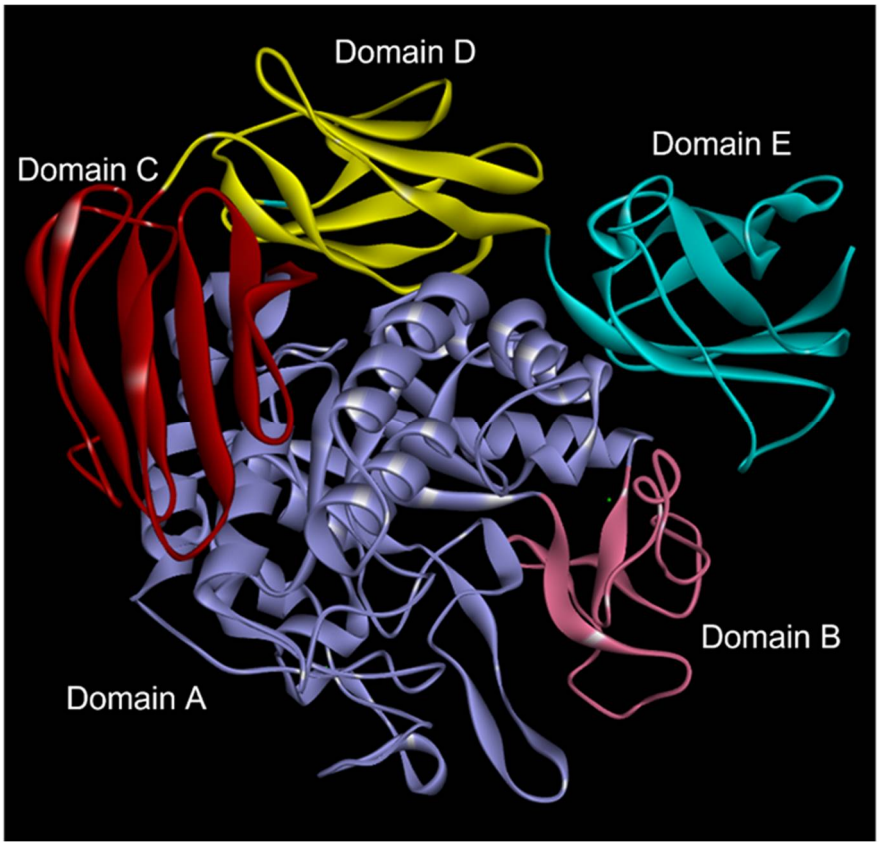

Figure 3: Structure of CGTase with its five conserved domains (PDB Id: 1CXK).

Protein Id	Region I	Region II	Region III	Region IV	Region V	Region VI	Region VII
	◆◆	★ ◆◆	★	◆★	◆		
P31746	DFTPNH	DGIRVDAVKH	FGEWFL	FIDNHDM	DLADY	GITALWISQP	GVPTIYYGT
P31797	DFAPNH	DGIRMDAVKH	FGEWFL	FIDNHDM	DLADL	GVTAIWISQP	GVPNIYYGT
P26827	DFAPNH	DGIRLDAVKH	FGEWFL	FIDNHDM	DLADL	GVTAIWISQP	GVPAIYYGT
P30920	DFAPNH	DGIRVDAVKH	FGEWFL	FIDNHDM	DLADF	GVTALWISQP	GVPAIYYGT
P05618	DFAPNH	DGIRVDAVKH	FGEWFL	FIDNHDM	DLADL	GITAIWISQP	GVPAIYYGS
P09121	DFAPNH	DGIRVDAVKH	FGEWFL	FIDNHDM	DLADL	GITAIWISQP	GVPAIYYGS
P43379	DFAPNH	DGIRMDAVKH	FGEWFL	FIDNHDM	DLADL	GVTAIWISQP	GVPAIYYGT
P30921	DFAPNH	DGIRMDAVKH	FGEWFL	FIDNHDM	DLADL	GVTAIWISQP	GVPAIYYGT
	:*	****:*****	******	*******	****	*:**:*****	*** ****:

Figure 4: CGTase sequences show the conserved regions among all. Red dot suggests Ca2+ binding site, star shows catalytic residues and blue square shows the conserved histidine residues present in all CGTase the source of CGTase: *Bacillus* sp. strain 1-1(P31797), *Geobacillus stearothermophilus* (P31797), *Thermoanaerobacterium thermosulfurigenes* (P26827), *Bacillus circulans* (P30920), *Bacillus* sp. strain 1011 (P05618), *Bacillus* sp. strain 38-2 (P09121), *Niallia circulans* (P43379), *Bacillus* sp. strain 17-1 (P30921).

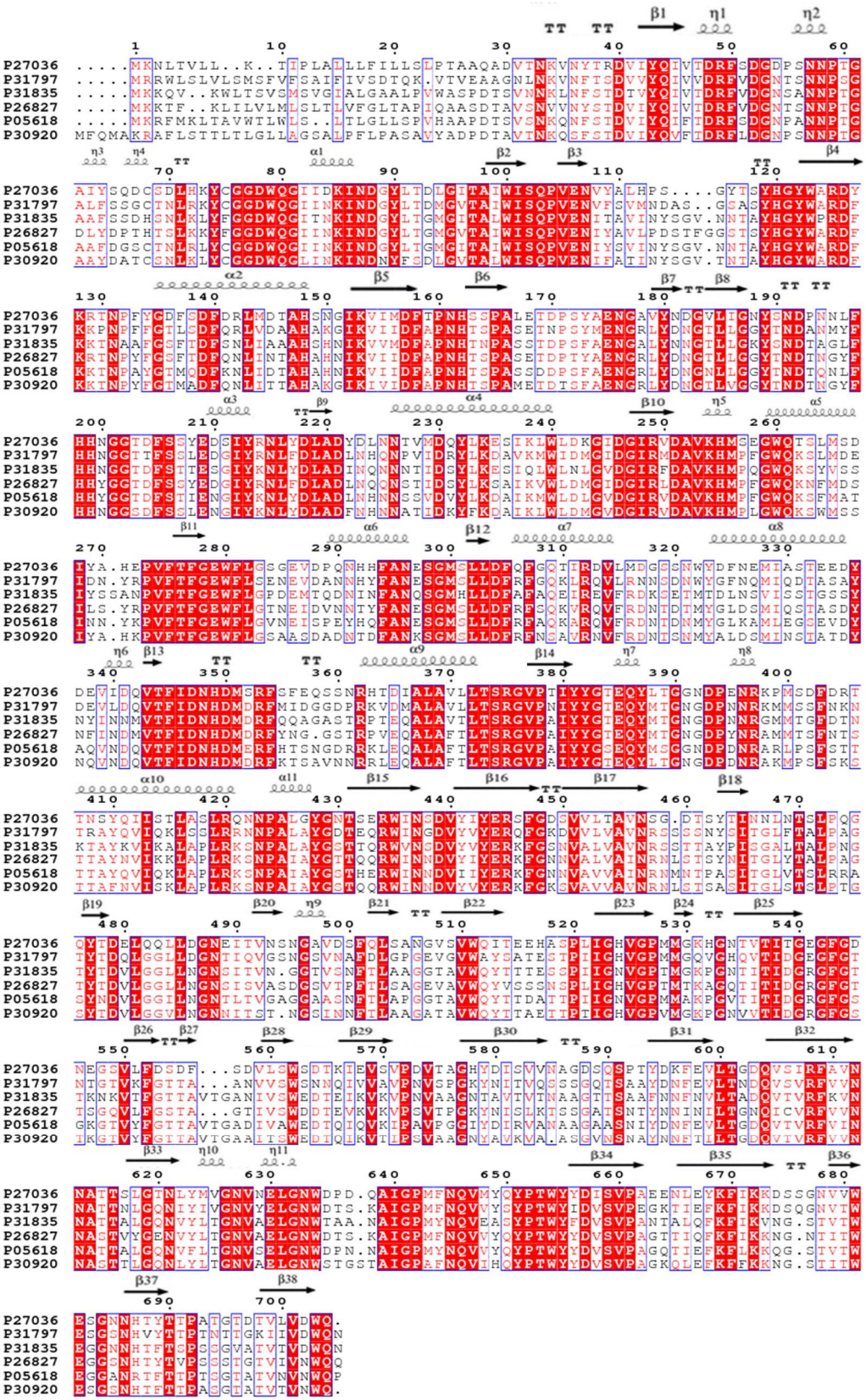

Figure 5: Multiple sequence alignment of CGTase with homologous CGTases from the UniProt database. Sources of the enzymes and accession numbers: *Bacillus ohbensis* (P27036), *G. stearothermophilus* (P31797), *Paenibacillus macerans* (P31835), *Thermoanaerobacterium thermosulfurogenes* (P26827), *Bacillus* sp. strain 1011 (P05618), *Bacillus* circulans strain 8 (P30920).

CONCLUSION

Genetic and chemical modification can be done to characterize the CGTase with specific activity like substrate specificity, product specificity, pH stability and thermal stability, suggesting the consequence of specific amino acid present in the structure of CGTase [21]. Catalytic sites and the residues involved in the enzymatic reaction can help in the ligand protein interaction prediction and product specificity of the enzyme [22]. Phylogenetic approach and sequence similarity for specific productivity as well conserved domains will help in identification of protein and molecular modification of novel or putative CGTase for downstream processing.

CONFLICTS OF INTERESTS

There aren't any conflicts of interest, according to the writers.

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