Cloning, overexpression, purification and characterization of maltooligosyltrehalose synthase from a cyanobacterium, *Anabaena 7120*

Trehalose is a non-reducing sugar with low energy (1 kcal mol$^{-1}$), withstands 100°C and is functional in the pH range 3.5–10 (ref. 1). Therefore, it is used as a stabilizer and preservative of foodstuff, cryoprotectant for cells in medicine, in cosmetics and various biotechnological purposes$^2$. It has also been reported as a signaling molecule of higher plants$^{3,4}$, and plays an important role in *Mycobacterium tuberculosis*–host interactions$^2$ and in Huntington’s and Alzheimer’s diseases$^5$. There are five types of trehalose biosynthetic pathways reported in various organisms$^1$. One of the pathways having maltooligosyltrehalose synthase (MTSase) (allo167) and maltooligosyltrehalose trehalohydrolase (MThase) (allo168) enzymes has superiority in trehalose production (up to 80–92%) in many bacteria$^1$. A gene cluster constituting trehalose biosynthesizing enzymes, MTSase and MThase, along with trehalase (allo168), has been reported in *Anabaena 7120* (ref. 9) and is maximally expressed in *Anabaena 7120* exposed to 150 mM NaCl (refs 10, 11). In addition, increase in trehalose yield may be achieved through manipulation of such enzymes.

The MTSase gene was PCR-amplified and cloned in pGEM-T Easy vector (Promega) and sub-cloned in pET-19b (Novagen) expression vector. The recombinant was transformed in *Escherichia coli* BL21 (DE3) and expressed optimally in isopropyl β-D-1 thiogalactopyranoside (IPTG) (1 mM)-induced cultures (14 h, 28°C). The recombinant MTSase enzyme purified by Ni$^{2+}$-NTA affinity chromatography showed maximum transglycosylation activity at pH 6 (40°C), with maltolhexaoase as the substrate. To our knowledge, there are no other reports on the expression, purification and characterization of MTSase from a cyanobacterium.

*Anabaena 7120* was grown in BG-11 medium$^{12}$ free from combined N-source under continuous tungsten plus fluorescent illumination (14.40 Wm$^{-2}$) at 28 ± 1°C. *E. coli* NM was used as the host for vectors. The amino acid sequence of MTSase of *Anabaena 7120* was compared with MTSase from different microbes. Protein sequence databases (Swiss Port, PIR and GenBank) were searched using the standard search algorithm, BLASTP (NCBI, NIH). The sequences were aligned and a phylogenetic tree was generated based on the neighbour-joining (NJ) algorithm using Clustal X$^{13}$.

Genomic DNA from *Anabaena 7120* was isolated using Marmur method$^{14}$, PCR-amplified allo167 from the genomic DNA using primers was designed from the nucleotide sequence (http://bacteria.kazusa.or.jp/cyanobase; GenBank accession code, Q8ZOD1). The forward primer (5′ GCG CAT ATG CGA ATT CCT AAA GCT AC 3′) and reverse primer (5′ GCG GGA TCC TTA TTC GGC GAT TAA CAG G 3′) contained the restriction


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Figure 1. Multiple sequence alignment of amino acids in maltooligosyltrehalose synthase (MTSase) of Anabaena PCC 7120 with the same enzyme of selected prokaryotes. *indicates conserved amino acids in ito, colors, high and, dots, low conservation. A.7120, Anabaena (Nostoc PCC 7120); N. punc, Nostoc punctiforme; A. vari, Anabaena variabilis, C.7424, Cyanothece PCC 7424; Synec., Synechococcus, Lyngb, Lyngbya; S. acid, Sulfolobus acidocaldarius; S. sulf., Sulfolobus solfataricus; P95869, M. hako, Metallosphaera hakonensis; Q6SZP7, C. glut, Corynebacterium glutamicum; M. smeg, Mycobacterium smegmatis; Rhiz., Rhizobium; X. oryz, Xanthomonas oryzae; B. helv, Brevibacterium helvolum, and Arthr, Arthrobacter.
sites (underlined) for NdeI and BamHI respectively, and the stop codon (bold bases). The PCR reaction mixture (100 μl) contained 25 pmol each of primer, 50 μg of genomic DNA, 0.2 mM each of 2-deoxynucleotide 5-triphosphate (dNTP) and 1 U of Taq polymerase. The programme included pre-denaturation at 95°C (5 min), denaturation at 95°C (1 min), annealing at 49°C (1 min) and extension at 72°C (1.3 min) for 30 cycles with a final extension at 72°C (15 min). Recombinant plasmid was ligated into pET-19b vector having His tag.

Overnight-grown culture (2 ml) was added to fresh LB medium containing ampicillin (100 μg ml⁻¹) and grown until OD₆₀₀ reached 0.6–0.8. The culture was centrifuged (10,000 rpm, 15 min, 4°C) and the supernatant and put on an orbital shaker temperature under standard conditions.

The purified enzyme in sodium phosphate buffer (pH 7.4) was spotted on agar plates with 0.5% starch. The plates were incubated at 37°C (48 h) and hydrolytic areas observed after spraying with iodine solution or in the presence of congo red (0.1%) representing the enzyme activity.

MTSase of Anabaena 7120 contained amino acid sequences corresponding to the four highly conserved regions in the α-amylase family along with other conserved regions and residues (Figure 1). The phylogenetic tree showed the cyanobacterial MTSase in a separate cluster (Figure 2 a). Multiple sequence alignment of translated amino acid sequences of MTSase from different organisms showed several conserved regions and specific amino acids such as D330, E357 and D600 residues of Anabaena 7120 (Figure 1) corresponding to D228, E255 and D443 residues of Sulfolobus acidocaldarius, as reported by Maruta et al., indicating its importance in specificity of MTSase, as mutations in such residues led to the loss of transglycosylation and hydrolytic activity. In a mutational analysis of the MTSase enzyme in Sulfolobus solfataricus, alterations in F405 residue (although in the non-conserved region) resulted in higher trehalose yield from starch. The F405 now corresponds to M442 of Anabaena 7120 after multiple sequence alignment, thus paving the way for putative change(s) that can be brought in MTSase of Anabaena 7120 leading to enhanced trehalose production.

The molecular weight of MTSase enzyme of Anabaena 7120 is 105.56 kDa (according to http://genome.kazusa.or.

Table 1. Comparison of maltooligosyltrehalose synthase activity in preparations from cells of Escherichia coli BL 21 (DE3) harbouring recombinant vector

<table>
<thead>
<tr>
<th>Source</th>
<th>Total protein (mg)</th>
<th>Total activity (U)</th>
<th>Specific activity (U mg⁻¹)</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell extract</td>
<td>160</td>
<td>40</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td>Ni²⁺-NTA column purified</td>
<td>1</td>
<td>25</td>
<td>25.0</td>
<td>100</td>
</tr>
</tbody>
</table>
Dumortierite from Susunia Hill, Bankura District, West Bengal, India

Susunia Hill (442 m high), famous for its holy spring, is located about 27 km northwest of Bankura town, in the Chhotanagpur gneissic plateau of West Bengal (Figure 1). The hill is chiefly composed of sillimanite and kyanite-bearing quartzites, which at places show evidence of shearing. The bedding planes dip generally 20°–25° towards 10°. However, owing to some folding the strike varies to the east, northeast and northwest.

An outcrop of blue-coloured pegmatitic rock trending N60°W–S60°E, covering few square metres area is found within the quartzite country rock of Susunia Hill (around 23°23′39″E, 86°59′11″N). Physically, the blue-coloured mineral which gives the overall blue hue to the rock, is similar to kyanite in appearance (Figure 2a and b). However, microscopic studies show that unlike kyanite this mineral is strongly pleochroic from colourless to azure blue (Figure 2c and d). The mineral is euhedral to subhedral, shows one set of perfect cleavage parallel to elongation and cross fractures and straight extinction with respect to cleavage. Petrographic study also reveals the presence of tourmaline and quartz in the pegmatite. Tourmaline grains are complexly zoned at places and vary considerably both in shape and size (Figure 2d).


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