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Cloning, expression and activity optimization of trehalose synthase from *Thermus thermophilus* HB27

Yan Li, Xinxiao Sun, Yue Feng, Qipeng Yuan*

State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, No. 15 East Road of North Third Ring, Chao Yang District, Beijing 100029, China

HIGHLIGHTS

- The trehalose synthase gene from *Thermus thermophilus* HB27 was successfully expressed in *Escherichia coli* Rosetta (DE3).
- The trehalose synthase protein solubility was enhanced by coexpressing molecular chaperone.
- Ca^{2+} and reductants could improve the activity of trehalose synthase.

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ABSTRACT

Five different sources of trehalose synthase genes and four plasmids have been used to construct recombinant plasmids for the highest trehalose bioconversion in *Escherichia coli* Rosetta (DE3). The results showed that plasmids with different promoters and copy numbers played an important role in the expression of trehalose synthase genes in *E. coli*. The trehalose synthase from *Thermus thermophilus* in the plasmid pET-22b was selected for the subsequent studies for its high activity in trehalose production, with 1.996 U/mg protein. We also coexpressed molecular chaperones sigma32, GroEL, GroES, DnaK and DnaJ with trehalose synthase gene tttreS and this greatly increased the solubility of the trehalose synthase protein. The enzymatic reaction conditions for the trehalose bioconversion was optimized, and the optimal temperature, pH, reaction time and substrate concentration were determined as 50 °C, 9.0, 10 h and 10% maltose solution, respectively. Following the addition of Ca^{2+} and reductant DTT and Vc, the activity of trehalose synthase was enhanced up to 19% by Ca^{2+} and 41% by DTT.

1. Introduction

Trehalose (*R*-D-glucopyranosyl-1,1-*R*-D-glucopyranoside) is a non-reducing disaccharide found in various organisms including bacteria, algae, fungi, yeasts, plants and some mammals (Elbein et al., 2003; Zhang et al., 2010). It could act as a protectant against a variety of physical and chemical stresses such as high temperature and desiccation in some plants and animals (Lillie and Pringle, 1980; Richards et al., 2002). Its water-holding capability makes it wide application as additives, stabilizers, and sweeteners in food, cosmetics and pharmaceutical industries (Guo et al., 2000).

Trehalose can be produced by many methods, among which enzymatic synthesis has been widely used. There are three main enzymatic routes involved in trehalose biosynthesis (Richards et al., 2002): (1) trehalose-6-phosphate synthetase and trehalose-6-phosphate phosphatase (Siebers et al., 2004; Carpinelli et al., 2006) (2) maltooligosyltrehalose trehalohydrolase and maltooligosyltrehalose synthase (Carpinelli et al., 2006; Zhou et al., 2006), (3) trehalose synthase (TreS) (Pan et al., 2004; Lee et al., 2005). Among these routes, the conversion of maltose into trehalose by TreS is a simple, fast, and low-cost approach (Wu et al., 2011; Liang et al., 2013). Thus, an increasing number of TreS genes from different sources have been heterologously expressed and purified, such as *Pimelobacter* sp. R48, *Thermus aquaticus*, *Mycobacterium smegmatis* and other strains (Chen et al., 2006; Ma et al., 2006; Asker et al., 2009; Wu et al., 2009). In addition, the yields of the exogenous enzymes expressed in *Escherichia coli* have also been studied and optimized (Liang et al., 2013). However, the current studies on TreS have only been limited to gene cloning and enzymatic property characterization (Li et al., 2012; Ryu et al., 2010; Zdziebło and Synowiecki, 2006; Wei et al., 2013; Kushwaha et al., 2011; Yoshiyama et al., 2014) and little is known about the transcription and translation process of the TreS genes. Moreover, there are no reports on the comparison of the expression of TreS

* Corresponding author. Tel.: +86 10 64437610; fax: +86 10 64437610.
E-mail address: yuanqp@mail.buct.edu.cn (Q. Yuan).

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genes from different sources and expression vectors with distinct promoters.

E. coli is the most extensively used host for recombinant expression of heterologous proteins. However, the recombinant proteins in *E. coli* often fail to properly fold and are overexpressed as inclusion bodies. The inclusion bodies could be reduced by lowering expression temperature, changing weak promoter or ribosome binding site (RBS), co-expressing with molecular chaperones, and improving the

Table 1
The details of strains and plasmids.

Plasmid or strain	Description[a]	Reference or sources
Strains		
E. coli DH5α	F$^-$ψ80d*lacZ*△ (*lacZYA-argF*) U169 *endA1 recA1 hsdR17* (r_k^-, m_k^+) *sup*E44λ-*thi*-1 *gyrA96 relA1 phoA*	TransGen
E. coli Rosetta (DE3)	F$^-$*ompT hsdS*$_B$(r_B^-, m_B^-) *galdcmlac*Y1 (DE3) pRARE (*arg*U, *arg*W, *ilex*, *glyT*, *leu*W, *pro*L) (Cm^r)	TransGen
E. coli Rosetta (DE3) -1	*E. coli* Rosetta (DE3) harboring pET-22b-pptreS	This study
E. coli Rosetta (DE3) -2	*E. coli* Rosetta (DE3) harboring pMAL-pptreS	This study
E. coli Rosetta (DE3) -3	*E. coli* Rosetta (DE3) harboring pCS27-pptreS	This study
E. coli Rosetta (DE3) -4	*E. coli* Rosetta (DE3) harboring pZE12-pptreS	This study
E. coli Rosetta (DE3) -5	*E. coli* Rosetta (DE3) harboring pET-22b-cgtreS	This study
E. coli Rosetta (DE3) -6	*E. coli* Rosetta (DE3) harboring pMAL-cgtreS	This study
E. coli Rosetta (DE3) -7	*E. coli* Rosetta (DE3) harboring pCS27-cgtreS	This study
E. coli Rosetta (DE3) -8	*E. coli* Rosetta (DE3) harboring pZE12-cgtreS	This study
E. coli Rosetta (DE3) -9	*E. coli* Rosetta (DE3) harboring pET-22b-sctreS	This study
E. coli Rosetta (DE3) -10	*E. coli* Rosetta (DE3) harboring pMAL-sctreS	This study
E. coli Rosetta (DE3) -11	*E. coli* Rosetta (DE3) harboring pCS27-sctreS	This study
E. coli Rosetta (DE3) -12	*E. coli* Rosetta (DE3) harboring pZE12-sctreS	This study
E. coli Rosetta (DE3) -13	*E. coli* Rosetta (DE3) harboring pET-22b-tttreS	This study
E. coli Rosetta (DE3) -14	*E. coli* Rosetta (DE3) harboring pMAL-tttreS	This study
E. coli Rosetta (DE3) -15	*E. coli* Rosetta (DE3) harboring pCS27-tttreS	This study
E. coli Rosetta (DE3) -16	*E. coli* Rosetta (DE3) harboring pZE12-tttreS	This study
E. coli Rosetta (DE3) -17	*E. coli* Rosetta (DE3) harboring pET-22b-tmtreS	This study
E. coli Rosetta (DE3) -18	*E. coli* Rosetta (DE3) harboring pMAL-tmtreS	This study
E. coli Rosetta (DE3) -19	*E. coli* Rosetta (DE3) harboring pCS27-tmtreS	This study
E. coli Rosetta (DE3) -20	*E. coli* Rosseta (DE3) harboring pZE12-tmtreS	This study
E. coli Rosetta (DE3) -21	*E. coli* Rosetta (DE3) harboring pET-22b-tttreS and pCS27-sigma 32	This study
E. coli Rosetta (DE3) -22	*E. coli* Rosetta (DE3) harboring pET-22b-tttreS and pCS27-GroeL-GroeS	This study
E. coli Rosetta (DE3) -23	*E. coli* Rosseta (DE3) harboring pET-22b-tttreS and pCS27-DnaK-DnaJ	This study
E. coli Rosetta (DE3) -24	*E. coli* Rosetta (DE3) harboring pET-22b-tttreS and pCS27-sigma 32-GroeL-GroeS	This study
E. coli Rosetta (DE3) -25	*E. coli* Rosetta (DE3) harboring pET-22b-tttreS and pCS27-sigma 32-DnaK-DnaJ	This study
E. coli Rosetta (DE3) -26	*E. coli* Rosseta (DE3) harboring pET-22b-tttreS and pCS27-GroeL-GroeS-DnaK-DnaJ	This study
E. coli Rosetta (DE3) -27	*E. coli* Rosetta (DE3) harboring pET-22b-tttreS and pCS27-sigma 32-GroeL-GroeS-DnaK-DnaJ	This study
Plasmids		
pET-22b	p_{T7} expression vector, PBR322origin, Amp^r	Novagen
pMAL-c2x	p_{Tac} expression vector, PBR322/f1 origin, malE, Amp^r	NEB
pCS27	$P_L$lacO1 expression vector, P15A origin, Kan^r	Ro et al. (2006)
pZE12-luc	$P_L$lacO1 expression vector, colE origin, luc, Amp^r	Lutke-Eversloh and Stephanopoulos (2007)
pET-22b-pptreS	pET-22b vector containing pptreS from *pseudomonas putida*	This study
pMAL-c2x-pptreS	pMAL vector containing pptreS from *pseudomonas putida*	This study
pCS27-pptreS	pCS27 vector containing pptreS from *pseudomonas putida*	This study
pZE12-pptreS	pZE12-luc vector containing pptreS from *pseudomonas putida*	This study
pET-22b-cgtreS	pET-22b vector containing cgtreS from *Corynebacterium glutamicum*	This study
pMAL-c2x-cgtreS	pMAL vector containing cgtreS from *Corynebacterium glutamicum*	This study
pCS27-cgtreS	pCS27 vector containing cgtreS from *Corynebacterium glutamicum*	This study
pZE12-cgtreS	pZE12-luc vector containing cgtreS from *Corynebacterium glutamicum*	This study
pET-22b-sctreS	pET-22b vector containing sctreS from *Streptomyces coelicolor*	This study
pMAL-c2x-sctreS	pMAL vector containing sctreS from *Streptomyces coelicolor*	This study
pCS27-sctreS	pCS27 vector containing sctreS from *Streptomyces coelicolor*	This study
pZE12-sctreS	pZE12-luc vector containing sctreS from *Streptomyces coelicolor*	This study
pET-22b-tttreS	pET-22b vector containing tttreS from *Thermus thermophilus*	This study
pMAL-c2x-tttreS	pMAL vector containing tttreS from *Thermusthermophilus*	This study
pCS27-tttreS	pCS27 vector containing tttreS from *Thermus thermophilus*	This study
pZE12-tttreS	pZE12-luc vector containing tttreS from *Thermus thermophilus*	This study
pET-22b-tmtreS	pET-22b vector containing tmtreS from *Thermotoga maritima*	This study
pMAL-c2x-tmtreS	pMAL vector containing tmtreS from *Thermotoga maritima*	This study
pCS27-tmtreS	pCS27 vector containing tmtreS from *Thermotoga maritima*	This study
pZE12-tmtreS	pZE12-luc vector containing tmtreS from *Thermotoga maritima*	This study
pCS27-sigma32	pCS27 vector containing sigma 32 from *E. coli* BL21	This study
pCS27-GroeL-GroeS	pCS27 vector containing GroeL and GroeS from *E. coli* BL21	This study
pCS27-DnaK-DnaJ	pCS27 vector containing DnaK and DnaJ from *E. coli* BL21	This study
pCS27-sigma32-GroeL-GroeS	pCS27 vector containing sigma 32 and GroeL and GroeS from *E. coli* BL21	This study
pCS27-sigma32-DnaK-DnaJ	pCS27 vector containing sigma 32 and DnaK and DnaJ from *E. coli* BL21	This study
pCS27-GroeL-GroeS-DnaK-DnaJ	pCS27 vector containing GroeL and GroeS and DnaK and DnaJ from *E. coli* BL21	This study
pCS27-sigma32-GroeL-GroeS-DnaK-DnaJ	pCS27 vector containing sigma 32 and GroeL and GroeS and DnaK and DnaJ from *E. coli* BL21	This study

[a] Amp^r, ampicillin resistant; Kan^r, kanamycin resistant.