

## EXPRESSION AND CHARACTERIZATION OF RECOMBINANT TREHALOSE SYNTHASE IN *Bacillus subtilis*

Nguyen Ngoc Trieu<sup>1</sup>, Nguyen Thi Quynh<sup>1</sup>, Nguyen Duc Hoang<sup>3</sup>,  
Nguyen Manh Dat<sup>4</sup>, Tran Duc Long<sup>1,2</sup>, Nguyen Thi Hong Loan<sup>1,2,\*</sup>

<sup>1</sup>Key Laboratory of Enzyme and Protein Technology, VNUHN-University of Science

<sup>2</sup>Faculty of Biology, VNUHN-University of Science

<sup>3</sup>Faculty of Biology and Biotechnology, VNUHCM-University of Science

<sup>4</sup>Food Industries Research Institute, Ministry of Industry and Trade

Received 17 April 2020, accepted 28 September 2020

### ABSTRACT

Trehalose synthase (TreS, EC 2.4.1.245) is a potential catalyst for synthesis of trehalose, an important natural disaccharide. In this study, the *treS* gene of *Pseudomonas putida* (VTCC 12263) was cloned into pHT01 plasmid at *Bam*HI-*Xba*I position, expressed in *Bacillus subtilis* (*B. subtilis*) 1012, and characterized. The recombinant TreS had molecular weight of 68 kDa when fused with 8xHis tag at the C-terminus. catalyzed conversion of maltose to trehalose in optimal conditions had specific activity of 1.664 U/g. Expression of TreS was highest when *B. subtilis* 1012 harboring pHT01-*treS* was cultured in TB medium at 30 °C, induced with 1.0 mM IPTG when OD<sub>600</sub> reached 0.8 and harvested after 10 hours of induction. The recombinant TreS purified by Ni-sepharose chromatography had specific activity of 41.700 U/g and formed a single band on Western blot with monoclonal antibody against His-tag. The recombinant TreS had optimal activity at 37 °C in 100 mM pH 7.4 PBS and 300 mM maltose. It was inhibited by NaCl, KCl and MgCl<sub>2</sub> (retaining 45% or 75% specific activity in buffer containing 5 mM KCl or 5 mM MgCl<sub>2</sub>, respectively) and stimulated by imidazol (with specific activity increasing by 30–200%).

**Keywords:** *Bacillus subtilis*, cloning, expression of recombinant protein, enzyme characteristics, trehalose synthase.

---

*Citation:* Nguyen Ngoc Trieu, Nguyen Thi Quynh, Nguyen Duc Hoang, Nguyen Manh Dat, Tran Duc Long, Nguyen Thi Hong Loan, 2020. Expression and characterization of recombinant trehalose synthase in *Bacillus subtilis*. *Academia Journal of Biology*, 42(4): 51–60. <https://doi.org/10.15625/2615-9023/v42n4.14990>

\*Corresponding author email: loannguyen@hus.edu.vn

©2020 Vietnam Academy of Science and Technology (VAST)

## INTRODUCTION

Trehalose (1- $\alpha$ -D-glucopyranosyl- $\alpha$ -D-glucopyranoside), a useful non-reducing disaccharide with two glucoses linked by a  $\alpha$ , $\alpha$ -1,1-glycoside linkage, is commonly found in yeasts, bacteria, invertebrates, plants and insects. Trehalose plays important roles as a carbon storage, and a component of the cell wall. It also has several applications in production and preservation of food, pharmaceuticals, cosmetics, and agricultural products (Liu et al., 2019).

Trehalose synthase (TreS) catalyzes the synthesis of trehalose from maltose in a single step and is considered to be a convenient, economical, and practical biocatalyst for industrial production of trehalose owing to simple reaction and inexpensive substrate (Wang et al., 2014). TreS from different bacterial strains, such as *Pseudomonas stutzeri*, *Corynebacterium glutamicum*, *Arthrobacter aureescens* and *Meiothemus ruber*, has been expressed for trehalose production (Lee et al., 2005; Chen et al., 2006; Wu et al., 2009; Yue et al., 2009; Kim et al., 2010).

*Pseudomonas putida* (*P. putida*), a non-pathogenic member of the genus *Pseudomonas*, colonizes many different environments and is well known for its metabolic and genetic diversity. This strain has also been extensively used as a host for gene cloning and expression of heterologous genes from gram-negative bacteria in soil. *P. putida* has been used in production of bioplastics, fine chemicals, as well as in plant growth promotion and plant pest control,... (Nogales et al., 2008). TreS from *P. putida* has been expressed but its activity was low (Ma et al., 2006; Li et al., 2016). *treS* isolated from *P. putida* KT2440 was expressed under the control of the *T7* promoter in *E. coli* BL21 (DE3) and the conditions for producing of TreS at 10 L fermentation scale were optimized (Wang et al., 2014). However, *E. coli* is pathogenic and not safe for food production.

Compared with *E. coli*, *B. subtilis* is a safe expression system. Since the method of transforming *B. subtilis* with plasmid DNA was discovered, *B. subtilis* has become an effective host for the expression of foreign genes. The advantages of *B. subtilis* include its non-pathogenic nature, being safe to use, its ability to secrete extracellular proteins directly into culture medium, easy genetic manipulation and rapid growth rate (Sarvas, 1995). In Vietnam, Nguyen et al. (2018) isolated a number of bacterial strains and found that *P. putida* (VTCC B2263) synthesized trehalose, however, *treS* has neither been cloned nor expressed.

In this study, the *treS* gene was cloned from *P. putida* (VTCC 12263) and expressed in *B. subtilis* strain 1012 under the control of the promoter *pgrac* of pHT01 vector. This research provides the basis for production of safe recombinant TreS, which can be used in the food processing industry.

## MATERIALS AND METHODS

*P. putida* (VTCC 12263) was bought from Vietnam Type Culture Collection, Institute of Microbiology and Biotechnology, Vietnam National University, Ha Noi. *B. subtilis* 1012 and pHT01 vector were from Nguyen et al. (2007).

### Construction of recombinant expression vector

The cloning primers *treS*-F cggGGATCCATGACCCAGCCCGACCCGT C (the *Bam*HI cleavage site is underlined and the start codon is in bold) and *treS*-R cggTCTAGATCAGTGATGGTGATGGTGATGGTGATGAACATGCCCGCTGCTGTTGA (the *Xba*I cleavage site is underlined, the stop codon is in bold, and 24 nucleotides encoding 8 histidine are italic) were designed based on the *treS* gene sequence of *P. putida* KT2440 (Accession No: NC\_002947.4). The *treS*-F and *treS*-R primers were used to amplify *treS* gene from *P. putida* (VTCC 12263) using Phusion High-Fidelity PCR Master Mix (Thermo Scientific). The PCR product (2106

bp in size, of which 2064 bp is specific to *treS*) was digested with *Bam*HI (NEB) and *Xba*I (NEB) and then ligated to pHT01, which had been digested with the corresponding restriction enzymes and dephosphorylated by QuickCIP (NEB).

The ligation product was transformed into *E. coli* DH5 $\alpha$  competent cells and bacteria were plated on LB agar medium supplemented with ampicillin 100  $\mu$ g/mL. The recombinant pHT01 containing *treS* gene (pHT01-*treS*) was verified by PCR with cloning primers or pHT01-F TACGATCTTTCAGCCGACTC and pHT01-R ATCTCCATGGACGCGTGAC primers flanking pHT01 multiple cloning site and then sequenced. The pHT01-*treS* was transformed into *B. subtilis* 1012 to express recombinant TreS, which has a predicted molecular weight of approximately 68 kDa (67 kDa of TreS and ~1 kDa of 8xHis-tag) (Wang et al., 2014).

#### Expression of recombinant TreS

The recombinant *B. subtilis* 1012 harboring pHT01-*treS* was pre-cultured in 50 mL LB broth supplemented with 10  $\mu$ g/mL chloramphenicol for 14–16 hours at 37 °C, shaking at 200 rpm (Phan et al., 2017). The starter culture was then diluted into four media: LB (15 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, and 300  $\mu$ l 3 M NaOH), LB containing 1% (w/v) glucose, terrific broth (TB; 12 g/L tryptone, 24 g/L yeast extract, 2.2 g/L KH<sub>2</sub>PO<sub>4</sub>, 9.4 g/L K<sub>2</sub>HPO<sub>4</sub>, and 8 mL/L glycerol) supplemented with 1% (w/v) glucose, and 2YT medium (16 g/L tryptone, 10 g/L yeast extract, 5 g/L NaCl) to OD<sub>600</sub> of 0.05.

Bacteria were grown at 18–37 °C under vigorous shaking (200 rpm) until OD<sub>600</sub> reached 0.4–1.0. Expression of *treS* was induced by IPTG at different concentrations of 0.1–1 mM and cells were harvested 3–16 h after induction by centrifugation at 6000 rpm, 4 °C for 10 minutes. Cell mass was resuspended in 2 mL ice-cold buffer A (PBS pH 7.4 with 50 mM NaCl, 1 mM PMSF),

sonicated and centrifuged at 12,000 rpm, 4°C for 30 minutes to obtain the soluble fraction of the enzyme. Insoluble fraction was resuspended in 2 mL of 1X sample buffer containing SDS, and  $\beta$ -mercaptoethanol to completely dissolve insoluble protein.

#### Purification of recombinant TreS using Ni-sepharose

Immobilized metal affinity chromatography was used to purify recombinant TreS. One milliliter of Nickel-sepharose was packed into a column (7  $\times$  1 cm), saturated with 4 mL of 50 mM NiCl<sub>2</sub> flowing at a rate of 15–20 mL/h and equilibrated with 10 mL buffer A containing 5 mM imidazole at 20–30 mL/h. Weakly bound proteins were washed by buffer A containing 5 mM imidazole until A<sub>280</sub> < 0.05. The bound proteins were then eluted from the column using buffer A containing 250 mM imidazole. Collected protein fractions were analyzed by SDS-PAGE and blotted with anti-6xHis-tag monoclonal antibodies.

#### Western blot using anti-6xHis-tag monoclonal antibodies

Western blot was carried out as previously described by Mahmood & Yang (2012). Crude extract of *B. subtilis* 1012 harboring pHT01-*treS* or purified protein fractions were separated by SDS-PAGE and proteins were transferred onto PVDF (Polyvinylidene fluoride) membrane in Tris-Glycine containing 10% methanol. The membrane was blocked in PBS pH 7.4 containing 3% BSA at 4 °C overnight or 30–60 minutes at room temperature with constant agitation and then washed 3 times in PBS, pH 7.4 containing 0.1% Tween 80.

After that, membrane was incubated with the primary anti-6xHis-tag monoclonal antibody (Clontech, USA) for one hour at room temperature, rinsed and incubated with secondary alkaline phosphatase (AP) conjugated antibody. Alkaline phosphatase converts colorless NBT (p-nitro blue

tetrazolium chloride) and BCIP (5-bromo-4-chloro-3-indolyl phosphate) substrates in 0.1 M Tris-HCl pH 9.5 containing 5 mM MgCl<sub>2</sub> and 0.1 M NaCl to colored substances, thus visualizing recombinant TreS. The reaction was stopped by incubating the membrane in 1x PBS pH 7.1 containing 20 mM EDTA.

### TreS activity assay

Crude extracts of *B. subtilis* harboring or not harboring plasmids were incubated with maltose of different concentrations ranging from 100 mM to 1000 mM in 100 mM PBS pH 6.0–8.0 at 25–45 °C for 2 hours and then heated at 90 °C for 10 min to stop enzyme activity. Maltose will be converted to trehalose if TreS is present in the crude extract. Extracts of *B. subtilis* 1012, or *B. subtilis* 1012 harboring pHT01 vector or non-induced *B. subtilis* 1012 harboring pHT01-*treS* served as negative controls (without TreS). The amount of trehalose in the reaction product was measured by Trehalose Assay Kit (Megazyme, USA) following kit producer's instruction. One

unit of TreS was defined as the amount of enzyme that catalyzes the formation of 1 mg of trehalose in one hour. The relative enzyme activity (%) was defined as the percentage of enzyme activity in the control (Ma et al., 2006).

## RESULTS AND DISCUSSION

### Cloning of *treS* gene into pHT01 vector

The *treS* gene was amplified from genome of *P. putida* by PCR with *treS*-F/R (Fig. 1A, lane 2) and inserted into the pHT01 plasmid. The recombinant pHT01-*treS* plasmid was verified by PCR with *treS*-F/R or pHT01-F/R primers (Fig. 1B-C). PCR from the pHT01-*treS* produced a single DNA band of ~2 kb (Fig. 1B, lane 2) similar to PCR product from *P. putida* (Fig. 1A, lane 2) and a ~2,4 kb DNA band (~0,4 kb fragment around multiple cloning sites of pHT01 vector and ~2 kb of *treS*) (Fig. 1C, lane 5). Sequencing results of pHT01-*treS* confirmed that *P. putida treS* gene was cloned successfully into the pHT01 vector.

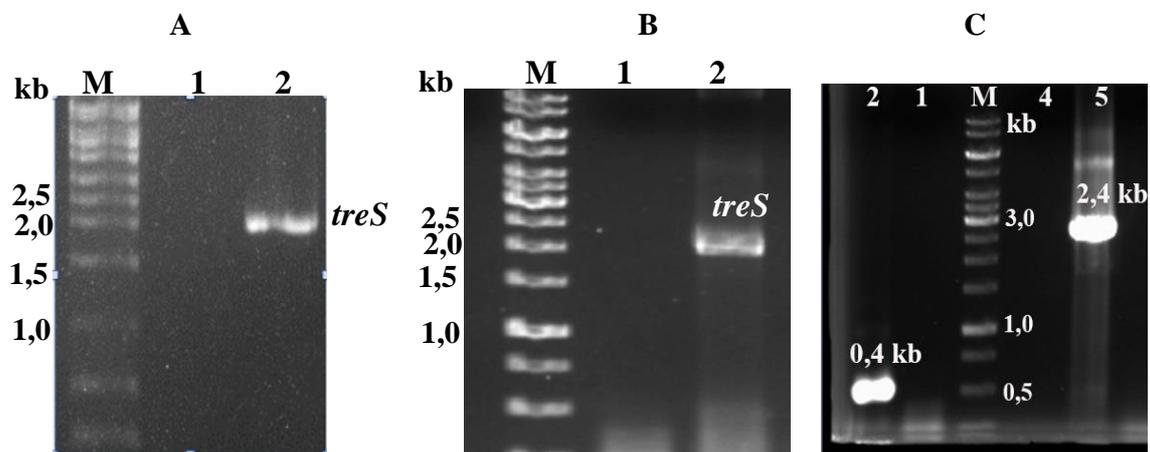


Figure 1. Cloning of *treS* gene into pHT01 vector. M: DNA marker 1 kb; A1, B1, C1 and C4: no template controls; A2: *treS* amplified from *P. putida* using *treS*-F/R primers; B2: *treS* amplified from pHT01-*treS* using *treS*-F/R primers; C2: fragment amplified from empty pHT01 with pHT01-F/R primers; C5: *treS* fragment amplified from pHT01-*treS* using pHT01-F/R primers

### Expression of TreS by pHT01-*treS* vector in *B. subtilis* 1012

Protein was extracted from *B. subtilis* 1012 harboring or not harboring plasmid and

analyzed by Western blot. The results indicated that recombinant TreS was only expressed in IPTG-induced *B. subtilis* 1012 harboring pHT01-*treS* in both soluble and insoluble forms (Fig. 2A, lanes 4 and 5). The recombinant TreS had a molecular weight of ~68 kDa as predicted, similar to TreS

expressed in *E. coli* BL21 by Wang et al. (2014). Furthermore, the recombinant TreS converted maltose to trehalose with the specific activity of 1120 U/g (Fig. 2B). While other protein extracts did not show any TreS activity (Fig. 2B).

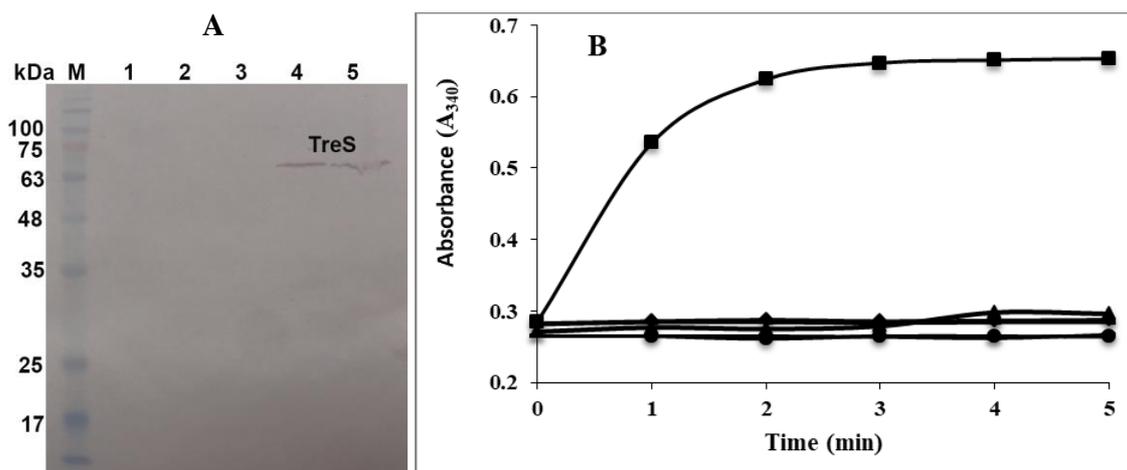


Figure 2. Western blot analysis (A) and activity plot (B) of extracts from *B. subtilis* 1012 harboring pHT01-*treS* vector

M: protein marker, 1: extract of *B. subtilis* 1012 (•), 2: extract of *B. subtilis* 1012 harboring pHT01 (♦); 3, 4: extracts of non-induced and induced *B. subtilis* 1012 harboring pHT01-*treS* (Δ), (■), respectively; 5: insoluble fraction of *B. subtilis* 1012 harboring pHT01-*treS* vector.

#### Suitable expression conditions of TreS using *B. subtilis* 1012 harboring pHT01-*treS* vector

To improve expression of TreS, different cultivating media and induction conditions were tested. In each experiment, only one condition (induction time in Fig. 3A, IPTG concentration in Fig. 3B, culture temperature after induction in Fig. 3C, cultivation duration in Fig. 3D, medium composition in Fig. 3E and lactose induction in Fig. 3F) was changed while the other conditions were kept constant. The results showed that TreS expression was highest when bacteria were induced by 1.0 mM IPTG at the mid-exponential phase ( $OD_{600} = 0.8$ ), subsequently cultured at 30 °C and harvested 10 hours after induction (Fig. 3A-D). Of the

4 media (LB, LB with 1% glucose (w/v), TB and 2YT) tested, the TB medium resulted in the highest TreS expression (Fig. 3E). The recombinant TreS was under the control of synthetic *Pgrac* promoter containing *E. coli lac* operator, however, lactose (up to concentration of 20 mM, which is twice the usual lactose concentration) failed to induce recombinant TreS expression (Fig. 3F).

In another study, Liu et al (2019) cultured *B. subtilis* WB800N in TB medium at 37 °C with extended cultivation time of 96 hours to express *P. putida* TreS. While in *E. coli* BL21, the highest expression of *P. putida* TreS was obtained when bacteria were grown in LB medium at 25 °C, induced by 0.6 mM IPTG at  $OD_{600} = 0.6$  and harvested after 6 hours adding IPTG (Wang et al., 2014).

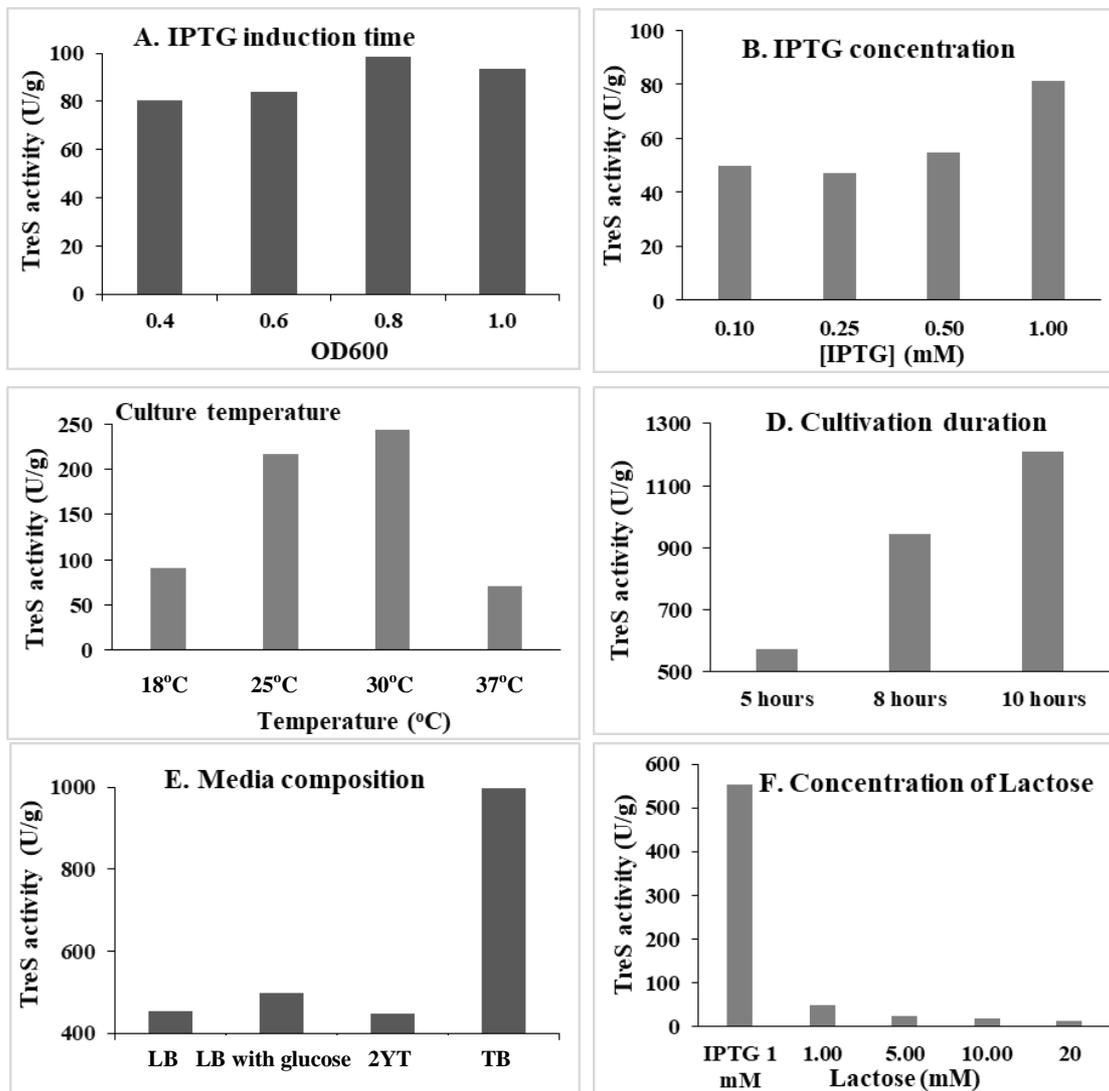


Figure 3. The effects of IPTG induction time (A), IPTG concentration (B), culture temperature after induction (C), cultivation duration (D), medium composition (E) and lactose concentration (F) on expression level of TreS using *B. subtilis* 1012 harboring pHT01-*treS* vector

### Initial purification of TreS from extract of *B. subtilis* 1012 harboring pHT01-*treS* vector

Extract of *B. subtilis* 1012 harboring pHT01-*treS* vector was loaded onto the Ni-sepharose affinity column in buffer A containing 5 mM imidazole. The elution profile of Ni-sepharose (Fig. 4) showed that unbound fractions had a wide peak (peak 1) while bound fractions eluted by buffer A

containing 250 mM imidazole had one narrow peak (peak 2). SDS-PAGE result showed a thick band at ~68 kDa besides several non specific protein bands (Fig. 5A, lane 5–6). This ~68 kDa band was also recognized specifically by anti-6xHis-tag monoclonal antibody (Fig. 5B, lane 5–6). In addition, these protein fractions converted maltose to trehalose with specific activity of 41.700 U/g, suggesting that the ~68 kDa band is truly recombinant TreS.

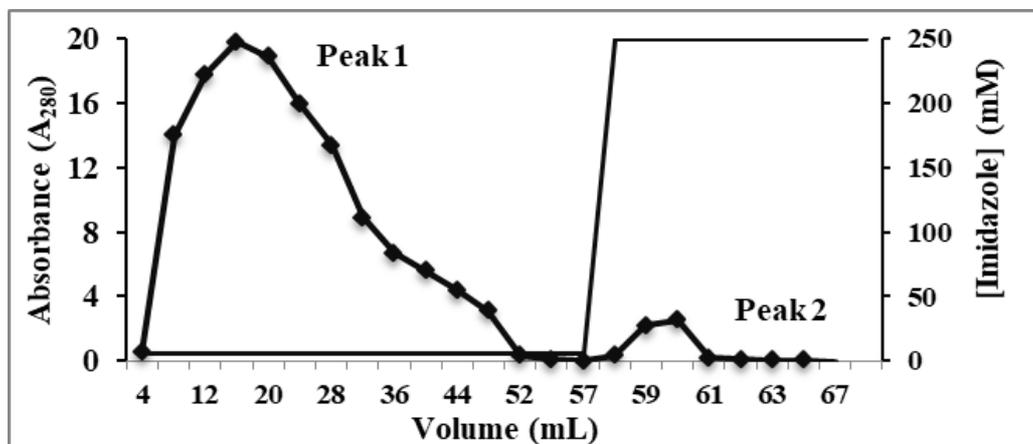


Figure 4. Elution profile of TreS from extract of *B. subtilis* 1012 harboring pHT01-*treS* vector using Ni-sepharose column

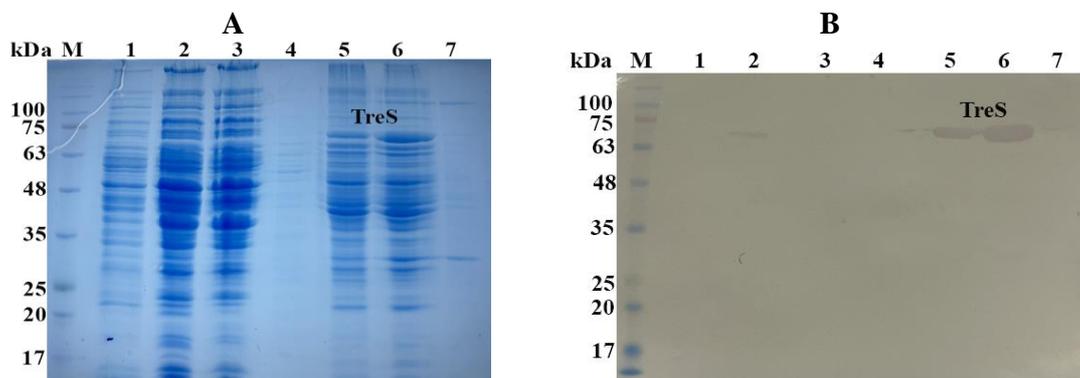


Figure 5. SDS-PAGE (A) and Western blot (B) of purified fractions from extract of *B. subtilis* 1012 harboring pHT01-*treS*

M: protein marker, 1-2: extracts of non-induced and induced *B. subtilis* 1012 harboring pHT01-*treS*, respectively; 3: Ni-sepharose unbound fraction, 4-7: fractions bound to Ni-sepharose eluted with imidazole at 58<sup>th</sup>-61<sup>st</sup> mL, respectively.

### Characteristics of TreS activity

Trehalose synthesis activity of recombinant TreS was tested under different conditions. The results showed that TreS was active in a wide range of temperature of 25–40 °C and most active at 37 °C (Fig. 6A). In addition, TreS activity was highest in pH 7.4–8.0 and significantly reduced when pH was lower than 7.4 (Fig. 6B). Maltose positively affected TreS in which TreS activity was increased when maltose concentration was increased from 100 mM to 1000 mM (Fig. 6C). Recombinant TreS reached ~80% of maximal activity at 300 mM maltose and

increased insignificantly at higher maltose concentration. NaCl, on the other hand, negatively affected TreS. Its activity was reduced by ~25% at 5 mM NaCl and almost completely inhibited at 50 mM NaCl (Fig. 6D). Similarly, KCl and MgCl<sub>2</sub> reduced TreS activity at concentration of 5 mM (Fig. 6E). Surprisingly, imidazole increased TreS activity with higher TreS activity at higher imidazole concentration (Fig. 6F). This is the first time such positive effect of imidazole on TreS has been reported. The characteristics of TreS activity in our study were similar to other studies. The enzyme exhibited an

optimal activity in temperature range of 35–37 °C and pH range of 7.4–7.5 (Ma et al., 2006; Yan et al., 2013; Wang et al., 2014; Liu et al., 2019); and was inhibited by  $Mg^{2+}$  and  $K^+$  (Yan et al., 2013). Under optimal conditions, TreS expressed in *B. subtilis* 1012 had the specific activity of 1.664 U/g, which was significantly higher than that of TreS isolated from *P. putida* H76 (Ma et al., 2006) and *Thermomonospora curvata* TreS expressed in *B. licheniformis* (Li et al., 2016) and as high as that of *P. putida* mutant TreS expressed on the surface of *B. subtilis* WB800N spores (Liu et al., 2019).

### CONCLUSION

The *P. putida treS* gene was cloned into pHT01 vector and expressed in *B. subtilis*. Recombinant TreS was fused with 8xHis-tag at

the C terminus, had molecular weight of ~68 kDa and specific activity of 1120 U/g. Expression of TreS was the highest when *B. subtilis* 1012 harboring pHT01-*treS* was cultured in TB medium at 30 °C with vigorous shaking, induced by 1.0 mM IPTG when OD600 reached 0.8 and harvested 10 hours after induction. Ni-sepharose purified TreS had specific activity of 41.700 U/g and generated a single band on Western blot with monoclonal antibody against 6xHis-tag. TreS had optimal activity at 37 °C in 100 mM PBS pH 7.4 buffer and 300 mM maltose. It was inhibited by NaCl, KCl and  $MgCl_2$  at concentration of 5 mM (remained activity from 75–45%) and activated by imidazole (activity increased by 30–200%). Under optimal conditions, TreS expressed in *B. subtilis* 1012 had specific activity of 1.664 U/g.

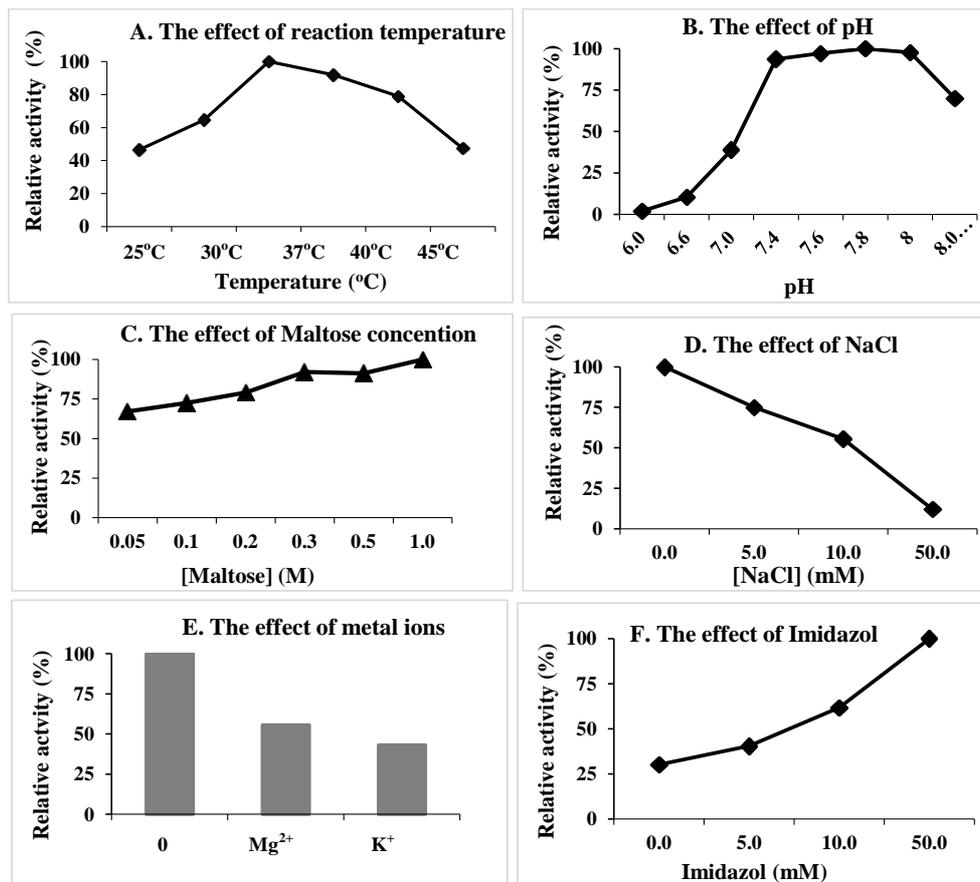


Figure 6. The effect of temperature (A), pH (B), maltose (C), NaCl (D),  $MgCl_2$ , KCl 5 mM (E) and Imidazole (F) on TreS activity

**Acknowledgements:** This research was financially supported by the Ministry of Science and Technology of Vietnam (project code ĐTĐLCN.11/18). The authors would like to thank Vietnam Type Culture Collection, Institute of Microbiology and Biotechnology, Vietnam National University, Ha Noi for providing *Pseudomonas putida* VTCC 12263 strain.

## REFERENCES

- Chen Y. S., Lee G. C., Shaw J. F., 2006. Gene Cloning, Expression, and Biochemical Characterization of a Recombinant Trehalose Synthase from *Picrophilus torridus* in *Escherichia coli*. *J. Agric. Food Chem.*, 54: 7098–7104.
- Kim T. K., Jang J. H., Cho H. Y., Lee H. S., Kim Y. W., 2010. Gene cloning and characterization of a trehalose synthase from *Corynebacterium glutamicum* ATCC13032. *Food Sci. Biotechnol.*, 19: 565–569.
- Lee J. H., Lee K. H., Kim C. G., Lee S. Y., Kim G. J., Park Y. H., Chung S. O., 2005. Cloning and expression of a trehalose synthase from *Pseudomonas stutzeri* CJ38 in *Escherichia coli* for the production of trehalose. *Appl. Microbiol. Biotechnol.*, 68: 213–219.
- Li Y., Gu Z., Zhang L., Ding Z., Shi G., 2016. Inducible expression of trehalose synthase in *Bacillus licheniformis*. *Protein Expr. Purif.*, <http://doi.org/10.1016/j.pep.2016.10.005>
- Lim H. K., Lee S. U., Chung S. I., Ung K. H., Seo J. H., 2004. Induction of the T7 Promoter Using Lactose for Production of Recombinant Plasminogen Kringle 1–3 in *Escherichia coli*. *J. Microbiol. Biotechnol.* 14: 225–230.
- Liu H., Yang S., Wang X., Wang T., 2019. Production of trehalose with trehalose synthase expressed and displayed on the surface of *Bacillus subtilis* spores. *Microb. Cell Fact.*, 18: 1–13.
- Ma Y., Xue L., Sun D.W. 2006., Characteristics of trehalose synthase from permeabilized *Pseudomonas putida* cells and its application in converting maltose into trehalose. *J. Food. Eng.*, 77: 342–347. <https://doi.org/10.1016/j.jfoodeng.2005.06.042>
- Mahmood T., Yang P. C. 2012., Western blot: technique, theory, and trouble shooting. *N. Am. J. Med. Sci.*, 4: 429–434.
- Nogales J., Palsson B., Thiele I., 2008. A genome-scale metabolic reconstruction of *Pseudomonas putida* KT2440: iJN746 as a cell factory. *BMC Systems Biology*, 2: 1–20. <https://doi.org/10.1186/1752-0509-2-79>
- Nguyen H. D., Phan T. T. P., Schumann W., 2007. Expression vectors for the rapid purification of recombinant proteins in *Bacillus subtilis*. *Current Microbiology*, 55: 89–93.
- Nguyen T. H. T., Nguyen T. T., Le T. H., Nguyen T. A. T., Nguyen M. D., Le D. M., Do T. T., 2018. Screening bacterial strains for production of maltooligosyl trehalose trehalohydrolase and maltooligosyl trehalose synthase. *Journal of Vietnamese Environment*, 9(2): 55–60.
- Phan T., Huynh P., Truong T., Nguyen H., 2017. A generic protocol for intracellular expression of recombinant protein in *Bacillus subtilis*. *Methods Mol. Biol.*, 1586: 325–334.
- Sarvas M., 1995. Gene expression in recombinant *Bacillus*. *Bioprocess. Technol.*, 22: 53–120.
- Wang T., Jia S., Dai K., Liu H., Wang R., 2014. Cloning and expression of a trehalose synthase from *Pseudomonas putida* KT2440 for the scale-up production of trehalose from maltose. *Canadian Journal of Microbiology*, 60(9): 599–604. <https://doi.org/10.1139/cjm-2014-0330>
- Xiuli W., Hongbiao D., Ming Y., Yu Q., 2009. Gene cloning, expression, and characterization of a novel trehalose

- synthase from *Arthrobacter aurescens*. *Applied microbiology and biotechnology*, 83(3): 477–482. <https://doi.org/10.1007/s00253-009-1863-5>
- Yan J., Qiao Y., Hu J., Ding H., 2013. Cloning, expression and characterization of a trehalose synthase gene from *Rhodococcus opacus*. *Protein J.*, 32: 223–229.
- Yue M., Wu X. L., Gong W. N., Ding H. B., 2009. Molecular cloning and expression of a novel trehalose synthase gene from *Enterobacter hormaechei*. *Microb. Cell Fact.*, 8: 34.