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CLONING, PURIFICATION AND CHARACTERIZATION OF A NOVEL RECOMBINANT TREHALOSE SYNTHASE (TreS) FROM *Acidiplasma sp.* MBA-1

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ABSTRACT

Trehalose, a nonreducing disaccharide, can be commercially produced using maltose from microorganisms. An intramolecular transglycosylase enzyme called trehalose synthase (TreS) can catalyze the conversion of maltose to trehalose in a single step reaction. Hence, in our study a novel gene TreS encoded with 562 amino acids was cloned from *Acidiplasma sp.* MBA-1 and expressed into *E. coli* BL21 (DE). HPLC results suggested that it could catalyze the conversion between maltose and trehalose in one step. The conversion of trehalose from maltose was about 43.62% in our study. At the same time, TreS produced about 23.85% glucose as a by-product after 10h of incubation. SDS page results showed that the purified recombinant enzyme has a molecular weight of 65.9kDa. The recombinant TreS showed its optimal activity at 40°C and its optimum pH was 6.5. Our study shows that the enzyme was not thermostable and its activity was increased by 1mM EDTA, Mn^{2+} and Li^{+} whereas $Cu^{2+}$and Ni^{2+} strongly inhibited the enzyme activity.

Keywords: Trehalose, Trehalose synthase, *Acidiplasma sp.* MBA-1, *Escherichia coli*, purification

INTRODUCTION

Trehalose, also known as α-D-glucopyranosyl, α-D-glucopyranoside, is a disaccharide molecule made up of two glucose molecules linked with a 1,1 glycosidic linkage. It is mostly found in a variety of organisms i.e., bacteria, fungi, invertebrates etc **(Himei 2008)**. Although trehalose was well known for carbon and energy source of plants and animals for years, the researches in the last two decades showed that trehalose is a multifunctional molecule as well. It is found in cell wall glycolipid as a structural component)**Arguelles, 2000; Richards, 2002)**. Trehalose is very stable under hot and acidic conditions. **Mizumoto *et al* (2004)** showed that trehalose can be used as a bulk agent due to its stability towards heat and hydrolysis. Moreover, it does not caramelize and undergoes Maillard reactions and it is safe for human consumption and widely accepted by the European regulation system **(Richards *et al.*, 2002; Schiraldi, Di Lernia, & De Rosa, 2002)**. It can also stabilize enzymes in vegetables)**Aga *et al.*,1988)**, suppress bitterness and enhance sourness)**Oku, 1995**(, suppresses foul odor **(Kubota, 2005)**, suppresses oxidation **(Himei, 2008)** reaction as well. **Colaco & Roser (1995)** reported that it can be used as an additive for food preservation. **Zdzieblo & Synowiecki(2006)** suggested that trehalose can be used for food processing because of its certain unique properties; mild sweetness, low carcinogenicity, good solubility in water, stability under low pH conditions, low hygroscopicity, depression of freezing point, high glass transition temperature and ability to protect proteins. Although trehalose is abundant in many microorganisms, it's commercial production for the industries has been a big challenge until several enzyme synthesis systems in microorganisms have been discovered)**Lama *et al.* 1990; Nakada *et al.*1996; Di Lernia *et al.* 1998**(which opened a new dimension in commercial trehalose production in the industries. **Avonce)2006**(reported that there are five main enzymatic pathways of trehalose biosynthesis has been identified so far. Two of which are very popular for commercial production of trehalose; MTS-MTH pathway (two novel enzymes maltooligosyl trehalose synthase and maltooligosyl trehalose trehalohydrolase convert maltodextrin into trehalose in a two-step reaction) and TreS pathway)Trehalose synthase isomerizes α1- α4 bond of maltose to α1- α1 bond resulting in trehalose(.The later one requires less energy, more simple, fast and cost-effective. *Acidiplasma sp.* MBA-1 is a novel acidophilic, cell wall-less archaeon, excretes a significant amount of trehalose into the culture media. A new gene for trehalose synthase has been identified from *Acidiplasma sp* MBA-1(GenBank). In this study, we hypothesized that the gene could be expressed into an *E. coli* expression system and using this enzyme trehalose could be produced commercially. We also aim to purify and characterize the trehalose synthase from *Acidiplasma sp* MBA-1. To the best of our knowledge, this is the first report on purification and characterization of trehalose synthase (TreS) from this bacterial strain.

MATERIALS AND METHODS

The column resin for recombinant *Acidiplasma sp.* MBA-1TreS purification, the chelating Sepharose Fast Flow, was obtained from GE (Uppsala, Sweden). Electrophoresis reagents were purchased from Bio-Rad. Isopropyl-β-D-1-thiogalactopyranoside (IPTG) and all chemicals for the assay were from Sigma-Aldrich (St. Louis, MO, USA). Standard trehalose was bought from Sinopharma Ltd., China. The reconstructed plasmid was synthesized by Generay Biotech Co., Ltd. (Shanghai, China).

Gene Cloning and expression of recombinant TreS

According to information from the NCBI, the whole genome of *Acidiplasma sp.* MBA-1 was sequenced by Bulaev A.G. in 2015 and was released into the Gen Bank (NCBI) with the accession number KJE50039.1. The target DNA gene (gene locus_tag: TZ01_03000) and the gene encoding the hypothetical protein of RDH (protein ID number WP_048101287.1) was synthesized and cloned into the pET-22b(+) vector with *NdeI* and *XhoI* sites and an in-frame fusion His6-tag sequence at the C-terminus was provided in the reconstructed plasmid. The plasmid was named pET-TreS and was transformed into *E. coli* BL21 (DE) for TreS overexpression. The *E. coli* BL21 (DE) cells harboring the pET-TreS plasmid were cultured in Luria Bertani medium supplemented with the antibiotic (kanamycin) to a final concentration of 100 μg mL^{-1} and incubated at 37 °C. After the culture reached an optical density of 0.6– 0.8 at 600 nm, IPTG was added to the culture to a final concentration of 1 mM, and TreS was induced at 28 °C for 6 h.

Purification of recombinant TreS

The cells were collected by centrifugation at 8,000 × *g* for 10 minutes and then washed with 50mM sodium phosphate buffer (PBS) with a pH of 7.5. The washed cells resuspended in the lysis buffer were disrupted by ultrasonication at

4 °C using a Vibra-Cell™ 72405 Sonicator (BioBlock Scientific, Illkirch, France). The disrupted cells were removed by centrifugation (10,000 × *g* for 30 minutes at 4 °C). The collected supernatant (crude enzyme) was loaded onto a chelating Sepharose Fast Flow resin column (1.0 × 10.0 cm) charged with Ni^{2+} and equilibrated with the binding buffer (50 mM PBS, 500 mM NaCl, pH 7.5). Unbounded proteins in the column were removed with the washing buffer (50 mM PBS buffer, 500 mM NaCl, 50 mM imidazole, pH 7.5). TreS was subsequently eluted with the elution buffer (50 mM PBS buffer, 500 mM NaCl, 500 mM imidazole, pH 7.5). The collected pure enzyme was dialyzed for 24 h at 4 °C against the dialysis buffer (50 mM sodium phosphate buffer, pH 7.5).

Activity assay of TreS

The activity was determined by measuring the amount of trehalose produced from maltose. The total volume of the standard reaction was 1ml consisting of 900 µl 50 mM sodium phosphate (pH 6.5) as a substrate solution (1% maltose) and 100 µl of purified enzyme. The mixture was incubated for 1h at 40°C. After that, the reaction mixture was heated at 100°C in boiling water for 10 minutes to stop the reaction.

Carbohydrate Analysis

Trehalose was detected by High-Performance Liquid Chromatography (HPLC) system equipped with a refractive index detector and an NH_2 column (Waters Spherisorb® 5µm, 46×250 mm). The flow rate of the mobile phase was 1ml/min. The mobile phase consists of 77.5% acetonitrile, 15% methanol and 7.5% ddH_2O.

Effect temperature on TreS

The effects of temperature on the activity of TreS was determined at various temperatures (20-60 °C). To check the stability of the TreS enzyme against temperature, 100 µl of purified TreS were preincubated with 50mM sodium phosphate buffer (pH 6.5) for 1h at different temperature ranging from 20-60 °C. Finally, a standard reaction was carried out at 40 °C for 1h adding 1% substrate (maltose) into the preincubated purified enzyme. The residual activity was measured by the HPLC system.

Effect of pH on TreS

The effect of pH on the activity of TreS was determined at various pH (5-8.5) using 50mM sodium phosphate buffer at 40 °C. The standard reaction was carried out for 1h. The residual activity was measured by the HPLC system.

Effect of Metal Ions and EDTA activity

The enzyme solution was incubated with various metal ions Mn^{2+}, Ni^{2+}, Cu^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}, Al^{3+}, Fe^{2+}, Li^{+}, Co^{2+} and a chelating reagent EDTA at a final concentration of 1mM. The residual activity was measured by the HPLC system. The measured activities were compared to the enzyme activity without the addition of metal ions (control) under the same conditions.

Total protein concentration

The total protein concentration was measured according to the Bradford method (Bradford, 1976). Bovine serum albumin was used as a standard.

Substrate specificity

Different sugars have been used as a substrate to check the substrate specificity of the enzyme TreS. We have used Glucose, Lactose, Sucrose, Fructose, Mannose, β-cyclodextrin, Starch, Cellobiose and Galactose as a substrate. The reaction was carried out at optimum conditions with 1% maltose as a substrate. The relative activity was determined by the HPLC.

Conversion rate

To determine the conversion rate of the TreS enzyme, a series of standard reactions were carried out using 1% maltose as a substrate. The standard 1ml reaction mixture containing 900 µlsodium phosphate buffers (50mM) as a substrate solution and 100 µl purified enzyme was used. The reaction was carried out at a different time (0-10h). The residual activity was measured by the HPLC system.

SDS page

As described by Laemmli, the subunit molecular weight of recombinant TreS was examined by using the denaturing conditions of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 5% stacking gel and a 12% separating gel. Gels were stained with Coomassie Brilliant Blue 250 and de-stained with an aqueous mixture of 10% (v/v) methanol/10% (v/v) acetic acid.

Sequence similarity

Acidiplasma sp MBA-1 amino acid sequence released from the gene sequence was compared with similar enzymes from other organisms using the NCBI web site tool BLAST and the sequence alignment tool ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html).

RESULTS

Gene Cloning and expression

The genome of *Acidiplasma sp.* MBA-1 released in the gene bank with accession number KJE50039.1 was analyzed and the existence of a trehalose synthase with protein ID WP_048101287.1 that convert maltose to trehalose was potentially identified. According to this analysis, the gene was synthesized and the target gene was sub-cloned into pET-22b (+) and named pET-TreS. This construct was transformed into *E. coli* BL21 (DE3(cells and *Acidiplasma sp.* MBA-1 trehalose synthase (`TreS`(overexpression was induced by IPTG. Using the NCBI amino acid sequence of the *Acidiplasma sp.* MBA-1 showed sequence identities of 89, 66, 63 and 59% with *Picrophilus torridus* [accession number is WP_011176870.1], *Bacteriam* JKG1 [accession number is WP_029315667.1], *Kouleothrix aurantiaca* [accession number is KPV52019.1], *Myxococcus xanthus* [accession number is WP_011553702.1], respectively (Fig.1).

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Acidiplasma     mlddgnklwyrdaifyevpvksfydsngdgigdfrgltmklgylknlgvdalwllpfyks
Picrophilus     -mldnnglwyrdavfyevpvksfydsnndgigdfngltmkldylkklgvdalwllpfyks
Bacterium       --mnsdelwykdaifyelhvkafqdsngdgigdfrglierldylqdlgvdciwllpfyps
Kouleothrix     --mtvenqwykdaifyelgvksfydsnadgigdfrgliekldylkelgvdciwvtpfyps
Myxococcus      --mdldplwykkaliyelhlrafhdsngdghgdipglieklplylqdlginclwllphyps
                     :  **:.*::**: :::* *** ** **: **  :* **:.**::.:*: *.* *

Acidiplasma     plkddgydisdyysilpeygtiddfkqfietahsmnirviadlvlnhvsdqhpwfveark
Picrophilus     plkddgydisdyysilpeygtiddfknfidtahsmnirviadlvlnhvsdqhpwfiesrs
Bacterium       plkddgydiadyygihpdygtlkdfkqflraahhrgmrviadlvinhtsdqhpwfqaara
Kouleothrix     plkddgydiadyygvhpdygtlqdtklfikeahrrglkvmfdlvmnhtsdqhawfqears
Myxococcus      plrddgydiadfygvhpdygtladfqrlvdeahkrdirvitelvvnhtsdqhpwfqears
                **:******:*:*.: *:***: * : ::  **   ::*: :**:**.**** **  :*

Acidiplasma     srdspkrnwfiwsdtpdkfkeariifidteksnwaydqesgqyyfhrfyssqpdlnydnp
Picrophilus     sidnpkrdwfiwsdtpekfkeariifidteksnwtydpetkqyyfhrfyssqpdlnydnp
Bacterium       drnspyrnwywwsdtpdkykeariifidteksnwtwdpvagqyywhrffshqpdlnydnp
Kouleothrix     dpksprrnyyywsdddqryndariifidtersnwtwdpaakkyfwhrffshqpdlnfdnp
Myxococcus      dpnspkrnwywwsdtdeqykgariifldtersnwtwdpvakqyfwhrffshqpdlnydnp
                . ..* *::::***  :::: *****:***:***::*  : :*::***:* *****:***

Acidiplasma     evreevkkiirywlnlgldgfradavpnlfkregtncenlpethnffkeirkmmdseypg
Picrophilus     dvrnevkkvirywldlgldgfradavpylfkrentncenlpethnffkeirkmmdedypg
Bacterium       kvrremlrvmnywldmgldgfrvdavpylferegtncenlpethaylkelrraidrryrg
Kouleothrix     rvrhsmfkimrhwldmgvdgfradaipylferdgtncenlpethaylkevrglmdreypg
Myxococcus      evqeamldvmrfwlnmgvdgfrcdavpylferegtncenlpethaflkrlrktidseykg
                 *:. :  ::..**::*:**** **:* **:*: ********** ::*.:*  :*  * *

Acidiplasma     tillaeanqwptdarayfgngdefhmafnfplmprifialakrdyypiediinqtlpvpd
Picrophilus     tillaeanqwptetkayfgngdefhmafnfplmprifialarsdyypimdiikqtlpipd
Bacterium       rillaeanqwpedllpyfgngdefhmafnfpvmprmfmalrreerrpiidiiqrtpdipe
Kouleothrix     rillaeanqwpadvrpyfgdgdefhmafnfpvmprifmalrredrtpieeiisqlpaipe
Myxococcus      kmllaeanqwpadvrvyfgdgdefhmgfhfpvmprlymavrredrtpiveimqqtpdipd
                 :********* :   ***:******.*.**:***:::*: : :  ** :*:.:   :*:

Acidiplasma     ncdwctflrnhdeltlemvtdaerdimfreyaklpkmrlnlgirrrlaplvdndintiel
Picrophilus     ncdwciflrnhdeltlemvtdserdimyreyakipkmrlnlgirrrlapladndintiel
Bacterium       ncqwclflrnhdeltlemvtdeerdymwaeyardprmklnlgirrrlaplldngrrriel
Kouleothrix     acqwclflrnhdeltlemvtdeerdymyheyakdarmkpnlgirrrlaplvdngrrriel
Myxococcus      ncqwaiflrnhdeltlemvtdedrdymyreyatdprmrinlgirrrlaplmdngrrriel
                 *:*. ************** :** *: ***   :*: ********** **  . ***

Acidiplasma     lnalifslpgtpiiyygdeigmgdniylgdrngvrtpmqwsydrnagfstadseelyspv
Picrophilus     lnalifslpgtpiiyygdeigmgdniylgdrngvrtpmqwsydrnagfsmadseqlyspv
Bacterium       lnamlfslpgspiiyygdeigmgdniflgdrngvrtpmqwsgdrnagfskadserlyapv
Kouleothrix     lnsllfslpgspiiyygdeigmgdniylgdrngvrtpmqwsgdrnggfsradaarlyspa
Myxococcus      mhsllftlpgtpvlyygdeigmgdniylgdrngvrtpmqwtgdrnagfsradyarlyapv
                :.:::*:***:*::************:*************: ***.*** **  .**:*.

Acidiplasma     itnpnynyesvnveaemrlnssllkwmikiinirkdykellgrgsikfinqknkrvlvyi
Picrophilus     itnpnyhyesvnveaelrlssslllnwmikiihvrkdykellgrgsikfieqgnkrvlsyi
Bacterium       iadpvygygavnveaqlrtpssllnwmkrlirirkryq-vfgrgsitflhpenqkvlayt
Kouleothrix     ivdavygygsinveaqlrdpssllnwmkrlirlrknyp-vfgrgdiaflhpenrkvlayi
Myxococcus      iadpvygygsinveaqervkssllhwvkrmirirqryp-vfslgnlrflqtdnrkvlafi
                *.:  * *:::****: *  ****.*: ::*.:*: *  ::. *.: *:.  *::** :

Acidiplasma     reyenqrmlclfnlsrsptyveldlheyaglkpieaitkaafprigdlnyfitmtprsff
Picrophilus     reyenqrmlclfnlsrnptyvelnlsdyiglkpieaitkaafprikddryfitmtprsff
Bacterium       rayqqehvlivnnlsrfsqpaeldlshyrgwtpvemfgetrfppigelpyfitlgphafy
Kouleothrix     rsgeqgtvlvvnnlsrfsqpveldlghyngwtpveifgetrfppigelpyflspgphgfy
Myxococcus      rewegqtvlvvcnlsrfaqpavldmrewegavpveligetpfprvselpyqlsmgpfmfl
                *  : : :* : ****    . *:: .: *  *:* : :: ** : :  * ::  *  *

Acidiplasma     wfnlivperddsfdlvddydqs
Picrophilus     wfnlivperddsydliged---
Bacterium       wfrleppasa------------
Kouleothrix     wfrleppaga------------
Myxococcus      wfrldrpqqvrsle--------
                **.*  *
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Figure 1 *Acidiplasma sp* MBA-1 amino acid sequence comparison with Trehalose synthase from different organisms. The amino acids marked by asterisks are sequence identical in all sequences. Amico acid marked by colons and dots are strongly and weakly conserved, respectively

SDS Page

TreS gene encodes a polypeptide of 562 residues with a calculated molecular mass of 66.09 kDa. Target recombinant protein purification was carried out using

nickel affinity column chromatography. SDS-PAGE analysis gave a strong protein band with a molecular mass of 65.9 kDa. The specific activity of this protein was 3.568 Umg-protein^{-1} in the purified enzyme.

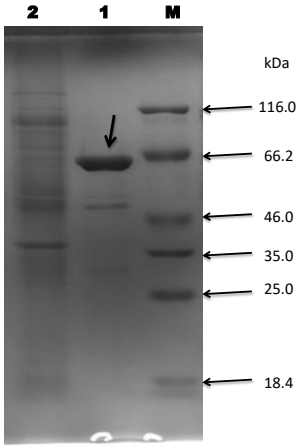

Figure 2 SDS-PAGE analysis of the recombinant protein. Lane 1 purified recombinant TreS (arrow indicates purified enzyme) Lane 2 Crude extract of the recombinant TreS. Lane M Molecular weight standards (116.0, 66.2, 46.0, 35.0, 25.0,18.4 kDa)

Effect of pH on recombinant TreS

Figure 3. shows the effect of pH on recombinant TreS activity. TreS showed the highest relative activity at pH 6.5 whereas the relative activity was 91.35% and 53.28% at pH 7.0 and 7.5, respectively. At higher pH value the activity of TreS dropped sharply.

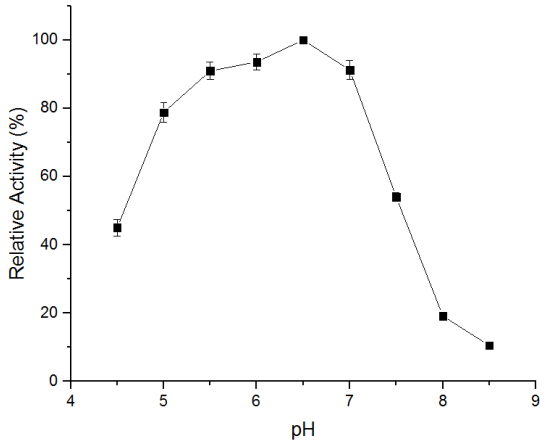

Figure 3 Effect of pH on recombinant TreS activity. Values are the means of three replicates ± standard deviation.

Effect of Temperature on recombinant TreS

As shown in figure 4, the enzyme showed maximum activity at a temperature of 40 °C, whereas at 35, 30 and 25 °C the relative activity decreased to 81.2%, 69.7%, and 42.4%, respectively. The enzyme activity increased up to 40 °C and then gradually decreased. The activity was dropped to 9.3% at 60°C.

The thermal stability of the enzyme was examined at pH 6.5 in a standard buffer (50mM Sodium phosphate buffer). As shown in figure 4, the relative activity of the enzyme was almost constant up to 40°C and the relative activity dropped significantly to 90, 71, 22 and 0% after incubation for 1h at 45, 50, 55 and 60 °C, respectively.

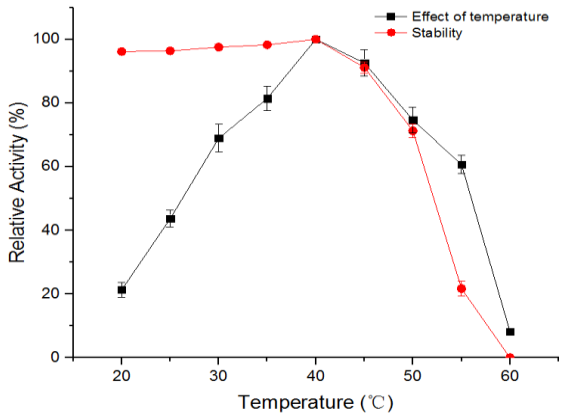

Figure 4 Effect of temperature on recombinant TreS activity. (■) effect of temperature on the enzyme activity (●(effect of temperature on the enzyme stability. To examine the thermal stability of TreS, the enzymes were pre-incubated at various temperatures (20–60 °C) for 1h at pH 6.5. The residual activities were measured at 40 °C. Values are the means of three replicates ± standard deviation.

Effect of metal ions on recombinant TreS

The recombinant TreS was assayed in the presence of various metal ions, which were incubated with an enzyme solution at a final concentration of 1 mM. The enzyme assay showed that magnesium, EDTA and Lithium raised the enzyme activity by 10.9%, 9.6%, and 6.7% respectively. Enzyme activity did not significantly change when incubated with manganese. The enzyme activity decreased significantly when incubated with cobalt, zinc, and nickel to 62.5%, 41.8%, and 33.34%, respectively, whereas copper completely inhibited the TreS enzyme activity.

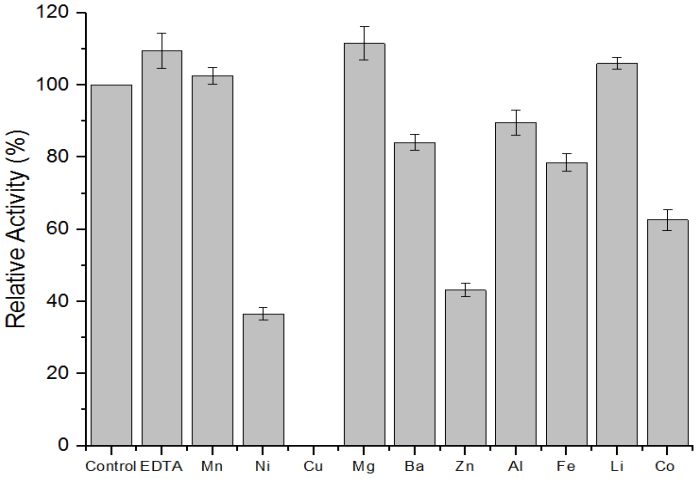

Figure 5 Effect of metal ions on recombinant TreS activity. Values are the means of three replicates ± standard deviation.

Substrate Specificity

Only maltose showed substrate specificity with TreS. The other substrates (Glucose, Lactose, Sucrose, Fructose, Mannose, β-cyclodextrin, Starch, Cellobiose and Galactose) showed no specificity (data not shown) for TreS as they did not produce any trehalose in the reaction.

Table 1 Substrate Specificity for TreS

Substrate	***Trehalose****
Glucose	×
Lactose	×
Sucrose	×
Fructose	×
Maltose	√
Mannose	×
Starch	×
Cellobiose	×
Galactose	×
β-cyclodextrin	×

**presence of Trehalose was checked in substrate specificity reaction*

Conversion profile

The purified enzyme)100 µl(was incubated in 900 µlsodium phosphate (50mM) buffer (pH 6.5) at 40 °C for 0–10 h, using 1% maltose as a substrate. All the reactions were stopped by boiling them for 10 min before the samples were analyzed by the HPLC system. After 9h of reaction, the conversion rate of trehalose and glucose were 43.62%, and 22.01% respectively.

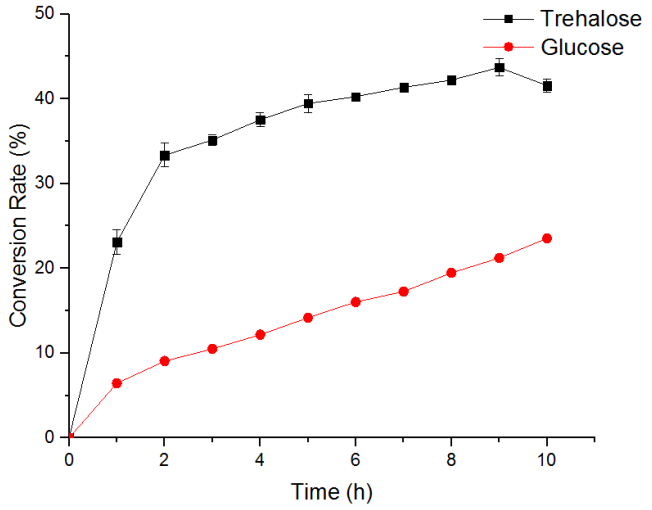

Figure 6 Conversion rate of trehalose and glucose from maltose with a different time (0-10h)

DISCUSSION

We have confirmed that the gene (gi= 765468230) from *Acidiplasma sp.* MBA-1. encoded a functional enzyme, trehalose synthase, and it could catalyze the conversion of maltose to trehalose. The optimum temperature of TreS found 40 °C which similar to those trehalose synthases coming from *Actinoplanes* SN223/29)**Lee *et al.*, 2008).** TreS maintained a high relative activity up to 45°C while checking the stability of the enzyme against temperature. The optimum pH was 6.5, similar to several trehalose synthases reported in past studies **(Liang *et al.*, 2013; Wu *et al.*, 2009)**. TreS activity was increased by Mg^{2+} and Li^{+}. The trehalose synthase from *Pseudomonas sp* **(Gao *et al.*, 2013)**, *Deinococcus sp.*)**Jiang *et al.*, 2013**(, *Deinococcus radiodurans*)**Filipkowski *et al.*, 201**2(is also reported to increase their activity by Mg^{2+}. EDTA has slightly enhanced the activity of the enzyme. We have not found any obvious reasons for that. It is most likely, EDTA is chelating metal ions that affect that binding site of TreS. TreS activity was strongly inhibited by Cu^{2+}. It is probably because copper ions were interfering with the binding site of TreS and making it inactive in the reaction. Other studies are also suggesting the same **(Yan *et al.*, 2013; Zhu *et al.*, 2010)**. Our experimental data showed that TreS could convert about 43.62% maltose to trehalose, accompanied by about 23.85% glucose as a byproduct after 10h of incubation. Other studies suggest that most TreS enzyme could produce glucose as a by product except *Pseudomonas stutzeri* CJ38 **(Lee *et al.*, 2005).** It is reported that glucose normally can inhibit the enzyme activity **(Chen *et al.* 2006)** and lowers the conversion rate from maltose to trehalose **(Wei *et al.* 2004).** Several other studies suggest that the trehalose synthases that produce less or no glucose as a byproduct have a higher production for trehalose of about 70% to 80% **(Lee *et al.* 2005; Chen et al., 2006; Nishimoto *et al.*, 1995,Nishimoto *et al.*, 1996).** As TreS possess a weak hydrolytic activity **(Zhu et al., 2010)**, it could be the reason as to why a high amount of glucose is produced. TreS could produce trehalose from maltose with a single step. Maltose is relatively cheap and this pathway could be an alternative method for industrial trehalose production. A number of Trehalose Synthase enzymes from different bacterial strains (**Nishimoto *et al.*, 1995; Nishimoto *et al.*, 1996 ; Chen *et al.*, 2006; Zdzieblo and Synowiecki, 2006; Wei *et al.*, 2004**; **Gao *et al.*, 2013**; **Yan *et al.*, 2013**; **Jiang *et al.*, 2013**; **Liang *et al.*, 2013**; **Filipkowski *et al.*, 2013**(have been identified and characterized. This study provided the characteristics of trehalose synthase from *Acidiplasma sp.* MBA-1 for the trehalose catalysis metabolism.

CONCLUSION

In our experiment, enzyme TreS produced from *Acidiplasma sp*MBA-1 can catalyze a considerable amount of maltose into trehalose in a single step reaction. We know that maltose is a relatively cheap substrate. Hence, TreS could be used as an alternative commercial enzyme to produce trehalose commercially. There is a drawback though. A significant amount of glucose is being produced as a byproduct which hinders the production of commercial trehalose. If it is possible to suppress glucose production by genetic modifications, it could enhance trehalose production. Beside, enzyme immobilization technique can be used to improve trehalose production further.

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