EUROPEAN ACADEMIC RESEARCH
Vol. IV, Issue 1/ April 2016

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Optimization of Trehalose Production from Maltose Using a Recombinant Trehalose Synthase (TreS) Enzyme from *Acidiplasma sp. MBA-1*

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Abstract:

Trehalose is an important disaccharide extensively used in pharmaceuticals and food industries. A novel enzyme called trehalose synthase (TreS) can convert maltose into trehalose with a single step reaction. Acidoplasma sp. MBA-1 has been used as a TreS source in our research work. The optimum condition for this enzymatic reaction has been identified. The optimum temperature and pH was found 40°C and 6.5 for 1h respectively. The highest transformation yield was found 10.57 mg/ml after 24h.

Key words: Trehalose, Trehalose synthase, *Acidiplasma sp. MBA-1*, Enzymatic assay, Transformation

1. INTRODUCTION

Trehalose is a naturally occurring disaccharide abundantly found in a variety of organisms i.e., bacteria, fungi,

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invertebrates [Himei, 2008] etc. It is also known as α-D-glucopyranosyl, α-D-glucopyranoside where two glucose molecules are liked with a 1,1glycosidic linkage. For a long time trehalose was known as a carbon and an energy source of plants and animals. A number of research in the past few decades showed that it is a multifunctional molecule. It has been identified as a structural component of the cell wall glycolipids [Arguelles, 2000][Richards, 2002]. Trehalose is stable under hot ad acidic conditions and it has affinity towards lipids and biological membranes and proteins. It can protect biological structures forming hydrogen bonds during freezing, desiccation and heating [Roser, 1991]. Due to its stability towards high temperature and hydrolysis it has been included as bulk agent in tablet applications [Mizumoto et al. 2004]. Colaco & Roser (1995) reported that trehalose can be used as an additive for food preservation. It can stabilize superoxide dismutase like activity of various vegetables [Aga et al. 1998]. It is completely non-toxic and capable of modifying flavors i.e., suppression of bitterness and enhancement of sourness [Oku, 1999][Richards & Dexter, 2001][Takeuchi & Banno, 2000]. Di methyl sulfide (DMS) is formed and degrades methionine and cystine residues during the heating of milk and it causes foul odor. Kubota (2005) reported that the addition of treahlose can suppress the formation of foul odor and reduced the amount of DMS. This is thought to occur through the direct interaction of trehalose with the amino acid groups, thus suppressing the formation of sulfur compounds [Ohtke & Wang, 2011]. Himei (2008) reported that trehaolse has the ability to suppress oxidation reaction as well. Trehalose can be used in the pharmaceuticals, cosmetics and food industries. The reason trehalose is a suitable molecule in food processing is its mild sweetness, low carcinogenicity, good solubility in water, stability under low pH conditions, and reduction of water activity, low hygroscopicity, depression of freezing point, high

glass transition temperature and ability to protect proteins [Zdzieblo & Synowiecki, 2006]. It is a multifunctional food ingredient that does not caramelizes and undergo 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].

Even though trehalose can be found in many organisms in nature, the commercial production of this rare sugar has always been a challenge to the researchers. The trehalose extraction from yeast and other organisms are very expensive for the industries. Recently, many researchers have been discovered several enzyme synthesizing systems in microorganisms [Lama et al. 1990][Nakada et al.1996][Di Lernia et al. 1998]. There are five main enzymatic pathways of trehalose biosynthesis have been identified so far [Avonce, 2006]. The first one is TPS/TPP pathway: a two step enzymatic pathway where TPS catalyzes the transfer of glucose from UDP glucose to glucose 6-Phospate forming trehalose 6-phosphate and UDP, while TPP dephosphorylates trehalose 6-phosphate to trehalose and inorganic phosphates [Elbein et al. 2003][De Smet, 2000]. The second biosynthetic pathway is MTS-MTH: it involves the conversion of maltodextrin (maltooligosaccharides, glycogen, starch) into trehalose through a two step enzymatic reaction. Two novel enzymes namely maltooligosyltrehalose synthase (MTS), coded by TreY gene, and maltooligosyl trehalosetre halohydrolase (MTH), coded by TreZ gene, are involved in this reaction. The MTS converts maltodextrin into an intermediate product called maltooligosyl trehalose. Later, the MTH enzyme converts maltooligosyltrehalose into free trehalose. These enzymes were reported in the thermophilic archaea of the genus sulfolobus [Nakada et al. 1996a][Nakada et al. 1996b]. The third one is TreP pathway: Trehalose Phosphorylase catalyses the reversible hydrolysis of trehalose in the presence of inorganic phosphate [Avonce, 2006]. The

fourth pathway is TreT: trehalose glycosyltransferring synthase catalyzes the reversible formation of trehalose from ADP-glucose and glucose [Qu et al. 2004]. The fifth biosynthetic pathwayis Trehalose synthase (TreS): TreS isomerizes α1- α4 bond of maltose to α1- α1 bond resulting in trehalose. It is first reported in *pimelobacter sp.* and orthologs of this protein have been foud in other eubacteria [Avonce, 2006][Nishimoto et al. 1995]. Among these pathways TreS is a simple, fast and low cost method for the industrial production of trehalose from maltose.

A number of bacterial strains have been reported to have a responsible gene that can produce trehalose synthase (TreS) enzyme upon fermentation with a suitable sugar sources. *Acidiplasma sp. MBA-1* has also a gene responsible for TreS production inside bacterial cells.

The objectives of our study are to find the potential of producing trehalose from maltose using a recombinant TreS enzyme from *acidiplasma sp.* and optimize the trehalose production conditions for further research. To the best of our knowledge, this is the first report on trehalose production using an *Acidiplasma sp. MBA-1*.

2. MATERIALS AND METHODS

All the chemicals required for the whole experiment were purchased from Sinopharma ltd., China. The genesequence of bacterial plasmid of A*cidiplasma sp. MBA-1*was obtained from GenBank; Accession no. KJE500039.1 and sent it to Generay Biotech Co., Ltd. (Shanghai, China) for plasmid synthesis. The reconstructed plasmid was cloned into *E. Coli BL21 (DE3)* in our laboratory.

2.1 Cultivation of Recombinant Strains

The recombinant *Acidiplasma sp. MAB-1* A has grown in 1000 ml of Luria Bertani (LB) mediawith 1µl /ml kanamycin (kan) at 37 °C until the OD_{600} is reached at 0.6. The fermentation was continued adding IPTG to the media with a concentration of 1 µl /ml at 28 °C degree temperature for 12 hours. The cells were harvested by centrifugation (8000×g for 10 minutes at 4°C). The cell pellets were re-suspended in 60ml of 50mM Sodium phosphate buffer (pH 7.5). The cells were disrupted by an ultra-sonicator using a Vibra-Cell™ 72405 sonicator (BioBlock Scientific, Illkirch, France). Crude enzyme was extracted by centrifugation (10000×g for 20 minutes at 4°C). Crude enzyme was stored at 4°C for further use.

2.2 Enzymatic Assay

The total volume of the reaction mixture was 1ml containing 900 µl 50 mM sodium phosphate buffers as a substrate solution (5% maltose) and 100 µl of crude enzyme. The standard reaction was carried out for an hour. Finally the reaction was stopped by heating in a boiling water bath at 100°C for 10 minutes. The residual activity of the standard reaction was checked in a HPLC system (Agilent 1260, CA,U.S.A.).

2.3 Optimum Temperature and pH

Optimum temperature was determined by carrying out a number of standard reactions at different temperatures ranging from 20-60°C for 1h.Similarly optimum pH was determined by carrying out a number of standard reactions at various pH ranging from 5.5-8.5 for 1h.

2.4 Carbohydrate Analysis

Trehalose was detected by High Performance Liquid Chromatography (HPLC)system equipped with a refractive index detector and a 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.

2.5 Total Protein Concentration

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

3. RESULT AND DISCUSSION

3.1 Effect of Temperature on TreS

The effects of temperature were showed in Fig. 1. The figure (1) showed that there is no significant difference in the relative activity from 35-°C-45°C temperature. It was a typical bell shaped curve. The optimum temperature of TreS was 40°C which is much higher than other TreS such as the one (25°C) coming from *Rhodococcusopacus* (Yan et al. 2013). The other TreS (35°C) from *Corynebacterium glutamicum* (Kim et al. 2010) and TreS (37°C) from *Pseudomonas sp P8005* (Gao et al. 2013) have the similar optimum temperature to this one. The relative activity reduced dramatically from 96.65% (45°C) to 79.78% at 50°C and 55.54% at 55°C. At 60°C, TreS showed no activity.

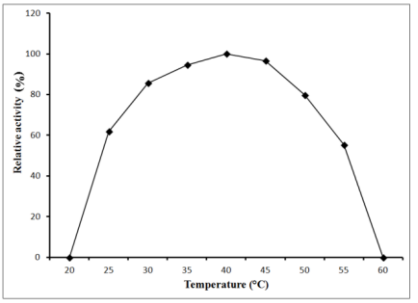

Figure 1. Effect of Temperature on TreS with maltose as the substrate

3.2 Effect of pH on TreS

The effects of pH were showed in Fig. 2. The optimum pH was found 6.5. There were slight differences (93.85-100%) in relative activity at lower pH (5.5-7). With the increase of pH value, the relative activity is increased gradually until it reached to pH6.5. The relative activity declined sharply (from 98.57% to 68.57%) after the neutral pH value. The relative activity reduced by 30%%. After that, relative activity gradually decreased to zero. The optimum pH of TreS was 6.5 which is similar to other TreS coming from *Rhodococcusopacus* (Yan et al. 2013).

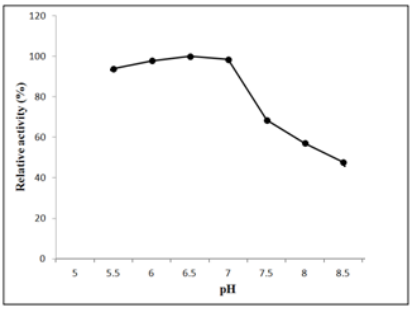

Figure 2.Effect of pH on TreS with maltose as a substrate

3.3 Transformation of Product

We determined the transformation of product over different time (0-48h).The higher the incubation time the greater the transformation yield. The highest transformation yield of trehalose was 10.57 mg/ml after 24h of incubation. After 36h of incubation the relative transformation of trehalose has dropped by 8.52% and the transformation yield was 9.67 mg/ml. Finally, after 48h of incubation the transformation yield was 8.94 mg/ml. The yield is reduced due to hydrolysis reaction by TreS.

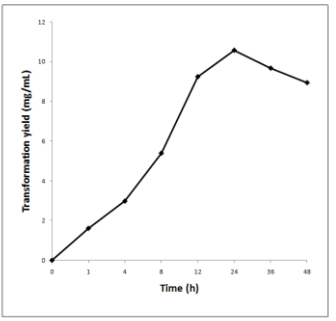

Figure 3.Transformation of product (trehalose) at different time.

3.4 Total Protein Concentration

The total protein concentration of the crude enzyme was found 5.23 mg/ml.

4. CONCLUSION

Crdue enzymes are easy to use and a cost effective for the production of rare sugar.And, considering the optimum reaction conditions and transformation yield of trehalose, TreS from *Acidiplasma sp. MBA-1* can be readily used for the commercial production of trehalose. Moreover, the purified TreS could provide a better yield than the crude enzyme, hence further research on the purification of TreS and its characterization is also highly recommended.

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