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Full Length Research Paper

Meiothermus sp. SK3-2: A potential source for the production of trehalose from maltose

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Trehalose has similar chemical formula as maltose. In terms of price, trehalose is much more expensive than maltose. A pink-pigmented bacterium identified as *Meiothermus* sp. SK3-2 was found to be able to convert maltose to trehalose. Based on fatty acids analysis, the *Meiothermus* sp. SK3-2 may be a new strain that produce trehalose synthase (TreS). *Meiothermus* sp. SK3-2 achieved a better biomass growth in MM medium containing maltose. Besides that, TreS activity yield was also higher in MM medium, approximately 3.5, 1.8 and 0.3 fold than that in PY medium, thermophilic *Bacillus* medium and Castenholz medium, respectively. The optimum working temperature and pH for *Meiothermus* sp. SK3-2 TreS was 65°C and pH 6.0, respectively. Ammonium chloride at 10 mM increased the activity significantly, while calcium chloride at 5 mM decreased the activity by about 80% and the activity was fully retarded by 10 mM $CaCl_2$. It was found that the product specificity of this TreS was influenced by factors like temperature, pH and buffer system used. Analysis of the nucleotide sequence revealed the presence of an open reading frame of 2,890 bp which encoded a 963 amino acid protein. In conclusion, *Meiothermus* sp. SK3-2 TreS could serve as an alternative source to trehalose production.

Key words: Maltose, *Meiothermus*, trehalose, trehalose synthase.

INTRODUCTION

Trehalose is a disaccharide of two glucose monomers that resembles maltose. Unlike maltose, trehalose is non-reducing and is found naturally in invertebrates, plants, yeasts, fungi and some prokaryotes bacteria. The presence of this disaccharide is known to increase the survival rate of some species typically during environment stress. Due to that, diverse research has been done to study the applications of trehalose.

Formulation of vaccines is an important determinant for the stability of the drug. Certainly in developing countries, the need of stable vaccines at room temperature during storage, handling and logistic is crucial (Amorij et al., 2008). Addition of trehalose in vaccine for an example in Newcastle disease (ND) strain I-2 (Wambura, 2009) has indeed proven its importance.

Besides that, trehalose has been reported as a stabilizing ligand or osmolytes for improving the stability of protein during storage. Supplements of trehalose at concentration of 10 to 30% improved the thermostability of bovine serum albumin (BSA) (Lavecchia and Zuorro, 2010), while protein secondary structure for thermolabile firefly luciferase was greatly stabilized by the addition of trehalose and magnesium sulfate (Ganjalikhany et al., 2009). Trehalose has also been found to stabilize amylolitic enzyme such as α-amylase (Yadav and Prakash, 2009) and glucose oxidase (Paz-Alfaro et al., 2009).

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Abbreviations: GPase, α-1,4-D-Glucan phosphorylase; **MTHase,** maltooligosyl trehalose trehalohydrolase; **MTSase,** maltooligosyl trehalose synthase; **PCR,** polymerase chain reaction; **TreS,** trehalose synthase; **TPase,** trehalose phosphorylase; **$T_{opt.}$,** optimum temperature.

Trehalose is also a good cryopreservative for animal cells (Shiva et al., 2010). Other function and applications of trehalose were earlier suggested elsewhere, (Higashiyama, 2002).

In spite of many usages, the conventional approach to obtain trehalose was extraction from yeast. In the 1990s, the cost for trehalose was USD$700/kg (Paiva and Panek, 1996). Since then, enzymatic approach to produce trehalose was preferred as the production cost is lower while yield is higher than the conventional approach. At least three enzymatic reactions were known to produce trehalose. In two-step reactions, enzyme GPase and TPase convert starch into intermediates which are further transform into trehalose. The second mechanism also involves two reaction steps in which combination of MTSase/TDFE and MTHase/TFE are able to produce trehalose from maltodextrins. To date, only trehalose synthase (TreS) converts maltose directly into trehalose in a single step reaction (Schiraldi et al., 2002). In this work, a locally isolated *Meiothermus* strain that exhibited TreS activity was reported. Characterization of the strain was described and the factors that affect the performance of this enzyme were reported. The strain was isolated from a famous geothermal spring in Malaysia. Sungai Klah (SK) is a streamer hot spring located at N 3°59'44", E:101°23'36" in Malaysia. It is one of the hottest springs with temperature range from 60 to 110°C.

MATERIALS AND METHODS

Sample source and isolation

The temperature and pH of the collected water sample was 70°C and pH 7.3, respectively. The collected water samples were kept at 4°C until use. Samples of 100 µl were spread on thermophilic *Bacillus* medium (pH 7.5) (Atlas, 2004) containing (g/L): peptone, 8.0; yeast extract, 4.0 and NaCl, 3.0; solidified with 1.0% (w/v) GELRITE and 0.1% (w/v) $CaCl_2 \cdot 2H_2O$. All the plates were incubated at 55°C for two days. Repeated streaking on the same solid medium was done until purified single colonies were obtained. Purity of the cultures was determined by colony morphology and microscopic observation.

Microscopic and phenotypic characterization

Cellular morphology was observed under a light microscope (Leica DMLS) at 1000× magnification. The microorganisms were observed according to their cellular shape, arrangement and Gram-staining reaction.

Fatty acid compositions

Analysis of cellular fatty acid methyl esters (FAME) was performed at the MIDI Sherlock, India (Royal Life Sciences Pvt. Ltd.). Comparison with established *Meiothermus* and *Thermus* sp. was done manually.

16S rDNA sequence and phylogenetic analysis

Genomic DNA was extracted using Yeastern Biotech Genomic DNA extraction kit after treating the cell wall with lysozyme solution. The 16S rDNA gene was amplified by PCR with YEAtaq DNA polymerase (Yeastern Biotech) using bacteria-specific universal forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1525R (5'-AAGGAGGTGATCCAGCCGCA-3') (Baker et al., 2003). The PCR product was cloned into pGEM-T system (Promega) and sequenced. A phylogenetic tree was constructed by neighbor-joining method with a bootstrap value of 1000 replicates using software package MEGA 4.0 (Tamura et al., 2007).

Biomass production using various media

A total of four media were used to screen for the best medium in terms of biomass production and TreS activity. The media recipe was in accordance with Atlas (2004), unless stated. The media were named as MM (Sinkiewicz and Synowiecki, 2009) (g/L): peptone, 5.0; yeast extract, 1.0; maltose, 5.0; the PY medium: peptone, 0.4; yeast extract, 0.2; starch, 1.0 and the Castenholz medium: nitrilotriacetic acid, 0.5; $CaSO_4$. $2H_2O$, 0.5; $MgSO_4.7H_2O$, 0.5; NaCl, 0.04; KNO_3, 0.5; $NaNO_3$, 3.4; $Na_2HPO_4.2H_2O$, 0.878; $FeCl_3.6H_2O$, 0.01; $ZnSO_4.7H_2O$, 0.0025; H_3BO_3, 0.0025; $CuSO_4.5H_2O$, 0.25; $Na_2MoO_4.2H_2O$, 0.25; $CoCl_2.6H_2O$, 0.45; tryptone, 5.0; yeast extract, 5.0. The original thermophilic *Bacillus* medium that was used to isolate the strain was compared as well. The growth was done simultaneously at 55°C.

Enzyme activity determination

After 48 h of culturing, the cells were lysed with sonicator and centrifuged to collect the crude enzyme. The crude enzyme was then reacted with maltose for 2 h at optimum temperature and pH. The column used for detecting the sugars of interest was WATERS® NH_2-column, while the mobile phase was 75:25 of acetonitrile and purified water. The flow rate was controlled at 0.6 ml/min. As for the standards, high purity grade of glucose, trehalose, maltose and maltotriose were prepared.

Optimum pH and temperature

The tested optimum pH range of the crude TreS activity was 5.5 to 8.0 using 20 mM sodium phosphate buffer, while the optimum temperature was determined by incubating the enzyme with maltose at different temperatures, ranging from 25 to 75°C.

Cloning of trehalose synthase gene

The isolated genomic DNA of *Meiothermus* SK3-2 was used as template. Degenerated forward primer 5'-GTGGAYCCYCTYTG GTACAAGG-3' and reverse primer 5'-TSKCCGGCCKKKKCCGK CCASGG-3' were synthesized by a local company; First Base Sdn. Bhd. Amplification using GoTaq polymerase (Promega) was conducted in 50 µl under the following condition: initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 50 s, 55°C for

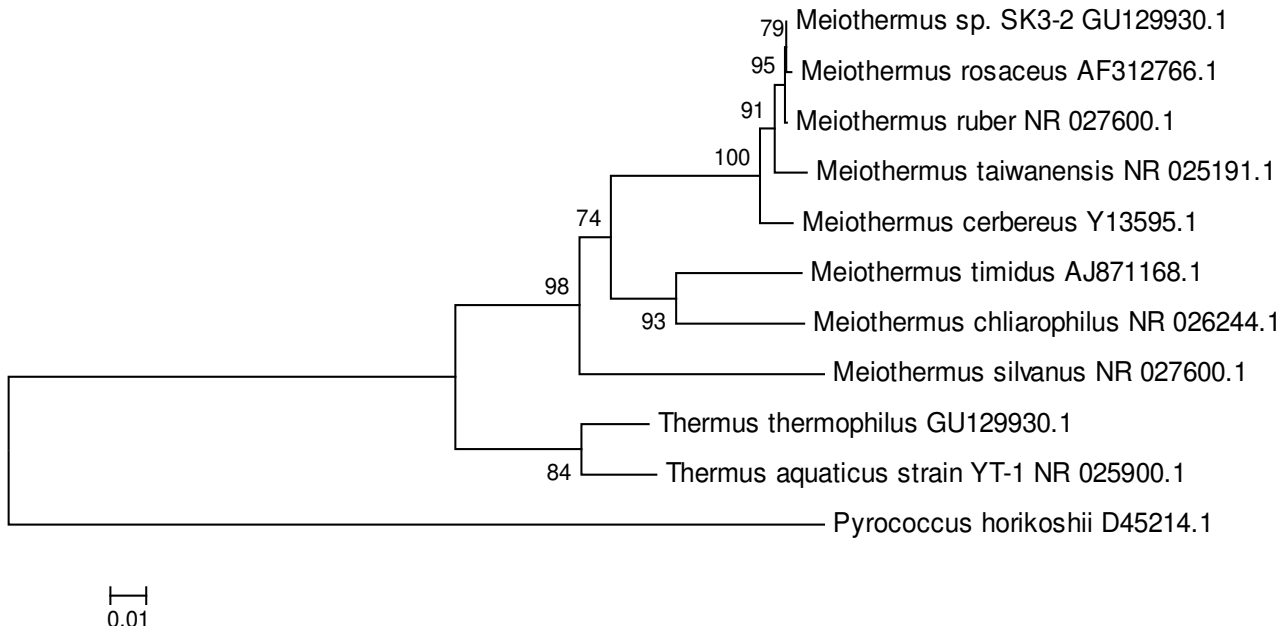

Figure 1. Phylogenetic analysis of *Meiothermus* sp. SK3-2 and other taxa from *Meiothermus* and *Thermus* species. *P. horikoshii* was chosen as an outgroup. Tree was generated with MEGA 4.0 program with bootstrap of 1000.

30 s, 72°C for 3 min and final extension at 72°C for 5 min.

RESULTS AND DISCUSSION

Morphological and biochemical analysis

Meiothermus sp. SK3-2 pure colonies appeared to be in circular forms, convex elevations, smooth margins and glistening surfaces. The size of the colonies was approximately 0.9 mm and they were pink pigmented. Under the light microscope with 1000× magnification, cells were fine, occurred in chains and stained Gram-negative. *Meiothermus* sp. SK3-2 had the optimum growth at 55 to 65°C with maximum tolerance at 70°C. The strain has a very broad range of growth pH, ranging from pH 6.5 to 10.0. However, the preferred growth pH was in the range of pH 7.5 to 8.5.

Phylogenetic analysis

Almost the complete 16S rDNA sequence of *Meiothermus* sp. SK3-2 was found to be 1482 bp in length and has been deposited with the accession number GU129930. Neighbor-joining statistical method with bootstrap replications number of 1000 was used to construct the phylogenetic tree (Figure 1). *Meiothermus* strain SK3-2 and other reported *Meiothermus* strains formed a sister line of descent with the species of *Thermus* genus. *Meiothermus* and *Thermus* are closely related genera inside the order of *Thermales* and were previously categorized as the same genus. *Meiothermus* sp. SK3-2 has close16S rDNA similarity with *Meiothermus rosaceus*, *Meiothermus ruber*, *Meiothermus taiwanensis* and *Meiothermus cerbereus*. The phylogeny showed that strain SK3-2 was more distantly related with *Meiothermus timidus*, *Meiothermus chliarophilus* and *Meiothermus silvanus*.

Mean fatty acid composition of *Meiothermus* sp. SK3-2

Meiothermus sp. SK3-2 fatty acids are predominantly iso- and anteiso-branched (Table 1). This is in good agreement with other known *Meiothermus* strains with iso- and anteiso-branched C15 and C17 fatty acid the major acylchains. Straight chain saturated fatty acids and unsaturated branched-chain fatty acids were found in minor concentrations.

Determination of the best medium for biomass production and enzyme activity

Four different media were used to grow *Meiothermus* SK3-2. As strain SK3-2 grew slowly, sampling was done every 8 h up to 48 h. According to Figure 2, *Meiothermus* SK3-2 grew moderately in thermophillic *Bacillus* medium, the medium that was previously used to isolate the strain.

Table 1. Fatty acids comparison of various *Meiothermus* species.

Fatty acid	1	2	3	4	5
13:0 iso	0.6	0.4	0.4	0.7	1.5
14:0 iso	1.4	0.6	1.3	0.7	2.6
15:0 iso	20.7	25.9	30.9	38.4	35.5
15:0 anteiso	30.8	22.5	6.5	2.9	6.2
15:0	-	0.2	3.3	2.0	2.0
16:1 ω7c alcohol	0.6	-	0.7	-	2.0
16:0 iso	3.9	1.6	4.8	2.6	4.1
16:0	7.1	5.5	4.9	6.1	5.1
15:0 iso 3OH	0.7	-	0.2	-	0.6
15:0 2OH	0.8	-	0.9	0.3	0.4
17:0 iso	10.3	12.7	16.5	17.4	6.0
17:0 anteiso	9.8	6.9	4.4	2.4	1.6
17:1 ω8c	1.6	-	0.6	-	0.7
17:0	0.5	0.3	2.1	1.7	0.4
17:0 iso 3OH	0.6	-	1.5	-	4.7

1, *Meiothermus* sp. SK3-2; 2, *M. silvanus*; 3, *M. ruber*; 4, *M. taiwanensis*; 5, *M. cerbereus*.

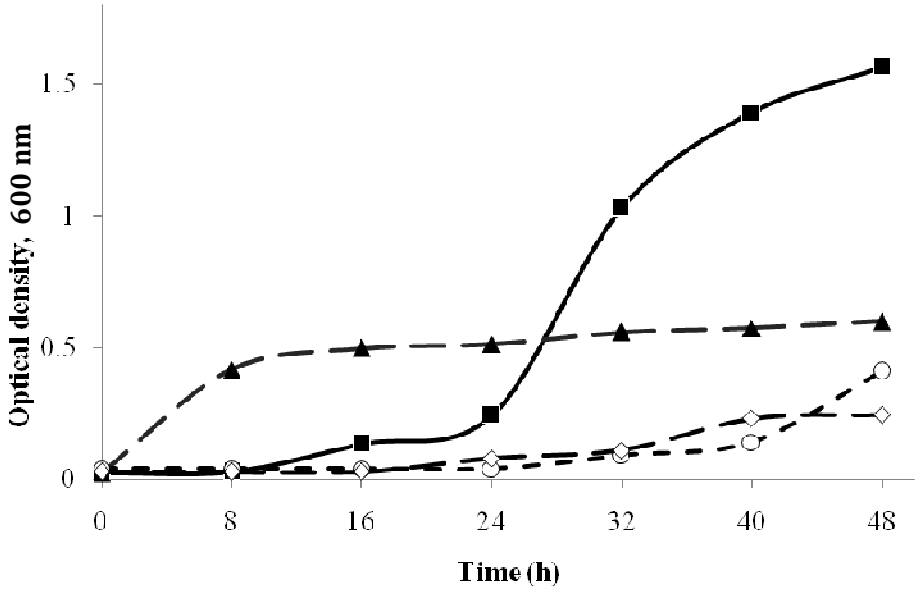

Figure 2. The effect of medium to cell growth of strain SK 3-2. Sampling was done every 8 hours. (■: MM medium, ▲: Castenholz medium, ○: PY medium, ◇: thermophilic *Bacillus* medium).

PY medium, which was supplemented with 0.1% starch, did not significantly promote the growth. Castenholz medium, on the other hand, minimized the lag phase of the strain and within 20 h, maximum growth rate was achieved. Although, the growth of cells in MM medium at 24 h was only half of the biomass using Castenholz medium, prolonged incubation of 48 h maximized the biomass up to approximately seven fold. The results imply that *Meiothermus* SK3-2 readily utilized maltose (in MM medium) but not starch as the main carbon source (PY medium). This suggests that *M.* SK3-2 is unable to hydrolyze starch. Some species such as *M. ruber* (Nobre et al., 1996) and *M. cerbereus* (Chung et al., 1997) were reported unable to utilized starch too;

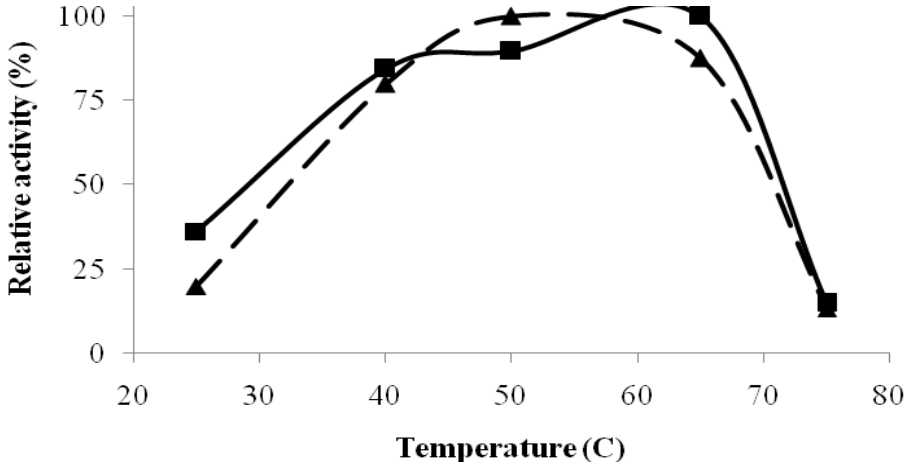

Figure 3. Effect of temperature to performance of TreS. Using maltose as substrate, trehalose forming activity was highest at 65°C while glucose was produced more at 50°C. (■: trehalose forming, ▲: glucose forming).

however, *M. chliarophilus* and *M. silvanus* could (Nobre et al., 1996).

Subsequently, equal volumes of Meiothermus SK3-2 cultures of the four media were centrifuged to collect cell pellets and were sonicated. Cell-free lysate that contained crude TreS was quantified using HPLC. For MM medium, besides promoting high biomass weight, *Meiothermus* SK3-2 exhibited the highest trehalose synthase activity. Castenholz, thermophillic *Bacillus* and PY media enabled the cells to exhibit comparatively lower activity of 77, 35 and 22%, respectively of the activity achieved in the MM medium. This suggested that, the production of enzyme is cell weight associated and the additional of maltose encouraged both cell propagation and enzyme yield.

Effect of temperature and pH on enzyme activity

The enzyme samples were subjected to five temperatures; 25, 40, 50, 65 and 75°C. The highest enzyme productivity obtained was at 65°C (Figure 3). When the reaction mixture was incubated in room temperature, the amount of trehalose was three times lesser than that incubated at the optimum temperature. The optimum temperature of 65°C is comparable with those of other TreS from thermopiles, such as *Thermus ruber* TreS (Sinkiewicz and Synowiecki, 2009) and *Thermus aquaticus* TreS (Nishimoto et al., 1996) and was higher than that of the thermophilic strain *Thermobifida fusca* (T_{opt}: 25°C) (Wei et al., 2004). A TreS gene from hyperacidophilic, thermophilic archaea *Picrophilus torridus* (Chen et al., 2006) was previously cloned. Its optimum temperature of 45°C was much lower than that of TreS from *Meiothermus* SK3-2. Other mesophilic trehalose synthase for example in *Corynebacterium nitrilophilus* NRC (Asker et al., 2009) and *Arthrobacter aurescens* (Xiuli et al., 2009) had optimum temperatures of 35 and 25°C, respectively. This suggests that, *Meiothermus* SK3-2 TreS may serve as a potential candidate for application as heat tolerant enzymes and are more feasible in industries.

It was found that *Meiothermus* SK3-2 TreS produced glucose as a byproduct of the intramolecular transglycosylation. The optimum temperature for this by-reaction was 50°C, however higher temperature is needed to produce trehalose. Therefore, it was suggested that product specificity was strongly determined by the reaction temperature.

The pH range of 5.5 to 8.0 was tested to determine the optimum activity for *Meiothermus* SK3-2 TreS. Transglycosylation reaction of *Meiothermus* SK3-2 TreS was significantly influenced by the pH of the reaction. The highest trehalose production happened at pH 6.0 and gradually decreased, as the pH was increased. In contrast, the transglycosylation reaction for glucose production as a by-product was highest at pH 7.0 (Figure 4). The results elucidate that, product specificity of TreS is greatly influenced by temperature and pH and such claim was not demonstrated clearly in earlier publications.

Effect of substrate concentrations and incubation time on production of trehalose

Three different concentrations of maltose; 30, 60 and 90 mM were prepared in sodium phosphate buffer at pH 6.0 (Figure 5). The reaction was carried out at 65°C up to 16 h. Trehalose formation was rapid and reached the maximum amount at an average of 7 h. When 30 mM

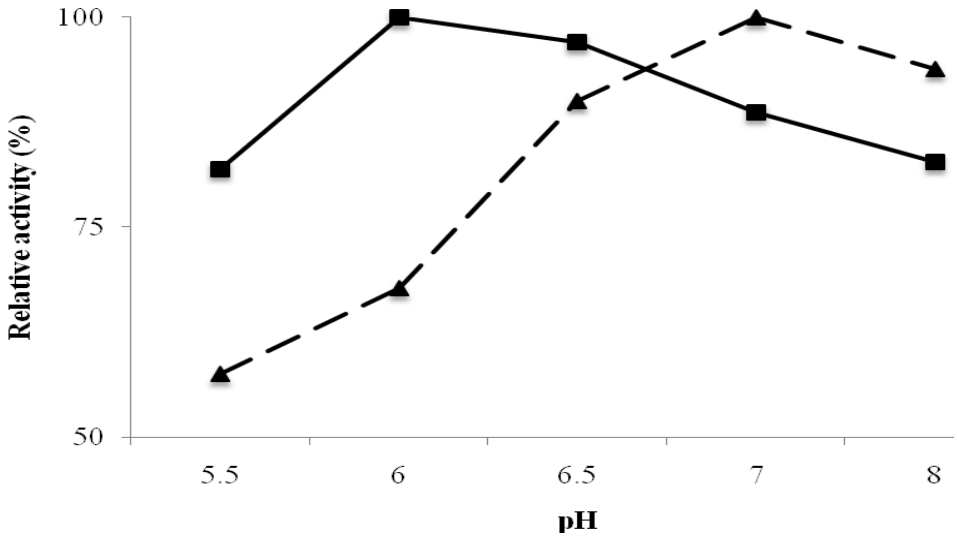

Figure 4. Effect of pH to optimum performance of TreS. Using maltose as substrate, trehalose forming activity was highest at pH 6.0 while glucose was produced more at pH 7.0. (■: trehalose forming;▲: glucose forming).

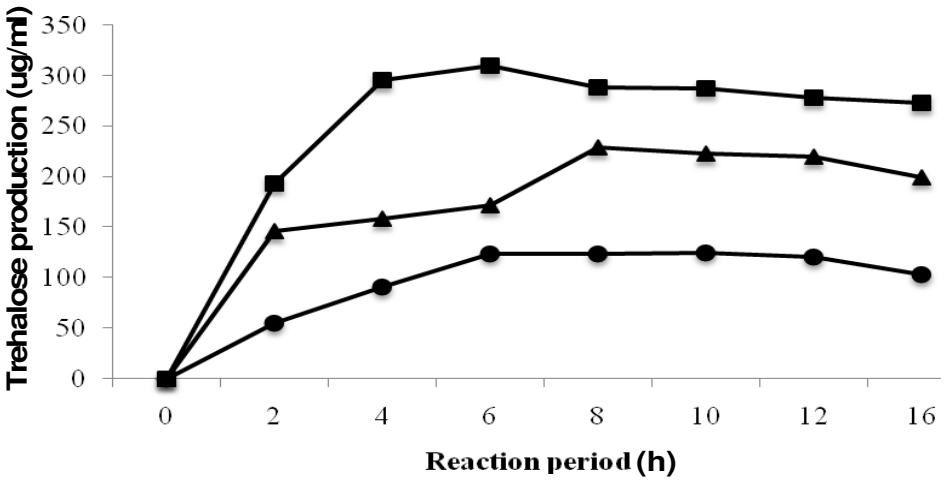

Figure 5. Production of trehalose using different concentrations of maltose (●, 30 mM maltose; ▲, 60 mM maltose; ■, 90 mM maltose).

maltose was used as substrate, the maximum of approximately 120 µg trehalose/ml was formed. The amount of trehalose formed was about 2.5 fold when 90 mM maltose was utilized.

Effect of supplements on TreS activity

The effects of various supplements on trehalose production are shown in Table 2. The control for this experiment was the normal reaction of TreS on maltose without any supplements. It was earlier mentioned that, glucose was a by-product of *Meiothermus* SK3-2 trehalose synthase. By additional of 1, 5 and 10 mM of glucose to the reaction, the trehalose-forming activity dropped and was only 65, 50 and 30%, respectively of that of the control. This finding is in agreement with *T. fusca* TreS (Wei et al., 2004) where glucose was reported as a competitive inhibitor. Most possibly, the glucose binds at the same location of the substrate binding site of the enzyme and therefore, causes a hindrance to the conversion of the substrate to product.

Besides glucose as a by-product, it was found that maltotriose was also formed as a by-product by

Table 2. Relative activity of TreS in various supplement.

Supplement	Relative activity (%)
Control	100
1 mM glucose	65
5 mM glucose	50
10 mM glucose	30
1 mM maltotriose	91
5 mM maltotriose	76
10 mM maltotriose	73
1 mM $CaCl_2$	66
5 mM $CaCl_2$	18
10 mM $CaCl_2$	0
5 mM NH_4Cl	114
10 mM NH_4Cl	132

Meiothermus TreS. However, maltotriose peaks on HPLC chromatogram was less significant during the first few hours of the reactions. In prolonged reaction, for example after 8 h, maltotriose peak was easily inspected on the chromatogram. Table 2 shows that, when 1, 5 and 10 mM maltotriose was added into the reaction tubes, the inhibition of *Meiothermus* SK3-2 Tres was found to be 9 to 27%. Comparison of glucose at equal concentration, with maltotriose showed that maltotriose had less inhibition effect. Maltotriose may binds more weakly to the binding pocket of TreS.

The additional of $CaCl_2$ is known to increase the thermostability or activity of many amylolytic enzymes. However, $CaCl_2$ had extremely negative effect on TreS. The relative activity was only 66% at 1 mM concentration, while the reaction was fully retarded at 10 mM $CaCl_2$. This finding is in good agreement with TreS from *P. torridus* (Chen et al., 2006); however, opposite observation was noticed for *A. aurescens* trehalose synthase (Xiuli et al., 2009).

Previously, comprehensive analysis of the effect of metal ions and reagents on the activity of TreS from *T. aquaticus*, *A. aurescens* and *P. torridus* were reported (Nishimoto et al., 1996; Chen et al., 2006; Xiuli et al., 2009). Nevertheless, trehalose synthase were found sensitive to most of the tested salts. For the first time, it was found that with the addition of 5 and 10 mM ammonium chloride, the relative activity of TreS increased to 114 and 132%, respectively.

Effect of different buffer systems on product specificity of trehalose synthase

Different buffer systems could offer different buffering and ionic strength and therefore, influence the activity, stability or even product specificity of enzymes. It was found that pH 6.0 was the optimum pH for *Meiothermus* SK3-2 TreS. At that pH, besides catalyzing the formation of trehalose from maltose, the in house TreS also produced glucose and maltotriose as by-products. The effect of various buffers to product specificity was studied also. Sodium phosphate, potassium phosphate, MES and citrate buffer of pH 6.0 were compared. Data shown in Figure 6 refers to the ratio of trehalose, glucose and maltotriose at the 8^{th} hour of the reaction period. Interestingly, most of the substrate maltose was converted into glucose and maltotriose. When sodium phosphate, potassium phosphate and citrate buffer were used for reaction, the percentage of trehalose produced was less than 20%. However, double increase in the percentage was observed for the reaction carried out in MES buffer. The actual reason behind this is unknown; however, MES buffer system may create a slight different protein structure conformation such as the active site or binding pocket that changes the product specificity profile 2,886 bp that encoded a 962 amino acid protein.

DNA and protein sequence of *Meiothermus* SK3-2 TreS

Gene that encodes for *Meiothermus* SK3-2 TreS was amplified using degenerated primers designed by comparing four deposited trehalose synthase genes in the NCBI database. The full-length gene of *Meiothermus* SK3-2 TreS has been submitted to Genbank with accession number HM587953. Analysis of the nucleotide revealed a large, uninterrupted, single open reading frame of 2,890 bp that encoded a 963 amino acid protein. The predicted pI and molecular weight was 5.17 and 110 kDa, respectively. The sequence size of *Meiothermus* SK3-2 TreS was much bigger than that of other cloned trehalose synthase, for example *A. aurescens* TreS with 1,797 bp or 598 amino acids (Xiuli et al., 2009). However, based on the public database, it seemed that TreS from thermophile bacteria, that is, *T. aquaticus* has also relatively longer sequence. Analysis for detecting repeat sequence was done and replication sequence was not identified for all the trehalose synthase from the thermophillic strains. Thus, this suggests that the mature sequence was indeed intact and probably monomeric. Representative TreS amino acid sequences from ten different bacteria source were aligned using Acceryls DS Align123 program. The lengths for this sequence varied; yet approximately, the first 490 amino acids shared similarity of more than 60%. Five highly conserved regions were identified and are summarized in Table 3. These regions were taken with the cutoff of five consecutive identical residues. Based on TreS *Meiothermus* SK3-2 numbering, the strictly conserved regions were

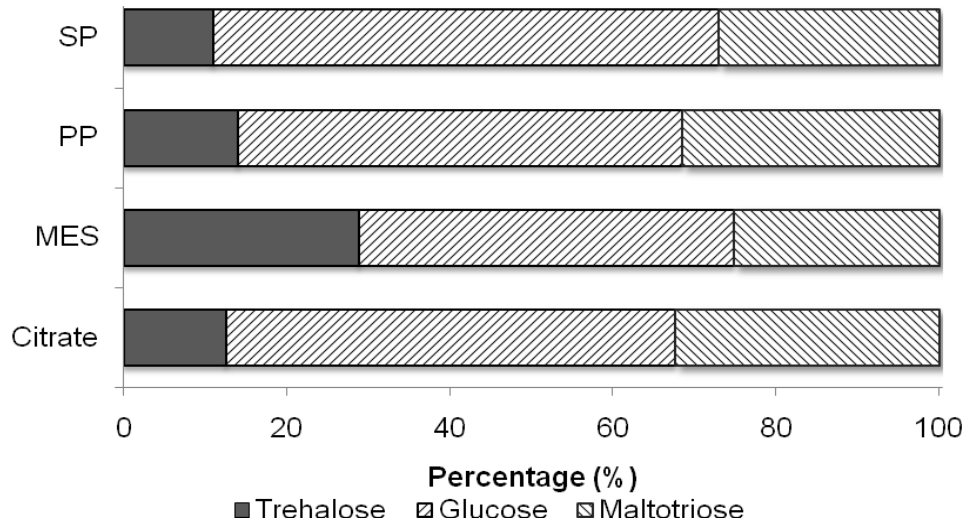

Figure 6. Effect of various buffer systems to product specificity of trehalose synthase (SP, sodium phosphate; PP, potassium phosphate; MES, 2-(*N*-morpholino) ethanesulfonic acid.

Table 3. Strictly conserved region in trehalose synthase from various sources: *Meiothermus* SK3-2 (this study); *A. aurescens* (ACL80570); *Pimelobacter* sp. (BAA11303); *Corynebacterium glutamicum* ATCC13032 (NP601502); *Sphaerobacter thermophilus* DSM20745 (YP003319350); *P. torridus* DSM_9790 (YP022847); *Salinibacter ruber* (YP003570903); *M. ruber* DSM1279 (YP003508484); *T. thermophilus* HB8 (YP143744) and *Thermus caldophilus* (AAD50660).

Origin of strain	Region 1	Region 2	Region 3	Region 4	Region 5
Meiothermus SK-32	^{168}QPDLN	^{304}FLRNHDELTLE	^{339}GIRRRL	^{372}YYGDEIGMGD	^{391}VRTPMQ
A. aurescens	^{194}QPDLN	^{331}FLRNHDELTLE	^{366}GIRRRL	^{399}YYGDEIGMGD	^{418}VRTPMQ
Pimelobacter sp.	^{178}QPDLN	^{322}FLRNHDELTLE	^{357}GIRRRL	^{390}YYGDEIGMGD	^{409}VRTPMQ
C. glutamicum	^{215}QPDLN	^{353}FLRNHDELTLE	^{388}GIRRRL	^{421}YYGDEIGMGD	^{440}VRTPMQ
S. thermophilus	^{181}QPDLN	^{316}FLRNHDELTLE	^{351}GIRRRL	^{384}YYGDEIGMGD	^{403}VRTPMQ
P. torridus	^{171}QPDLN	^{306}FLRNHDELTLE	^{341}GIRRRL	^{374}YYGDEIGMGD	^{393}VRTPMQ
Salinibacterruber	^{173}QPDLN	^{314}FLRNHDELTLE	^{349}GIRRRL	^{382}YYGDEIGMGD	^{401}VRTPMQ
M. ruber	^{167}QPDLN	^{303}FLRNHDELTLE	^{338}GIRRRL	^{371}YYGDEIGMGD	^{390}VRTPMQ
T. thermophilus	^{167}QPDLN	^{303}FLRNHDELTLE	^{338}GIRRRL	^{371}YYGDEIGMGD	^{390}VRTPMQ
T. caldophilus	^{167}QPDLN	^{303}FLRNHDELTLE	^{338}GIRRRL	^{371}YYGDEIGMGD	^{390}VRTPMQ

168 to 172, 304 to 314, 339 to 344, 372 to 381 and 391 to 396.

Conclusions

A new pink-pigmented *Meiothermus* strain was isolated from Malaysian's hot spring. It was found that trehalose synthase from this strain produced trehalose, maltose and maltotriose at different ratio and was greatly influenced by the reaction parameters. The gene that encodes the TreS protein was isolated. This enzyme had high optimum temperature which makes it a suitable candidate in the production of trehalose in single step reaction.

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