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Production of Trehalose from Maltose by Whole Cells of Permeabilized Recombinant *Corynebacterium glutamicum*

Zaiwei Man [1,2], Huihui Cui [3], Jin Li [3], Zhiqiang Cai [1,*] and Jing Guo [1,*]

[1] School of Biological and Food Engineering, Changzhou University, Changzhou 213164, China
[2] Zaozhuang Key Laboratory of Corn Bioengineering, Zaozhuang Science and Technology Collaborative Innovation Center of Enzyme, Shandong Hengren Gongmao Co., Ltd., Zaozhuang 277533, China
[3] School of Petrochemical Engineering, Changzhou University, Changzhou 213164, China
* Correspondence: zhqcai@cczu.edu.cn (Z.C.); guojing113@cczu.edu.cn (J.G.)

Abstract: Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is a stable and nonreducing disaccharide; can be used as sweetener, stabilizer, and humectant; and has many applications in the food, pharmaceutical, and cosmetic industries. Trehalose production from maltose catalyzed by trehalose synthase (TreS) is simple and economically feasible for industrial-scale application. Reducing the cost and enhancing the efficiency of TreS synthesis and the conversion of maltose to trehalose is critical for trehalose production. In this study, the homologous TreS was constitutively overexpressed in *Corynebacterium glutamicum* ATCC13032 by removing the repressor gene *lacI*^{q} fragment in the plasmid, and TreS expression could be exempt from the inducer addition and induction process. For cell permeabilization, Triton X-100 was used as a permeabilization agent, and the treatment time was 3 h. In the conversion system, the permeabilized cells of recombinant *C. glutamicum* were used as biocatalysts, 300 g/L maltose was used as a substrate, and 173.7 g/L trehalose was produced within 12 h under 30 °C and pH 7.0 conditions. In addition, the whole-cell biocatalysts showed promising reusability. This study provides a safe, convenient, practical, and low-cost pathway for the production of trehalose.

Keywords: *Corynebacterium glutamicum*; trehalose; trehalose synthase; constitutive overexpression; permeabilization

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1. Introduction

Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is a stable and nonreducing disaccharide. Trehalose is widespread in nature and is found to be present in bacteria, fungi, plants, and many invertebrates. Many studies have proved that trehalose can act as an active protectant of DNA, proteins, and cellular membranes in organisms and can improve their tolerance to adverse conditions, such as desiccation, extreme temperature, high osmolarity, and oxidative stress [1,2]. Because of its unique biochemical stability and biological function, trehalose can be used as a sweetener, stabilizer, and humectant and has many applications in the food, pharmaceutical, and cosmetic industries [3,4]. Trehalose synthase (TreS, EC 5.4.99.16) can catalyze the conversion of maltose to trehalose in a one-step conversion process, which has many advantages, such as simple reaction, low cost, and high substrate specificity. Therefore, enzymatic trehalose production by the TreS pathway has become appealing for industrial applications [3–5]. Obtaining TreS by safe, high-efficiency, and low-cost methods is critical for trehalose production by enzymatic conversion.

Many genes encoding TreS from different bacteria have been expressed in *Escherichia coli*. The TreS gene from *Corynebacterium glutamicum* was expressed in *E. coli* MC1061, the hexahistidine-tagged TreS was purified, the optimum reaction conditions of TreS from *C. glutamicum* were 35 °C and pH 7.0, and the preference temperatures for trehalose production were 25 °C and 30 °C. Under suitable conversion conditions, TreS could catalyze

the conversion of 0.5% maltose to trehalose with a maximum conversion yield of 69% [6]. The TreS gene from *Thermomonospora curvata* DSM 43183 was cloned and expressed in *E. coli* XL10-Gold, and the purified recombinant enzyme (TreS-T.C) could catalyze the conversion of maltose to trehalose with a maximum conversion yield of 70%; furthermore, TreS-T.C could efficiently convert sucrose into trehalulose without other disaccharides [7]. Similarly, the TreS gene from Marine *Pseudomonas* sp. P8005, *P. putida* ATCC47054, and *Thermus thermophilus* HB27 was also expressed in *E. coli*, and the recombinant TreSs could also efficiently convert maltose to trehalose [8–10]. However, the endotoxin produced in the *E. coli* expression system limits the application of produced trehalose in the food and medicine industry [3,11]. *Bacillus subtilis* does not contain endotoxins and is considered safe by both the European Food Safety Authority (EFSA) and the US Food and Drug Administration (FDA). Using *B. subtilis* as TreS and as a trehalose producer could be used to obtain pharmaceutical- and food-grade trehalose [3,5,11].

Some specific inducers must be added to the medium during cultivation when using inducible expression systems for gene overexpression and recombinant enzyme production. The addition of an inducer can increase the processing cost and complexity and is not ideal for industrial-scale protein expression [3,12,13]. In order to reduce the inducer cost during fermentation, an inexpensive and easily obtainable alternative inducer can be used. Lactose as a carbon source and autoinducer could be used to substitute the costly inducer isopropyl *β*-D-1-thiogalactopyranoside (IPTG) during recombinant TreS production by *E. coli* [4] and *B. subtilis* [5]. For some inducible expression systems, where the expression of the target gene in recombinant plasmid is controlled by an inducible promoter and repressor, the repressor can bind to the operator sequence in the inducible promoter and repress the gene expression. Therefore, the inducers need to be added to remove the binding of the repressor and activate the gene expression [12,14]. In *C. glutamicum*, the inducible promoter P_{tac} could transform into the constitutive promoter $P_{tac\text{-}M}$ by introducing mutations into the promoter sequence, and the improved plasmid with promoter $P_{tac\text{-}M}$ could constitutively express the target gene without inducer addition [14,15]. Besides promoter optimization, the repressor gene *lacI*^{q} in the plasmid could be deleted to inactivate the repressor and express the target gene without induction [12]. In *B. subtilis*, replacing the inducible promoter with constitutive promoter P_{43} in recombinant plasmid could allow for the constitutive overexpression of TreS [3].

For biocatalysis using free enzymes, the intracellular enzymes produced by recombinant strains are usually obtained by breaking the cells, and the biocatalysts are difficult to recycle [4,16]. To establish convenient and economical biocatalytic processes, the whole cells of recombinant strains can be applied as biocatalysts [17]. The permeabilized recombinant *E. coli* cells coexpressing *β*-glucosidase and chaperone genes could be used for the complete biotransformation of protopanaxadiol-type ginsenosides into 20-*O*-*β*-glucopyranosyl-20(*S*)-protopanaxadiol [18]. The permeabilized *Kluyveromyces lactis* cells pretreated by acetone could perform enantioselective synthesis of ethyl-*S*-3-hydroxy-3-phenylpropanoate from ethyl benzoyl acetate [19]. The whole cells of recombinant *E. coli* expressing TreS have exhibited a high ability to convert maltose to trehalose [4,17]. Permeabilizing the cells of recombinant *B. subtilis* expressing TreS with hexadecyltrimethylammonium bromide (CTAB) could improve mass transfer and enhance the catalytic action of the intracellular TreS [5].

C. glutamicum is a Gram-positive bacterium generally regarded as a safe (GRAS) organism, possesses outstanding performance in the production of L-amino acids [20,21], can grow relatively fast to high cell densities in a minimal medium under aerobic conditions, and has good robustness, such as osmotic pressure and phage resistance [22]. Due to its unique features, such as being non-pathogenic, not producing endotoxins, low nutrient need, and minimal protease activities, *C. glutamicum* is a promising expression system for protein production [23,24]. In order to establish a safe, convenient, and economical biocatalytic process for trehalose production, in this study, the GRAS strain *C. glutamicum*

was used for the recombinant expression of homologous TreS and trehalose production from maltose.

2. Materials and Methods

2.1. Microorganism and Cultivation Conditions

All strains and plasmids used in this study are listed in Table 1. *E. coli* strain JM109 was used for plasmid construction and aerobically cultured in Luria–Bertani (LB) medium (yeast extract 5 g/L, tryptone 10 g/L, NaCl 10 g/L) at 37 °C. The cultivations of *C. glutamicum* were carried out aerobically in LBG medium (LB medium supplemented with 5 g/L glucose) at 30 °C. Where appropriate, chloramphenicol (Cm) (15 mg/L) was added to the medium.

Table 1. Strains and plasmids used in this study.

Strain or Plasmid	Description	Reference or Source
Strains		
E. coli JM109	General cloning host	TaKaRa
C. glutamicum ATCC13032	Wild type strain	ATCC
C. glutamicum/pXMJ19-*Cgtrs*	*C. glutamicum* ATCC13032 derivative harboring pXMJ19-*Cgtrs*	This work
C. glutamicum/pXMJ19(-*lacI*^{q})-*Cgtrs*	*C. glutamicum* ATCC13032 derivative harboring pXMJ19(-*lacI*^{q})-*Cgtrs*	This work
Plasmids		
pXMJ19	Cm^r; shuttle vector between *E. coli* and *C. glutamicum*	Lab stock
pXMJ19(-*lacI*^{q})	The pXMJ19 derivative deleting the repressor gene *lacI*^{q}	This work
pXMJ19-*Cgtrs*	Derived from pXMJ19, for inducible overexpression of *Cgtrs* gene	This work
pXMJ19(-*lacI*^{q})-*Cgtrs*	Derived from pXMJ19(-*lacI*^{q}), for constitutive overexpression of *Cgtrs* gene	This work

2.2. Construction of Plasmids and Strains

The primers used in this study are listed in Table 2. The TreS gene *Cgtrs* from *C. glutamicum* was amplified via PCR from *C. glutamicum* ATCC13032 genomic DNA using primers *trs*F and *trs*R. The purified PCR product was double-digested by *Hin*dIII and *Xba*I restriction enzymes and ligated into the equally digested vector pXMJ19, resulting in plasmid pXMJ19-*Cgtrs*. This plasmid was used to transform *C. glutamicum* ATCC13032 by electroporation, as described previously [14], to obtain recombinant strain *C. glutamicum*/pXMJ19-*Cgtrs*.

Table 2. Primers used in this study.

Names	Sequences (5′→3′)	Restriction Sites
*trs*F	cccaagcttaaaggagggaaatcatgaattctcagccgagtgcag	*Hin*dIII
*trs*R	ctagtctagattattccatatcgtccttttcatcg	*Xba*I
Δ*lacI*^{q}F	ccgcgatatcgacaccggcatactctgcg	*Eco*RV
Δ*lacI*^{q}R	ccgcgatatcgtagtgggatacgacgataccg	*Eco*RV

For constructing the constitutive expression vector pXMJ19(-*lacI*^{q}), the repressor gene *lacI*^{q} in plasmid pXMJ19 was deleted. The *lacI*^{q} gene-deleted plasmid fragment was amplified using pXMJ19 as a template and primer pair of Δ*lacI*^{q}F and Δ*lacI*^{q}R, and the resulting fragment was single-digested by *Eco*RV restriction enzyme. Then, the single-digested fragment was self-ligated to obtain the pXMJ19(-*lacI*^{q}) plasmid. The construction of TreS constitutive expression vector pXMJ19(-*lacI*^{q})-*Cgtrs* was performed by cloning the *Cgtrs* gene into the pXMJ19(-*lacI*^{q}) plasmid according to the construction of plasmid pXMJ19-*Cgtrs*. This plasmid was used to transform *C. glutamicum* ATCC13032 and obtain recombinant strain *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs*.

2.3. TreS Expression and Enzymatic Activity Assays

For seed cultivation, one loop of *C. glutamicum*/pXMJ19-*Cgtrs* or *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* colonies from LBG agar slants was inoculated in 10 mL of LBG medium in 50 mL shake flasks and cultured at 30 °C and 200 r/min for 12 h. The seed culture (1 mL) was transferred into 50 mL of LBG medium in 250 mL shake flasks and cultured at 30 °C and 200 r/min. For TreS expression by *C. glutamicum*/pXMJ19-*Cgtrs* strain, 0.5 mmol/L isopropyl-*β*-D-thiogalactopyranoside (IPTG) was added as inducer at 3 h during cultivation, and the cells were harvested after a cultivation time of 12 h. For TreS expression by the *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* strain, the cells were also harvested after a cultivation time of 12 h without addition of inducer.

The cells were washed twice with 50 mM ice-cold potassium phosphate buffer (pH 7.0) and resuspended using the same buffer. Then, cells were lysed by sonication on ice (SCIENTZ-IID, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China). After centrifugation at 4 °C and 10,000× *g* for 10 min, the cell-free supernatants were used to determine the protein concentrations and TreS activities. The protein concentration was determined by Bradford method [25]. The TreS activity was quantified by measuring the trehalose yield produced from maltose. The reaction system comprised a certain amount of TreS, 100 g/L maltose, and 50 mM potassium phosphate buffer (pH 7.0). After incubation at 30 °C for 30 min, the enzyme reaction was terminated by incubation at 100 °C for 10 min. The trehalose concentration was measured by high-performance liquid chromatography (HPLC) method [4]. One unit (U) of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μg of trehalose per minute under the described assay conditions. All experiments were performed in triplicate, and data are presented as the means and standard deviations of the results.

2.4. Cell Permeabilization and Production of Trehalose by Whole-Cell Biocatalysis

The *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* strain was used for TreS expression and trehalose production. To simplify the cell permeabilization process, the permeabilization agents were directly added to the cultivation solution after the cultivation time of 12 h. The permeabilization treatment was still at 30 °C and 200 r/min, and the treatment time was 2 h. After permeabilization treatment, the cells were harvested by centrifugation at 4 °C and 4000× *g* for 10 min, resuspended with 50 mM potassium phosphate buffer (pH 7.0), and the cell concentration (OD_{600}) was adjusted to 30. The production of trehalose using whole-cell biocatalysis was carried out by adding 100 g/L maltose into the cell suspension and incubating at 30 °C and 120 r/min for 30 min. Whole-cell biocatalysis was terminated using incubation at 100 °C for 10 min, and the cell permeabilization was evaluated for trehalose production. The cells of the *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* strain without permeabilization treatment were also harvested after the cultivation time of 12 h and similarly resuspended to a cell concentration (OD_{600}) of 30, and cell lysis was subsequently performed by sonication on ice. Trehalose production using crude cell lysates under the same conditions was used as a control. After choosing the appropriate permeabilization agent, the optimization of additive amount and treatment time was performed.

For efficient production of trehalose and the reusing of permeabilized cells, the maltose concentration was increased by linear gradients, and the cell concentration (OD_{600}) was maintained at 30. The reaction systems were incubated at 30 °C and 120 r/min. After bioconversion, cells were harvested by centrifugation at 4 °C and 4000× *g* for 10 min and resuspended with 50 mM potassium phosphate buffer (pH 7.0). New bioconversion was started by adding maltose and performed under the same conditions. All experiments were performed in triplicate, and data are presented as the means and standard deviations of the results.

3. Results and Discussion

3.1. TreS Overexpression by Recombinant C. glutamicum

For TreS overexpression, *C. glutamicum*/pXMJ19-*Cgtrs* and *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* were constructed. Sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) was used to assess the expression of TreS. The TreS protein bands were found at approximately 66 kDa (Figure 1a), which were consistent with the calculated molecular weight. TreS activities from *C. glutamicum*/pXMJ19-*Cgtrs* and *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* were 276.9 U/mg protein and 258.2 U/mg protein (Figure 1b). This result indicated that deleting the repressor gene *lacI*^{q} in the pXMJ19 plasmid could obtain the constitutive expression vector. Compared with *C. glutamicum*/pXMJ19-*Cgtrs*, the nearly identical overexpression level of TreS without induction was achieved by *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs*. *C. glutamicum* is a GRAS strain that can be used to produce pharmaceutical- and food-grade products. In a simple medium, *C. glutamicum* can grow relatively fast to high cell densities under aerobic conditions, and the recombinant protein can be expressed at a high level [23,24]. Therefore, using the *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* strain for TreS constitutive expression could implement biocatalyst production in a safe, simple, and low-cost operation [4].

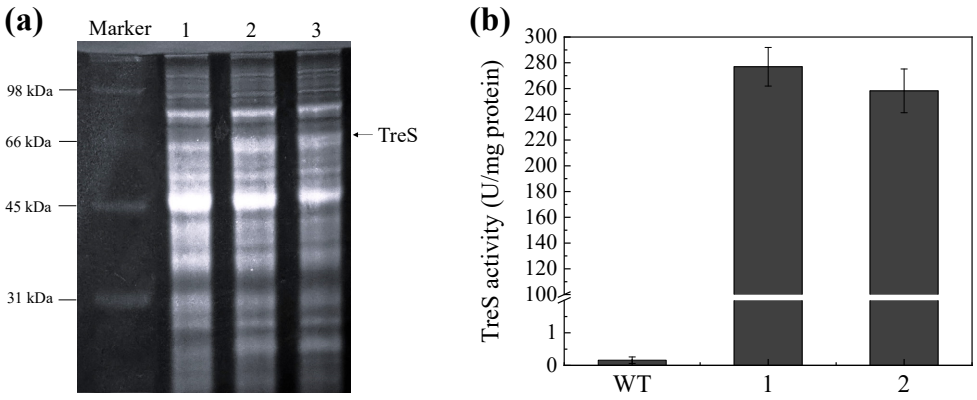

Figure 1. (**a**) SDS-PAGE analysis of recombinant TreS expression: Lane 1, the crude extract of the *C. glutamicum* ATCC13032; lane 2, the crude extract of the *C. glutamicum*/pXMJ19-*Cgtrs* with IPTG induction; lane 3, the crude extract of the *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* without induction. (**b**) TreS activities from *C. glutamicum* ATCC13032 (WT), *C. glutamicum*/pXMJ19-*Cgtrs* (1), and *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* (2). The error bars represent the standard deviation of three independent replicates.

3.2. Cell Permeabilization of C. glutamicum/pXMJ19(-lacI^q*)-Cgtrs*

Tween-80, Triton X-100, and cetyltrimethylammonium bromide (CTAB) are surfactants that can lead to the perforation of the membrane and enhance the permeability of the cell membrane. Therefore, the transfer of reaction substrates and products across the cell membrane can be enhanced by cell permeabilization using surfactants [5,18,26]. Tween-80, Triton X-100, and CTAB were used as permeabilization agents. Permeabilization effects when the additive amount of permeabilization agent was 10 g/L and the treatment time was 2 h are shown in Figure 2. Triton X-100 was the most effective permeabilization agent. The cells of *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* without permeabilization could also catalyze the conversion of maltose to trehalose, but the catalytic efficiency was low.

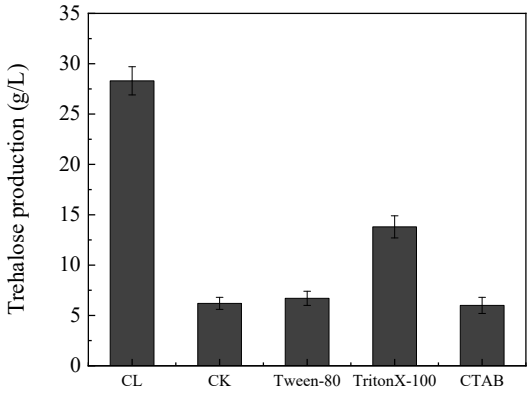

Figure 2. The effects of permeabilization agents on cell permeabilization. CL represents trehalose production by crude cell lysates; CK represents trehalose production by cells without permeabilization. The error bars represent the standard deviation of three independent replicates.

The effects of the Triton X-100 additive amount are shown in Figure 3. Trehalose production was increased along with the increase in Triton X-100 additive amount when the treatment time was 2 h. Meanwhile, trehalose production was almost the same when Triton X-100 additive amount was 15 g/L and 20 g/L. Therefore, the Triton X-100 additive amount was set at 15 g/L.

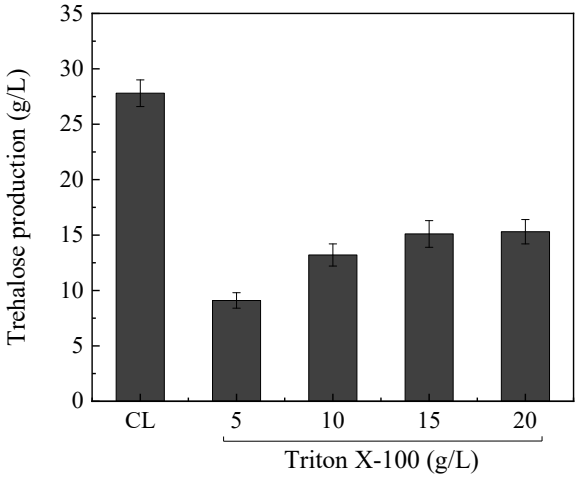

Figure 3. The effects of Triton X-100 additive amount on cell permeabilization. CL represents trehalose production by crude cell lysates. The error bars represent the standard deviation of three independent replicates.

The treatment time had a significant effect on cell permeabilization and trehalose production (Figure 4). Trehalose production was increased along with the increase in treatment time, and a treatment time of 3 h was enough for cell permeabilization. Thus, the treatment time was set at 3 h. Meanwhile, the trehalose production by whole-cell biocatalysis was lower than that catalyzed by cell lysates (Figure 4). This may be because *C. glutamicum* is a Gram-positive bacterium that has a thick cell wall. The cell wall still played a certain barrier function that limited the diffusion of the substrate and product through the permeabilized cell [19,22].

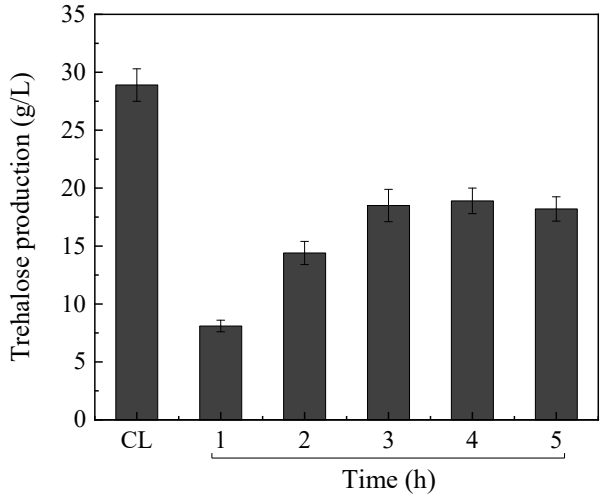

Figure 4. The effects of treatment time on cell permeabilization by Triton X-100. CL represents trehalose production by crude cell lysates. The error bars represent the standard deviation of three independent replicates.

3.3. Production of Trehalose by Whole-Cell Biocatalysis and Reusability of the Cell

For efficient production of trehalose, the concentration of substrate maltose was increased, and the conversion processes are shown in Figure 5. The initial production rate of trehalose in the first 2 h was increased along with the increase in the initial maltose concentration. Glucose was produced in the biocatalysis process, and the glucose concentration was gradually increased throughout the process (Figure 6). Trehalose production was stopped when trehalose concentrations reached a certain level, and the biocatalysis mixtures contained trehalose, maltose, and glucose. This is because TreS catalyzes the reversible interconversion of maltose and trehalose, and the bioconversion of maltose to trehalose will approach equilibrium when the accumulation of trehalose reaches a certain ratio [2,5].

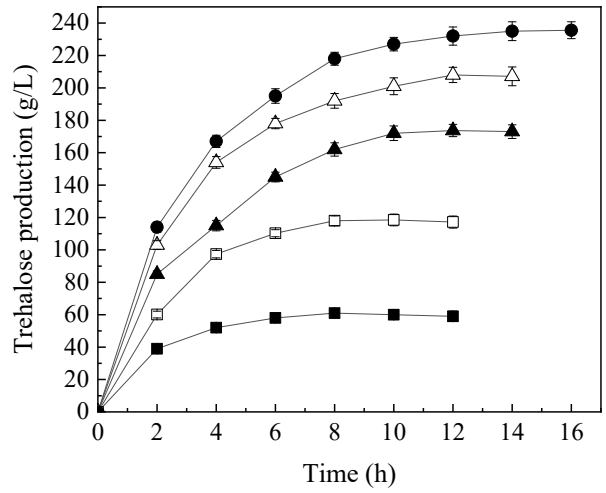

Figure 5. The production of trehalose by whole-cell biocatalysis with different initial maltose concentrations. The initial maltose concentrations are represented by different signals: filled square, 100 g/L; empty square, 200 g/L; filled triangle, 300 g/L; empty triangle, 400 g/L; filled circle, 500 g/L. The error bars represent the standard deviation of three independent replicates.

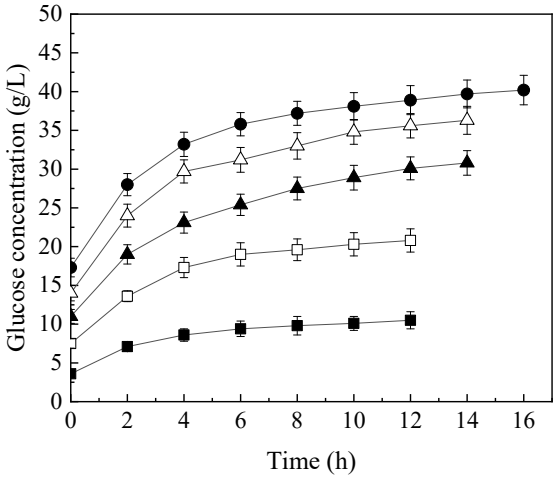

Figure 6. Glucose concentration in the production of trehalose by whole-cell biocatalysis with different initial maltose concentrations. The initial maltose concentrations are represented by different signals: filled square, 100 g/L; empty square, 200 g/L; filled triangle, 300 g/L; empty triangle, 400 g/L; filled circle, 500 g/L. The commercial maltose contains approximately 3.5% glucose. The error bars represent the standard deviation of three independent replicates.

Trehalose production was increased along with the increase in initial maltose concentration; the highest productions were 60.8, 118.5, 173.7, 208.2, and 235.6 g/L when the initial maltose concentrations were 100, 200, 300, 400, and 500 g/L, respectively (Figure 5). However, the yield of trehalose from maltose decreased with the increase in the initial maltose concentration (Figure 7). This may be because the byproduct glucose can inhibit trehalose synthesis (with TreS) from maltose, and the catalytic formation of trehalose will be dramatically inhibited when the glucose concentration reaches a certain level [4,5,27]. Although the high initial maltose concentration could bring high trehalose production, the accumulation of glucose also increased (Figure 6) and led to stronger inhibition. Therefore, under a high initial maltose concentration, the catalytic formation of trehalose was terminated prematurely due to the high concentration of byproduct glucose, and the yield of trehalose from maltose was decreased. When the initial maltose concentration was above 300 g/L, the yield of trehalose from maltose decreased. Therefore, the initial maltose concentration for trehalose production was set at 300 g/L based on the comprehensive consideration of production and yield, and the trehalose production and yield from maltose reached 173.7 g/L and 57.9%, respectively, at 12 h.

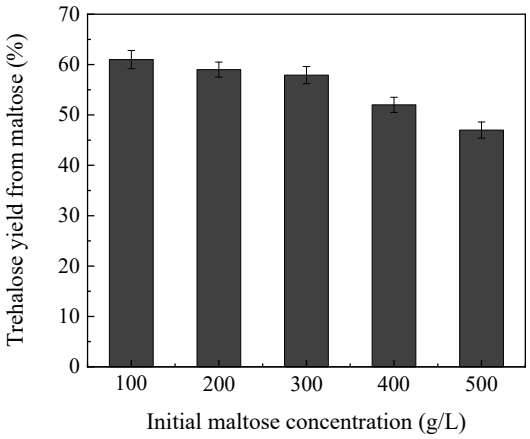

Figure 7. The yield of trehalose from maltose in the production of trehalose by whole-cell biocatalysis with different initial maltose concentrations. The error bars represent the standard deviation of three independent replicates.

The repeated use of whole-cell biocatalysts for the production of trehalose from maltose is shown in Figure 8. The production and productivity of trehalose in the first batch of conversion were 176.2 g/L and 14.7 g/L/h, respectively. The trehalose production showed almost no decrease during the first five cycles, when the reaction time for every batch was 12 h. After seven cycles, the trehalose production was reduced by approximately 20% to 143.6 g/L. The decrease in trehalose production was probably due to TreS inactivation during long-term high-intensity catalysis. In addition, the TreS loss caused by cell lysis and enzyme leakage during cell-recovery steps could also have reduced production.

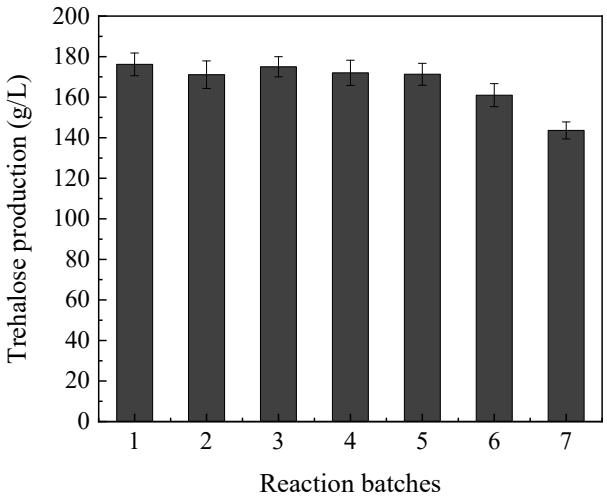

Figure 8. Repeated use of the whole-cell biocatalysts for the production of trehalose. The reaction time for every batch was 12 h. The error bars represent the standard deviation of three independent replicates.

The overview of enzymatic trehalose production by the TreS pathway in recent years is shown in Table 3. Using the biocatalyst reusing method for trehalose production, in contrast to trehalose production by *E. coli* and *B. subtilis* whole cells [4,5], in this study, the trehalose yield from maltose was relatively low, but the production and productivity of trehalose were relatively high. Therefore, using whole cells of the permeabilized recombinant *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* strain could also carry out the efficient production of trehalose. The *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* strain could overexpress TreS without induction, and the biocatalyst production for producing trehalose from maltose could be exempt from the inducer addition and induction process. Using whole cells of the recombinant *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* strain as biocatalysts for trehalose synthesis could eliminate the cost for cell lysis and implement the easy reusing of biocatalysts. In addition, compared to *E. coli*, *C. glutamicum* is a GRAS strain that can be used for producing pharmaceutical- and food-grade products [5,23,24]. Therefore, the production of trehalose by recombinant *C. glutamicum* in this study is a safe, convenient, practical, and low-cost pathway. In addition, *C. glutamicum* can use a variety of carbon sources for cell growth and product synthesis, such as lignocellulosic hydrolysates [28]. In future work, optimization of the fermentation medium and the use of lignocellulosic hydrolysates as a carbon source for cell growth and TreS expression can be performed, and the cost of production of trehalose could possibly be further reduced.

Table 3. Overview of enzymatic trehalose production by TreS pathway in recent years.

Host Strain	Inducer	Biocatalyst	Trehalose Production	Productivity	Yield from Maltose	Biocatalyst Reusing	Reference and Year
E. coli	IPTG	Whole cells	96.0 g/L	16.0 g/L/h	64.0%	No	[17] 2015
E. coli	Lactose	Cell lysate	193.5 g/L	8.1 g/L/h	64.5%	No	[10] 2018
E. coli	Lactose	Whole cells	134.5 g/L [1]	9.6 g/L/h [1]	90.5% [1]	Yes	[4] 2018
B. subtilis	Lactose	Whole cells	136.0 g/L [1]	8.1 g/L/h [1]	67.8% [1]	Yes	[5] 2020
C. glutamicum	None	Whole cells	176.2 g/L [1]	14.7 g/L/h [1]	58.7% [1]	Yes	This study

[1] The results of the first batch reaction in the biocatalyst-reusing method for trehalose production.

4. Conclusions

In this study, the GRAS strain *C. glutamicum* was used as a host for the recombinant expression of homologous TreS, and constitutive overexpression was carried out by the removal of the repressor gene *lacI*^{q} fragment in the plasmid. The obtained recombinant *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* strain could efficiently express TreS for trehalose synthesis without induction. For cell permeabilization, Triton X-100 was an effective permeabilization agent. Treating the cells of the recombinant *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* strain with 15 g/L of Triton X-100 for 3 h could obtain permeabilized cells. The whole cells were able to act as high-performance biocatalysts, which could convert 300 g/L maltose to 173.7 g/L trehalose within 12 h. In addition, the whole-cell biocatalysts had promising reusability, and trehalose production showed almost no decrease during the first five cycles. The biocatalytic process using the *C. glutamicum*/pXMJ19(-*lacI*^{q})-*Cgtrs* strain in this study is a safe, convenient, practical, and low-cost pathway for producing pharmaceutical- and food-grade trehalose. For future work, using lignocellulosic hydrolysates as a carbon source in a medium for strain cultivation and TreS expression is a promising approach for further reducing the cost of trehalose production.

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