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RESEARCH PAPER

High-efficiency expression of *Sulfolobus acidocaldarius* maltooligosyl trehalose trehalohydrolase in *Escherichia coli* through host strain and induction strategy optimization

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Abstract

Maltooligosyl trehalose trehalohydrolase (MTHase, EC 3.2.1.141) catalyzes the release of trehalose, a novel food ingredient, by splitting the α-1,4-glucosidic linkage adjacent to the α-1,1-glucosidic linkage of maltooligosyl trehalose. However, the high-yield preparation of recombinant MTHase has not yet been reported. In this study, a codon-optimized synthetic gene encoding *Sulfolobus acidocaldarius* MTHase was expressed in *Escherichia coli*. In initial expression experiments conducted using pET-24a (+) and *E. coli* BL21 (DE3), the MTHase activity was 10.4 U/mL and a large amount of the expression product formed inclusion bodies. The familiar strategies, including addition of additives, co-expression with molecular chaperones, and expression with a fusion partner, failed to enhance soluble MTHase expression. Considering the intermolecular disulfide bond of MTHase, expression was investigated using a system comprising plasmid pET-32a (+) and host *E. coli* Origami (DE3), which is conducive to cytoplasmic disulfide bond formation. The MTHase activity increased to 55.0 U/mL, a 5.3-fold increase. Optimization of the induction conditions in a 3-L fermentor showed that when the lactose was fed at 0.2 g/L/h beginning at an OD_{600} of 40 and the induction temperature was maintained at 30 °C, the MTHase activity reached a maximum of 204.6 U/mL. This is the first report describing a systematic effort to obtain high-efficiency MTHase production. The high yield obtained using this process provides the basis for the industrial-scale production of trehalose. This report is also expected to be valuable in the production of other enzymes containing disulfide bonds.

Keywords Maltooligosyl trehalose trehalohydrolase · Recombinant expression · *Escherichia coli* · Fermentation optimization

Introduction

Maltooligosyl trehalose trehalohydrolase (MTHase, EC 3.2.1.141) catalyzes the conversion of maltooligosyl trehalose into trehalose and a maltooligosaccharide with lower molar mass by splitting the α-1,4-glucosidic linkage adjacent to the α-1,1-glucosidic linkage. It is among the key enzymes involved in the production of trehalose from starch [1]. Trehalose is a non-reducing disaccharide composed of two glucose molecules linked by an α-1,1-glucosidic bond. Because it protects proteins and lipid membranes from desiccation, heat, frost, and osmotic changes, trehalose is used as a preservative or stabilizer for cells, medicines, food, and cosmetics. Thus, trehalose is used in a variety of applications in many different industries [2–5]. Because the number of industrial applications of trehalose is gradually increasing, highly efficient, low-cost production of the enzymes used in trehalose production is attracting more attention.

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MTHases have been reported in a variety of microorganisms, including *Sulfolobus acidocaldarius, Sulfolobus solfataricus, Sulfolobus shibatae, Arthrobacter* sp., *Arthrobacter ramosus, Rhizobium* sp., and others [2, 6–8]. Some researchers developed MTHase production systems based upon the isolated wild-type microorganisms [9, 10]. However, the low activities obtained with these systems generally restricted their use for commercial MTHase preparation. Higher MTHase yield would be more easily obtained through genetically engineered microorganisms constructed using recombinant DNA technology [11, 12].

Escherichia coli has been the most commonly used host for MTHase expression, but only a few researchers reported the MTHase yield; no systematic strategies for enhancing MTHase production have been explored [7, 13, 14]. Donatella de Pascale's group expressed the MTHase from *Sulfolobus solfataricus* MT4 in *E. coli* Rb791 using a pTrc expression vector. The yield was 3630 U/L of medium [13]. Min Chang introduced the gene encoding MTHase from *Corynebacterium glutamicum* into *E. coli* BL21 (DE3) pLysS. In this system, soluble recombinant MTHase accounted for about 40% of total cell protein, which was 0.27 mg/mL (five times concentrated). The remainder of the recombinant protein was expressed as inclusion bodies [15].

Many proteins, especially cytoplasmic proteins, readily form insoluble aggregates when expressed as recombinant proteins using *E. coli* as the host. Several techniques have been developed to resolve this problem, including reducing the protein synthesis rate, adding various additives to the culture medium, optimizing the host and plasmid, co-expressing the protein with molecular chaperones, and expressing the protein with fusion partners [16]. For example, adding ethanol to the culture media induces the expression of chaperones and enhances the solubility of recombinant proteins [17, 18]. Some carbohydrates also enhance the expression of recombinant proteins by inducing osmotic stress responses [19, 20]. Betaine supplementation favors the native folding of recombinant proteins [21]. Fusion partners commonly used to solve solubility and folding issues include small ubiquitin-like modifier (SUMO), thioredoxin A (TrxA) and glutathione *S*-transferase [22–25].

The MTHase from *S. acidocaldarius*, which has excellent thermostable and acidophilic properties, displays its highest activity at pH 5.5–6.0 and 75 °C [10]. This MTHase can catalyze the production of trehalose from starch at high yield when coupled with the maltooligosyl trehalose synthase from the same strain. The high reaction temperature, which accelerates the rate of the transformation process and reduces the risk of bacterial contamination, is beneficial for its use in industrial applications. However, the high-yield preparation of recombinant MTHase has not yet been reported. In this study, the gene encoding *S. acidocaldarius* MTHase was cloned and expressed in *E. coli*. Different strategies were investigated to identify a system that produced MTHase in good yield. Then fermentation conditions were optimized to enhance the yield of this MTHase (Fig. 1).

Materials and methods

Bacterial strains, vectors and materials

Escherichia coli strain JM109 and the pMD™18-T Vector Cloning Kit (Takara, Dalian, China) were used for gene cloning. *E. coli* strains BL21 (DE3), Origami (DE3) and the plasmids pET-24a (+), pET-32a (+) were used

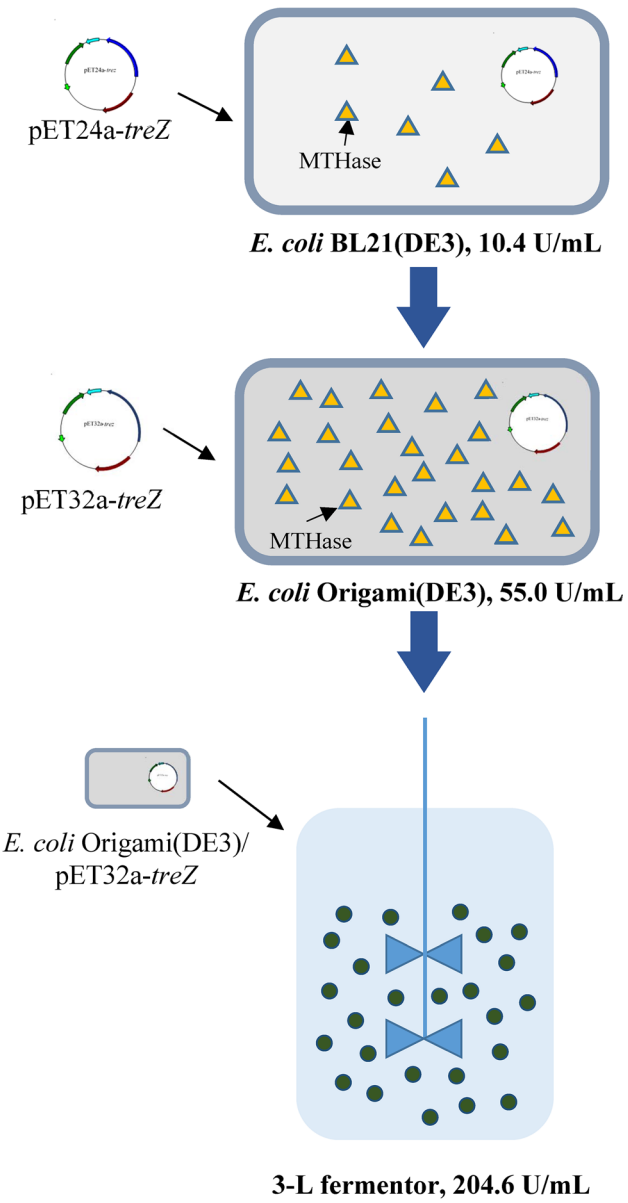

Fig. 1 The experimental scheme of MTHase expression

for protein expression. The plasmid pG-Tf2, the enzymes used for DNA manipulations, and agarose were purchased from Takara (Dalian, China). Plasmid mini preparation kit agarose, and gel DNA purification kit were purchased from TIANGEN Biotech Co., Ltd (Beijing, China). Primer synthesis was performed by Shanghai Generay Biotech Co., Ltd (Shanghai, China). DNA sequencing was performed by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd (Shanghai, China). Other analytically pure reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Construction of engineered *E. coli* for MTHase expression

The gene *treZ* encoding the *S. acidocaldarius* MTHase (NCBI accession number WP_011278268) was analyzed, optimized for expression in *E. coli*, and synthesized by Shanghai Generay Biotech Co., Ltd (Fig. S1). Rarely used codons were replaced with codons preferred by *E. coli* while preserving the amino acid sequence, and the mRNA free energy and GC content of the gene were also optimized for protein expression. The synthetic gene, which was flanked by *Nde*I and *Hin*dIII sites (up- and downstream, respectively), was inserted into the plasmid pET-24a (+) to generate pET24a–*treZ* (Fig. S2). The genes encoding MTHase and the SUMO family protein SMT3 (NCBI accession number NP_010798.1) were amplified using pET24a–*treZ* and pET24a-*sumo* (laboratory stock) as template, respectively, then overlapping PCR was utilized to create a gene encoding an N-terminal fusion of *sumo* to *treZ*. The following primers were used to construct this gene. CCATATGTCGGACTCAGAAGTCAAT CAAGAAGC, TTGCCGCCAAAGCTAAACATATAC GTAGCACCACCAATC, GATTGGTGGTGCTACGTA TATGTTTAGCTTTGGCGGCAA, and AAGCTTATT CCAGTTGATACACGC, where the underlined bases represented the *Nde*I and *Hin*dIII restriction enzyme sites. After ligation into the pMD18-T simple vector and verification of the resulting plasmid with DNA sequencing, *sumo–treZ* was liberated by *Nde*I and *Hin*dIII digestion and ligated into similarly digested pET-24a (+) to form plasmid pET24a–*sumo–treZ* (Fig. S3). Finally, the gene encoding MTHase, amplified from pET24a–*treZ* using primers CGGAATTCATGTTTAGCTTTGGCGGCAA and CCAAGCTTATTCCAGTTGATACACGC (*Eco*R I and *Hin*dIII restriction enzyme sites underlined), was ligated into pET-32a (+) using the approach described above, to form the expression plasmid pET32a–*treZ*. Chemically competent *E. coli* were transformed with pET24a–*treZ*, pET24a–*sumo–treZ*, or pET32a–*treZ* to produce candidate MTHase expression systems.

Cultivation conditions for engineered *E. coli*

Shake-flask culture

A 10-μL sample of frozen glycerol stock was used to inoculate 10 mL of Luria–Bertani medium (g/L: yeast extract, 5.0; tryptone, 10.0; NaCl, 10.0). After cultivation at 37 °C in a rotary shaker (200 rpm) for 8 h, the seed culture (4% [v/v]) was used to inoculate 40 mL of Terrific Broth medium (g/L: yeast extract, 24.0; tryptone, 12.0; KH_2PO_4, 2.3; and K_2HPO_4, 16.4; glycerol, 5.0; and ampicillin 0.1). The resulting culture was shaken at 37 °C in a rotary shaker (200 rpm) until the optical density at 600 nm (OD_{600}) reached 1.5; then, isopropyl β-D-thiogalactopyranoside (IPTG) was added to the culture at a final concentration of 0.1 mM to induce MTHase expression. At this point, the incubation temperature was reduced to 25 °C.

3-L fermentor

Escherichia coli Origami (DE3) cells harboring pET32a–*treZ* were cultivated using a fed-batch pattern in a 3.6-L fermentor (Labfors 5; Infors-HT Co., Ltd). The seed culture, prepared as described above, was used to inoculate a 1-L initial batch of semisynthetic medium (g/L: tryptone, 30.0; yeast extract, 20.0; K_2HPO_4, 14.6; $MgSO_4{\cdot}7H_2O$, 2.0; $(NH_4)_2$-H-citrate, 1.0; glycerol, 8.0; ampicillin 0.1; and trace metal solution, 1.0 mL/L) at 37 °C. The trace metal solution contained (g/L) $FeSO_4{\cdot}7H_2O$ 10.0, $ZnSO_4{\cdot}7H_2O$ 5.3, $CaCl_2$ 2.0, $CuSO_4{\cdot}5H_2O$ 3.0, $MnSO_4{\cdot}4H_2O$ 0.5, $Na_2B_4O_7{\cdot}10H_2O$ 0.2, $(NH_4)_6Mo_7O_{24}$ 0.1. During the entire fermentation process, the pH was maintained at 7.0 by automatic addition of 25% ammonia solution. The dissolved oxygen was maintained at about 30% of air saturation under a cascaded control of agitation and aeration rates, and the use of oxygen-enriched air. When the dissolved oxygen content and pH of the resulting culture suddenly spiked, which indicated the initial glycerol was completely consumed, feeding solution (g/L: tryptone, 50.0; yeast extract, 50.0; $MgSO_4{\cdot}7H_2O$, 3.4; and glycerol, 500.0) was continuously added to the culture. When the OD_{600} of the culture reached a specified level (30, 40, or 50), lactose was fed at a constant rate (0.1, 0.2, 0.4, or 0.8 g/L/h) to induce MTHase expression. The temperature was kept at 37 °C during the growth phase, and at a specified value (25 °C, 30 °C, and 35 °C) during the induction phase.

Determination of biomass

A specified amount of the culture broth was collected, and the optical density at 600 nm (OD_{600}) was measured using a spectrophotometer. If the OD_{600} value was higher than 0.8, the sample was diluted with 0.9% (w/v) sodium chloride solution. The dry cell weight (DCW) was measured

as follows. Culture broth (10 mL) was collected and centrifuged at 13,200×*g* for 10 min. The supernatant was discarded, and the pellet was washed twice with 0.9% (w/v) sodium chloride solution. Finally, the washed pellet was dried to constant weight at 105 °C in a drying oven.

MTHase activity assay

Maltopentaose was dissolved to a concentration of 1% in 20 mM phosphate–citrate buffer (pH 6.0). *S. acidocaldarius* maltooligosyltrehalose synthase (MTSase) solution (10 µL), prepared in our previous study [26], was added to 0.48 mL of maltopentaose solution to produce maltotriosyl trehalose. After 2-h incubation at 60 °C, the reaction was stopped by heating for 10 min in boiling water. The product solution was incubated at 60 °C, 10 µL of appropriately diluted MTHase was added, and the mixture was allowed to react for exactly 10 min. Then reaction was terminated by heating for 10 min in boiling water. The amount of maltotriose released was measured using the 3,5-dinitrosalicylic acid reagent, as previously reported [27]. One unit (U) of MTHase activity was defined as the amount of MTHase needed to produce 1 µmol of trehalose per min under the assay conditions.

Results and discussion

Expression of *S. acidocaldarius* MTHase in *E. coli* BL21 (DE3)

An *E. coli* codon-optimized synthetic gene (*treZ*) encoding *S. acidocaldarius* MTHase was prepared by Shanghai Generay Biotech Co., Ltd. To ensure optimal expression, rare codons in the coding sequence were replaced with preferred codons and the free energy of mRNA was decreased 13.5 kcal/mol. Engineered *E. coli* BL21 (DE3) harboring the plasmid pET24a–*treZ*, which expresses *treZ* with a T7 promoter, was cultivated in a shake flask. MTHase expression was induced through the addition of 0.05 mmol/L IPTG and further cultivation for 24 h. SDS-PAGE analysis showed a band around 59 kDa, which is consistent with the calculated molar mass of MTHase, in both the soluble and insoluble intracellular fractions (Fig. 2). The MTHase activity was determined to be 10.4 U/mL (228.8 U/g wet cell) in the soluble intracellular fraction, which was much higher than the expression level (3.4 U/g wet cell) previously reported [12].

Although MTHase was successfully expressed in *E. coli* BL21 (DE3), the MTHase in the soluble fraction was unacceptably low; much of the target protein was located in insoluble inclusion bodies. Further experiments were conducted to attempt to address this issue. A series of additives including ethyl alcohol (2%, v/v), glycine betaine (20 mmol/L), sorbitol (1%, w/v), saccharose (0.4 mol/L), and dithiothreitol

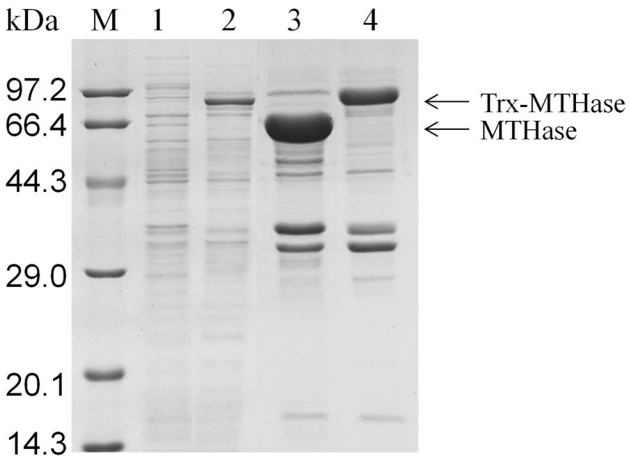

Fig. 2 SDS-PAGE analysis of recombinant MTHase in shake flasks. M, molecular mass standard proteins; 1, intracellular soluble fraction of *E. coli* BL21 (DE3)/pET24a–*treZ*; 2, intracellular soluble fraction of *E. coli* Origami (DE3)/pET32a–*treZ*; 3, intracellular insoluble fraction of *E. coli* BL21 (DE3)/pET24–*treZ*; 4, intracellular insoluble fraction of *E. coli* Origami (DE3)/pET32a–*treZ*

(DTT, 1 mmol/L), were added to the culture medium. Only the addition of sucrose increased both cell growth (by 5.2%) and MTHase activity (by 5%). Other additives had no effect on MTHase production (Fig. S4). The plasmid pG-Tf2 harboring the gene sequence *groES–groEL–tig* was inserted into *E. coli*/pET24a–*treZ* for co-expression of MTHase with the chaperones GroES, GroEL and trigger factor. Unfortunately, the MTHase activity was 10.3 U/mL, almost the same as that obtained without chaperone co-expression. Finally, an N-terminal fusion of SUMO with MTHase was prepared. Once again, expression of MTHase activity was not increased. Thus, it seemed that the typical strategies used to improve recombinant protein expression in *E. coli* failed to improve MTHase production in *E. coli* BL21 (DE3).

Expression of *S. acidocaldarius* MTHase in *E. coli* Origami (DE3)

The inability to produce soluble MTHase in high yield in *E. coli* BL21 (DE3) and the failure of typical solubilization strategies suggested that MTHase may be not suitable for expression in *E. coli* BL21 (DE3), and that this was related to some property of MTHase. The native form of MTHase is a dimer covalently linked by a single intermolecular disulfide bond between the cysteine-297s of the two molecules. Because the reducing environment of the *E. coli* cytoplasm is not conducive to disulfide bond formation [28], it seemed reasonable that the inability to form the proper disulfide bond caused the formation of inclusion bodies.

To address disulfide bond formation, a commercial plasmid bearing the TrxA tag, pET-32a (+), was used in this study. TrxA is a low molecular weight protein (109 aa) that

catalyzes the formation of disulfide bonds and improves protein folding [28]. Use of this plasmid was paired with use of the *E. coli* Origami strain. This strain contains mutant thioredoxin reductase (*trx*B) and glutathione reductase (*gor*) genes, which enable recombinant proteins to form disulfide bonds more efficiently in the cytoplasm [28, 29]. The codon-optimized synthetic gene MTHase encoding was inserted into pET-32a (+) to form a gene encoding an N-terminal TrxA fusion. This plasmid was inserted into *E. coli* Origami (DE3). Initial expression experiments were conducted in shake flasks. SDS-PAGE analysis of the soluble fraction of the engineered *E. coli* displayed a single major band at approximately 71 kDa, which is consistent with the calculated molar mass of the Trx–MTHase fusion. This band was thicker than that obtained from *E. coli* BL21 (DE3), while the inclusion body content obviously decreased (Fig. 2). Correspondingly, the MTHase activity was 42.0 U/mL, fourfold higher than that obtained from the engineered *E. coli* BL21 (DE3). When the N-terminal TrxA was removed using enterokinase, the MTHase activity increased to 55.0 U/mL, 5.3-fold greater than that obtained using *E. coli* BL21 (DE3). The combined use of pET-32a (+) and *E. coli* Origami (DE3), which is more beneficial for the formation of cytoplasmic disulfide bonds than the previous expression system, efficiently improved the production of active MTHase, suggesting that formation of the disulfide bond plays an important role in the correct folding of MTHase.

To clarify whether the improvement in soluble MTHase expression was caused by the thioredoxin fusion or oxidative cytoplasmic expression in *E. coli* Origami (DE3), *E. coli* BL21 (DE3) harboring pET-32a (+), and *E. coli* Origami (DE3) harboring pET-24a (+) were constructed. The MTHase activities obtained from the two strains were 17.9 U/mL and 38.8 U/mL, respectively, indicating that the improvement in soluble MTHase expression depended on a synergistic effect of the two mechanisms and that oxidative cytoplasmic expression in *E. coli* Origami (DE3) played a larger role in the process.

Enhancing MTHase production in *E. coli* Origami (DE3) by optimizing induction in a 3-L fermentor

MTHase production in *E. coli* Origami (DE3) was scaled up in a 3-L fermentor. The fermentation was performed using a fed-batch cultivation process used in a previous study to achieve high cell density [30]. As with all proteins produced using an inducible expression system, the method of induction was considered the primary factor determining the yield of the target protein [31]. Many reports have shown that optimal induction, which is closely related to the balance between cell growth and protein expression, occurs under different conditions for different proteins [31–33]. In this study, the point at which induction was initiated, the lactose concentration used, and the induction temperature were investigated in an effort to optimize MTHase production.

Effect of induction point on cell growth and MTHase production

Protein expression generally places a metabolic burden on cells. This burden decreases biomass production and protein yield, especially when the induction is performed too early. Late induction also limits induction efficiency and protein production. Therefore, induction is usually initiated when the biomass in the fermentor reaches a certain level. The optimized induction point is generally at the early- or mid-log phase [30, 33]. In addition, according to the previous study [21, 30, 31], high cell density (an OD_{600} of more than 100) could usually be achieved using the semisynthetic medium and fed-batch cultivation. To identify the optimal induction point for MTHase production, the inducer lactose was fed into the culture when the OD_{600} reached 30, 40, and 50, respectively. As shown in Fig. 3, cell growth was similar during the growth phase, but differences in the biomass gradually appeared when induction was initiated at different biomass levels. Earlier induction gave rise to lower biomass, as expected. The highest DCW (70.8 g/L) was obtained when induction was begun at an OD_{600} of 50. This DCW was 43.0% greater than that observed when induction was initiated at an OD_{600} of 30 and 6.9% higher than that observed when induction was initiated at an OD_{600} of 40. The lowest MTHase activity (143.4 U/mL) was also observed at the earliest induction point. When induction was delayed until the biomass reached OD_{600} 40 or 50, the MTHase production improved. A slightly higher value (184.0 U/mL) was seen at an OD_{600} of 40 than at an OD_{600} of 50, but the difference was only 5.1%. The specific MTHase activities were also close, which were 16.4 U/mg and 15.8 U/mg, respectively. This range of induction points available for high-yield MTHase production is beneficial for controlling the fermentation process under practical production conditions.

Effect of induction temperature on cell growth and MTHase production

Induction temperature is another important factor for both cell growth and protein production [21]. In this study, three induction temperatures, 25, 30, and 35 °C, were investigated. As shown in Fig. 4, the cell growth rate and biomass increased with increasing induction temperature. Cell growth was obviously inhibited when the induction temperature was 25 °C; the peak DCW was only 42.6 g/L. The highest DCW (73.2 g/L), obtained when the induction temperature was 35 °C, was 1.7- and 1.1-fold of that obtained at 25 °C and 30 °C, respectively. However, high biomass did not cause high MTHase production. Among

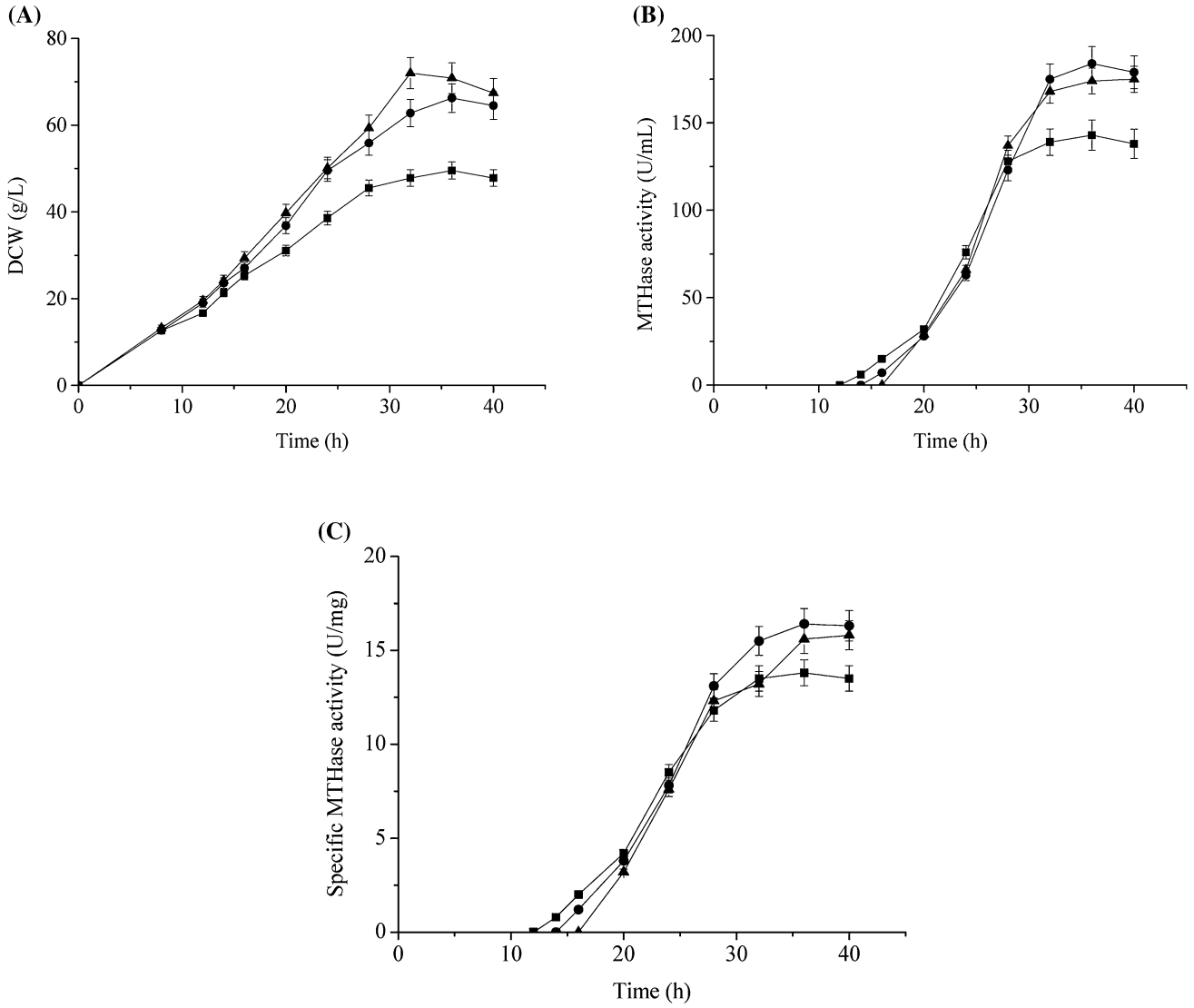

Fig. 3 Effect of induction point (OD_{600}) on cell growth and MTHase production. **a** Cell growth, **b** MTHase activity, **c** specific MTHase activity. Filled squares: 30, filled circles: 40, filled triangles: 50

the three induction temperatures, the highest MTHase activity (184.0 U/mL, 16.4 U/mg) was observed at 30 °C. At 25 °C, the MTHase activity (142.5 U/mL, 19.8 U/mg) equated to a production of 3.3×10^3 U per g DCW, 17.8% higher than that observed at 30 °C. Because lower induction temperatures decrease the protein synthesis rate and help appropriate protein folding, these temperatures help to generate the soluble form of recombinant proteins [16, 21]. This is probably why the lower temperature led to higher MTHase production for per g DCW. However, the low induction temperature also resulted in a lower final biomass, which hampered the final yield of the target protein. Thus, induction at 25 °C resulted in the lowest MTHase yield. The final biomass value was highest when protein expression was induced at 35 °C. However, the final MTHase activity was only 94.6% of that seen when expression was induced at 30 °C. Higher induction temperatures are widely considered to make the folding of newly synthesized polypeptides too fast. Rapid folding leads to folding errors that cause the formation of insoluble inclusion bodies and limit the production of active enzyme [21]. In this study, induction of MTHase at 35 °C had only a slightly negative effect on MTHase production, compared with induction at 30 °C. Considering the cost of cooling required to maintain the lower temperature, an induction temperature between 30 and 35 °C should be acceptable under practical production conditions.

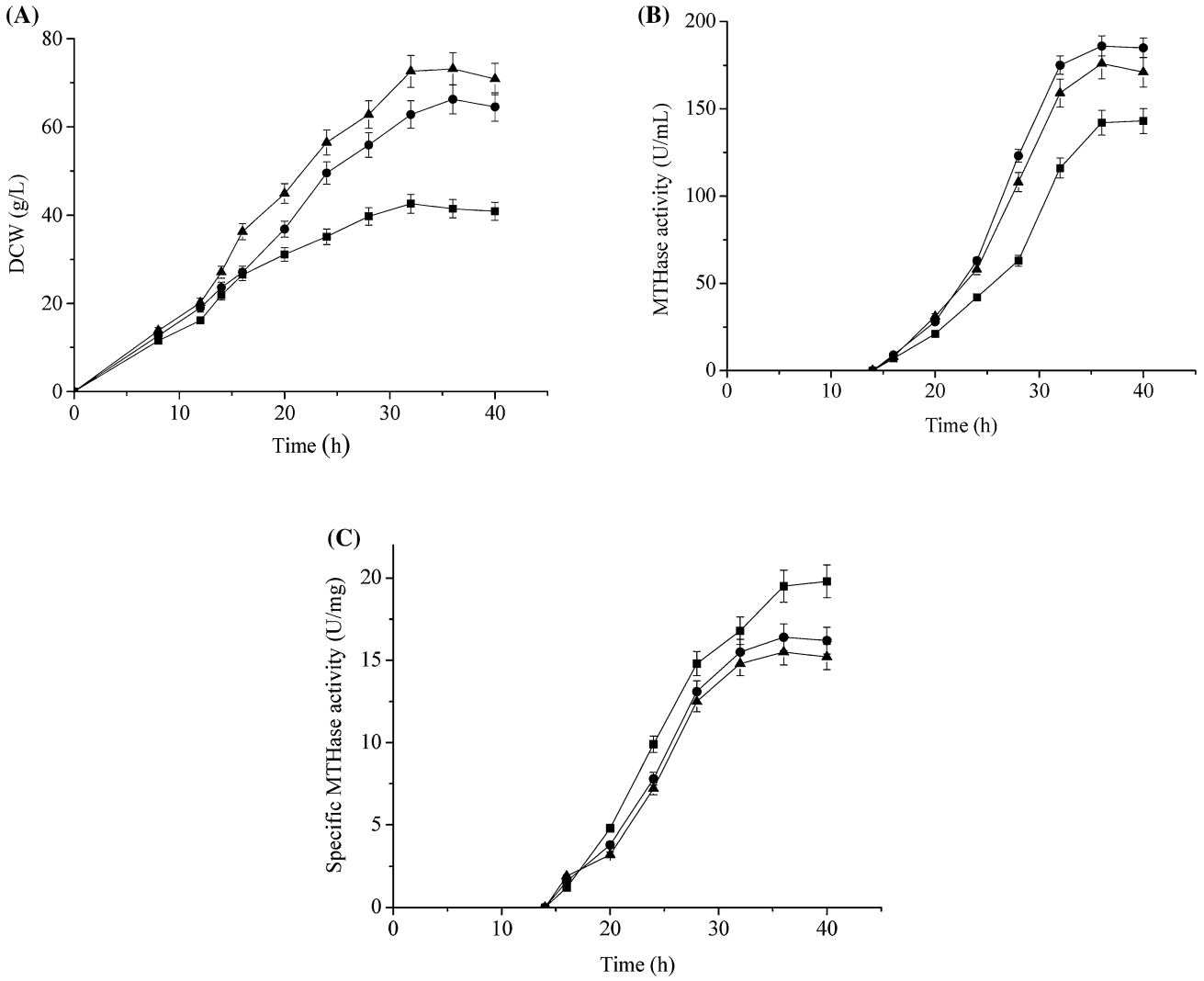

Fig. 4 Effect of induction temperature on cell growth and MTHase production. **a** Cell growth, **b** MTHase activity, **c** specific MTHase activity. Filled squares: 25 °C, filled circles: 30 °C, filled triangles: 35 °C

Effect of the inducer lactose feeding rate on cell growth and MTHase production

Lactose is a low-cost, non-toxic inducer used with T7 promoter-based expression systems. Expression from the T7 promoter can normally be fully induced using high concentrations of lactose. However, cell growth and protein should be balanced to obtain the maximal level of protein production when expression is induced with lactose [34, 35]. In this study, lactose feeding was performed at four different feeding rates, 0.1, 0.2, 0.4 and 0.8 g/L/h, to determine the optimal feeding rate. As shown in Fig. 5, the biomass reached its highest value (69.1 g/L) when the lactose feeding rate was the lowest (0.1 g/L/h). Increasing the lactose feeding rate reduced the biomass to some extent; the lowest DCW (52.4 g/L) was seen at 0.8 g/L/h. The highest MTHase activity, 204.6 U/mL (19.5 U/mg), was obtained at a lactose feeding rate of 0.2 g/L/h. This activity was 1.6-fold of that seen at a lactose feeding rate of 0.1 g/L/h. Higher lactose feeding rates (> 0.2 g/L/h) decreased the final MTHase activity, especially 0.8 g/L/h. This phenomenon may have been caused by the metabolic burden or the presence of misfolded proteins caused by excessive induction [31, 36].

Conclusion

Sulfolobus acidocaldarius MTHase was expressed in *E. coli*, and the MTHase activity obtained using the pET-32a/*E. coli* Origami (DE3) system was 5.3-fold greater than that obtained using the pET-24a/*E. coli* BL21 (DE3)

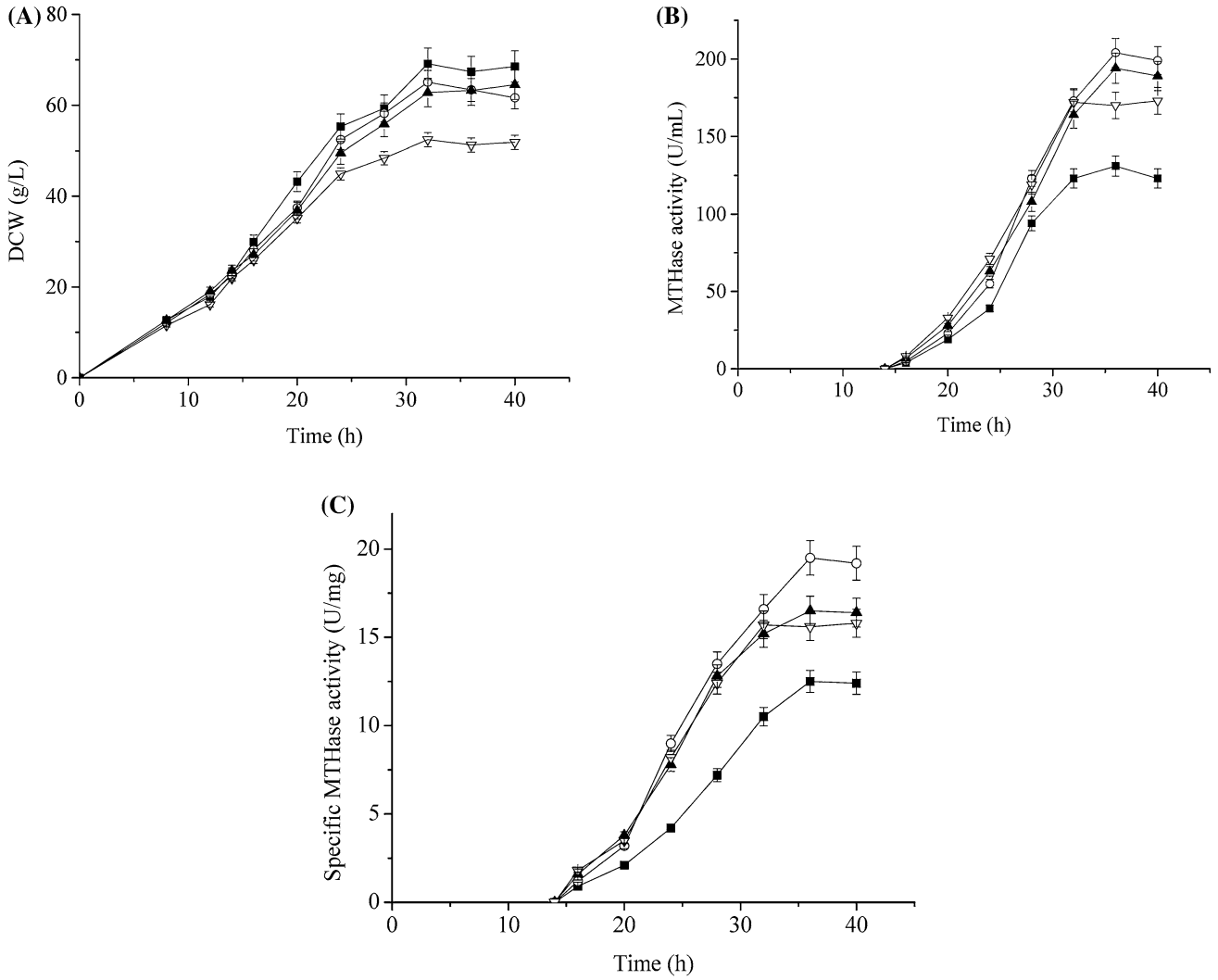

Fig. 5 Effect of inducer lactose feeding rate on cell growth and MTHase production. **a** Cell growth, **b** MTHase activity, **c** specific MTHase activity. Filled squares: 0.1 g/L/h, open circles: 0.2 g/L/h, filled upright triangles: 0.4 g/L/h, unfilled downward triangles: 0.8 g/L/h

Table 1 Comprehensive comparison of the MTHase heterologous expression in shake flask and 3-L fermentor

Mode of cultivation	Host	Plasmid	Induction point (OD_{600})	Induction temperature (°C)	Lactose feeding rate (g/L/h)	Biomass (g/L)	MTHase activity (U/mL)
Shake flask	*E. coli* BL21 (DE3)	pET-24a (+)	1.5	25	IPTG 0.1 mM	7.3	10.4
		pET-32a (+)				7.6	17.9
	E. coli Origami (DE3)	pET-24a (+)				7.8	38.8
		pET-32a (+)				7.2	55.0
3-L fermentor	*E. coli* Origami (DE3)	pET-32a (+)	30	30	0.4	49.5	143.4
			40	30	0.4	66.2	184.0
			50	30	0.4	70.8	175.1
			40	25	0.4	42.6	142.5
			40	35	0.4	73.2	176.0
			40	30	0.1	69.1	131.3
			40	30	0.2	65.1	204.6
			40	30	0.8	52.4	172.8

system. This supports that disulfide bond formation plays an important role in the proper folding of overexpressed cytoplasmic MTHase. When the fermentation conditions were optimized, the MTHase activity reached 204.6 U/mL (Table 1). To the best of our knowledge, this is the first report describing a systematic effort to obtain high-efficiency MTHase production. This report is also expected to be valuable in the production of other enzymes containing disulfide bonds.

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