



High-level production of maltobionic acid from high-maltose corn syrup by genetically engineered *Pseudomonas taetrolens*

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ABSTRACT

Maltobionic acid (MBA) has recently emerged as an important material in various industries. Here, we showed that quinoprotein glucose dehydrogenase (GDH) from *Pseudomonas taetrolens* could convert maltose into MBA by heterologously expressing this enzyme in MBA non-producing *Escherichia coli*. We homologously expressed GDH in *P. taetrolens* to improve intracellular maltose-oxidizing activity and MBA production. We optimized culture conditions, then applied these conditions to batch fermentation by recombinant *P. taetrolens* in a 5-L bioreactor. The MBA production, yield, and productivity of batch fermentation using high-maltose corn syrup (HMCS), an inexpensive maltose source, were 200 g/L, 95.6 %, and 6.67 g/L/h, respectively. Although the MBA productivity from HMCS was 70.1 % of that compared with pure maltose as the substrate, HMCS was a better substrate for commercial MBA production, considering the cost was 1.1 % of that of pure maltose. The present findings provide an economically feasible strategy with which to produce MBA.

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1. Introduction

Sugar acids are organic acids derived from the direct oxidation of mono- or oligosaccharides such as gluconic acid, glucaric acid, xylonic acid, and lactobionic acid (4-*O*- β -galactopyranosyl-D-gluconic acid; LBA) [1]. Among these sugar acids, lactobionic acid, a type of aldonic acid, has received considerable attention because it can chelate metal, and is biocompatible, biodegradable, antioxidant, antimicrobial, moisturizing, and nontoxic [2,3]. Lactobionic acid is used in the cosmetic, pharmaceutical, food, and chemical industries [4,5].

At present, LBA is mainly synthesized by chemical oxidation, which requires high-energy metal catalysts that are costly, harmful, and generate unwanted byproducts [6,7]. Biological method of producing LBA by microbial fermentation has been

intensively investigated as an alternative to chemical oxidation because of its high selectivity, efficiency, and eco-friendliness [2,4]. Nonpathogenic *P. taetrolens* does not generate byproducts from lactose and it can produce large quantities of LBA [4,8].

Maltobionic acid (4-*O*- α -D-glucopyranosyl-D-gluconic acid, MBA) is another aldonic acid obtained from maltose oxidation. It is a stereoisomer of LBA with similar physicochemical characteristics to those of LBA, such as biocompatible, biodegradable, antioxidant, metal chelating, nontoxic, and moisturizing properties [9] that are useful to the food, cosmetic, and pharmaceutical industries [10–12]. However, the applications of MBA are somewhat narrower than those of LBA because the period of application research of MBA is shorter than that of LBA and the production volume of MBA is lower. Therefore, the development of an efficient production process is important for expanding the applications of MBA, which has also been chemically produced like LBA [13]. Because the problems associated with the chemical production of MBA and LBA are similar, microbial fermentation method of MBA production is of interest. However, microbial MBA production has not been investigated in detail. To date, two reports have described microbial MBA production by *Pseudomonas sp.* and *Pseudomonas fragi*, but the production was much lower (94.7 g/L) than that of LBA (400 g/L) [14–16].

We previously characterized the substrate specificity of *P. taetrolens* for various saccharides and found that it could produce

Abbreviations: DCIP, 2,6-dichlorophenol indophenol; GDH, quinoprotein glucose dehydrogenase; HMCS, high-maltose corn syrup; HPLC, high-performance liquid chromatography; IPTG, isopropyl- β -D-1-thiogalactopyranoside; LB, Luria-Bertani; LBA, lactobionic acid; MBA, maltobionic acid; NB, nutrient broth; OD, optical density; PQQ, pyrroloquinoline quinone; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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not only LBA but also MBA (submitted for publication). We also found that the glucose dehydrogenase (GDH, GenBank accession number WP048384179), which is one of the GDHs from *P. taetrolens*, could convert lactose into LBA. Some GDHs have been reported to convert lactose into LBA [17,18]. Up to now, all these GDHs have been originated from gram-negative bacteria and are known to quinoprotein GDH (EC 1.1.5.2) using PQQ as a cofactor. Moreover, some quinoprotein GDHs can also convert maltose into MBA [18,19].

Here, we aimed to enhance MBA production by optimizing the substrate concentration, growth temperature, aeration, and cell density of seed cultures using a genetically modified *P. taetrolens* strain, which homologously expresses the quinoprotein GDH. To our knowledge, the MBA titer, yield, and productivity from pure maltose or high-maltose corn syrup (HMCS) substrates were higher than those of a previous study of applying microorganisms to produce MBA [14,15] and comparable to those obtained in microbial LBA production [20].

2. Material and methods

2.1. Materials

Pure maltose (EP grade, 92 % purity) was purchased from Daejung Chemical and Metals Co., Ltd. (Siheung, Korea). The EP graded pure maltose reagent consists of 92 % maltose, 2% D-glucose, 4% maltotriose, and 2% other oligosaccharides. High-maltose corn syrup (HMCS, 55 % purity) was purchased from Ottogi Co., Ltd. (Anyang, Korea). The HMCS consists of 55 % maltose, 5% D-glucose, 28 % maltotriose, and 12 % other oligosaccharides. All other reagents were commercial products of analytical grade. All restriction enzymes, DNA-modifying enzymes, and related reagents used for DNA manipulation were purchased from New England Biolabs (Ipswich, MA, USA), Solgent (Daejeon, Korea), or Sigma-Aldrich (St. Louis, MO, USA).

2.2. Bacterial strains and plasmids

Escherichia coli JM109 (Promega Corporation, Madison, WI, USA) was the host strain for DNA manipulation and expression of the quinoprotein glucose dehydrogenase (GDH) gene. The strain was propagated in Luria-Bertani (LB) medium (10 g/L bactotryptone, 5 g/L yeast extract, and 5 g/L NaCl) at 37 °C for 24 h and stored frozen in 50 % glycerol at –80 °C. *Pseudomonas taetrolens* Haynes (ATCC 4683, KCTC 12501) was purchased from the Korea Collection for Type Cultures (KCTC, Jeongseup, Korea). The cells were propagated in nutrient broth (NB), 1 g/L beef extract, 2 g/L yeast extract, 5 g/L peptone, and 5 g/L NaCl containing 10 g/L lactose at 25 °C for 24 h and stored frozen in 50 % glycerol at –80 °C. Glucose dehydrogenase was expressed in *Escherichia coli* using the plasmid pKK223-3 and in *P. taetrolens* using the plasmid pDSK519, a broad-host range vector for gram-negative bacteria [21], under the control of the lac promoter induced by isopropyl-β-D-1-thiogalactopyranoside (IPTG) (Sigma Aldrich).

2.3. Culture medium and conditions

Single colonies of recombinant *E. coli* and *P. taetrolens* strains were cultivated in 10 mL of liquid LB broth or on LB agar plates containing appropriate antibiotics (50 μg/mL ampicillin or 30 μg/mL kanamycin) for gene cloning and protein expression. Seed cultures were inoculated to a final optical density (OD_{600nm}) of 0.1. The inoculum was cultured at 25 °C and 120 rpm for 24 h, and then employed as seed culture for the shake-flask experiments and bioreactor experiments.

Bacteria were cultured in 300-mL baffled or non-baffled flasks containing 50 mL of NB. Cultures were then transferred to 50 mL of NB medium containing 200 g/L maltose and shaken at 200 rpm for 24 h at 25 °C. We added 10 μM pyrroloquinoline quinone (PQQ), a cofactor of quinoprotein glucose dehydrogenase that is not synthesized by *E. coli* [22] to the broth to express GDH in *E. coli*. Expression of the recombinant gene was induced with 1 mM IPTG at the mid-log growth phase.

2.4. Plasmid construction

Chromosomal DNA of *P. taetrolens* was prepared using a genomic DNA extraction kit (Qiagen GmbH, Hilden, Germany) as described by the manufacturer. To express *gdh* (GenBank accession number WP048384179, 2.4 kb) in *E. coli*, pKK-GDH was constructed by inserting the GDH gene into the *EcoRI* and *HindIII* restriction sites of pKK223-3, which contained *tac* promoter. The GDH gene was amplified by PCR using the following primer pair: GDH_ EcoRI_F 5'-TAAGCAGAATTCATGAGTACGCAAGCGAAAGG-3' and GDH_HindIII_R 5'-TGCTTAAAGCTTCATCATTTCTCTTAGGATCG-3'. We constructed pDSK-GDH by inserting the GDH gene into the *PstI* and *HindIII* restriction sites of pDSK519, which contained *lac* promoter, to express *gdh* in *P. taetrolens*. The GDH gene was amplified by PCR using the following primers pair: GDH_PstI_F 5'-CTGCAGAGGAATATGATATGAGTACGCAAGCGAAAGGCTCAG-3' and GDH_ EcoRI_R 5'-GAATTCTCATTCTCTTTAGGATCGGGCAGCGG-3'. The primer pairs were inserted into restriction sites (underlined) for subsequent cloning into pKK223-3 and pDSK519. The genes were amplified by PCR using Pfu-X DNA polymerase (Solgent). The amplified PCR products were purified using (GeneAll® ExpresTM PCR purification kits (GeneAll Biotech Co., Ltd., Seoul, Korea) and digested with restriction enzymes. The products digested at each restriction site were ligated into pKK223-3 and pDSK519, and the resultant plasmids were transformed into *E. coli* JM109 and *P. taetrolens* using a standard protocol. *E. coli* was transformed by the recombinant plasmids using conventional heat shock method and *P. taetrolens* was transformed by the recombinant plasmids using electroporation method. The electroporation condition was 2.5 kV, 125 Ω, and 50 μF. To confirm that the GDH gene was cloned in frame, recombinant plasmids from the transformed *E. coli* JM109 and *P. taetrolens* were isolated and were sequenced at Macrogen Inc. (Seoul, Korea). The nucleotide and amino acid sequences were compared with entries in GenBankTM and EMBL protein databases using the FASTA and BLAST programs.

2.5. Enzyme assays

Equal amounts (volume × OD₆₀₀) of cultured cells were harvested by centrifugation at 4 °C, washed twice with phosphate buffered saline (PBS; 10 mM Na₂HPO₄, 2 mM KH₂PO₄, 137 mM NaCl, 2.7 mM KCl, pH 7.4), then suspended in an equal volume of PBS. The suspension was sonicated on ice then separated by centrifugation at 13,000 ×g for 10 min at 4 °C. Protein concentrations of the enzyme in the supernatant were determined using the Bradford method [23] with bovine serum albumin as the standard. The maltose-oxidizing activity was estimated by measuring the change of absorbance at 530 nm, which was caused by the enzymatic reduction of 2, 6-dichlorophenol indophenol (DCIP). The substrate (1.35 mL), comprising 0.075 mM DCIP, and 5 mM maltose in 100 mM acetate buffer (pH 5.5), was mixed with 150 μL of enzyme solution, and incubated at 30 °C. One unit of activity was defined as the amount of enzyme required to oxidize 1 μmol of maltose per min [24]. All results are shown as the means of at least three independent experiments.

2.6. Effects of initial maltose concentration on MBA production

We investigated the effects of the initial maltose concentration on MBA production by *P. taetrolens* (pDSK-GDH) as follows. *P. taetrolens* cells (initial density at OD_{600nm}, 0.1) were cultivated in 300-mL baffled flasks containing 50 mL of NB supplemented with 30 g/L CaCO₃, to maintain the pH of the culture medium near pH 6.0, with 50, 100, 150, 200, 300, 400, and 500 g/L maltose at 200 rpm and 25 °C. Error bars represent the standard deviation (SD) of three independent experiments.

2.7. Effects of temperature on MBA production

We investigated the effects of temperature on MBA production by *P. taetrolens* (pDSK-GDH) as follows. *P. taetrolens* cells (initial cell density at OD_{600nm}, 0.1) were cultivated in 300-mL baffled flasks containing 50 mL of NB supplemented with 200 g/L of maltose and 30 g/L CaCO₃ at 20 °C, 25 °C, 30 °C, and 35 °C. Error bars represent the SD of three independent experiments.

2.8. Effects of aeration on MBA production

We examined the effects of aeration on MBA production in *P. taetrolens* (pDSK-GDH) as follows. *P. taetrolens* cells (initial cell density at OD_{600nm}, 0.1) were cultivated in 300-mL baffled and non-baffled flasks containing 50 mL of (NB) supplemented with 200 g/L of maltose and 30 g/L CaCO₃ at 200 rpm and 25 °C. Error bars represent the SD of three independent experiments.

2.9. Effect of seed culture cell density on MBA production

We investigated the effects of the density of seed cultures on MBA production by *P. taetrolens* (pDSK-GDH) as follows. Cultured *P. taetrolens* cells at densities at OD_{600nm} of 0.1, 0.3, 0.5, and 1.0 were inoculated into 50 mL of NB supplemented with 200 g/L maltose and 30 g/L of CaCO₃, then cultivated at 25 °C. Error bars represent the SD of three independent experiments.

2.10. Bioreactor conditions

Fermentation proceeded in a 5-L bioreactor (BioCNS, Daejeon, Korea) at a working volume of 2 L. The composition of the fermentation medium was 1 g/L beef extract, 2 g/L yeast extract, 5 g/L peptone, 5 g/L NaCl, and 200 g/L of pure maltose or HMCS. The antibiotic kanamycin (30 µg/mL) was added to the culture media as required. The agitation speed was controlled from 300 to 600 rpm to maintain 30 % dissolved oxygen (DO), and the air supply was maintained at 1 vvm. Fermentation proceeded at 25 °C, and the pH was controlled with 30 g/L CaCO₃.

2.11. Analytical methods

Sampling was performed by withdrawing 1 mL of culture broth during the cultivation of the recombinant *P. taetrolens* to analyze the cell growth, maltose and MBA concentration. Three-hundred µL of culture broth was used for measuring cell growth and the rest of culture broth (700 µL) was centrifuged at 15,000 rpm for 10 min to prepare the culture supernatant for assaying maltose and MBA concentration. Cell growth was measured using a UV-2600 model spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at a wavelength of 600 nm (OD_{600nm}). Concentrations of maltose and MBA in the culture medium were measured using an Agilent 1260 HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA). Samples for HPLC were prepared after centrifugation at 15,000 rpm for 3 min and filtration (pore size, 0.2 µm). Cell-free samples were diluted 10-fold with distilled water then analyzed by

HPLC using a RID detector with an ICsep ICE-ION-300 column (Transgenomic Inc., New Haven, CT, USA). The mobile phase was 0.5 mM H₂SO₄ applied at a flow rate of 0.3 mL/min, and the column temperature was 75 °C.

3. Results and discussion

3.1. Heterologous expression of quinoprotein glucose dehydrogenase in *E. coli* to confirm maltose conversion into maltobionic acid

In our previous study, we found that the quinoprotein GDH (GenBank accession number WP048384179) from *P. taetrolens* could convert lactose into LBA, as described in detail in another manuscript (submitted for publication). Some quinoprotein GDHs can not only convert lactose into LBA, but also convert maltose into MBA [18,19]. To examine whether the GDH from *P. taetrolens* could also convert maltose into MBA, we tried to heterologously express GDH genes in bacterium unable to produce MBA and confirm formation of MBA in the recombinant bacterium. In our previous work, we found that *E. coli* strain, which the quinoprotein glucose dehydrogenase (GCD) gene was inactivated, could not produce MBA from maltose. Thus, we used *E. coli* Δ gcd strain to verify the GDH from *P. taetrolens* could produce MBA from maltose. The GDH gene was cloned into pKK223-3 to generate pKK-GDH, which was then transformed into *E. coli* Δ gcd to construct recombinant *E. coli* Δ gcd (pKK-GDH). This recombinant strain was cultivated to assess the formation of MBA from maltose by HPLC. Fig. 1 shows that *E. coli* Δ gcd (pKK-GDH) converted maltose into MBA. This result indicated that the GDH from *P. taetrolens* had maltose-oxidizing activity and could produce MBA from maltose.

3.2. Effects of GDH expression on maltose-oxidizing activity and MBA production in *P. taetrolens*

We cloned *gdh* into pDSK519 to generate pDSK-GDH, which was then transformed into *P. taetrolens*. Wild-type and recombinant *P. taetrolens* (pDSK-GDH) strains were incubated in non-baffled flasks for 12 h. The crude intracellular maltose-oxidizing activity of *P. taetrolens* (pDSK-GDH) was approximately 38 % higher than that of wild-type *P. taetrolens* (1.98 ± 0.11 vs. 1.44 ± 0.21 U/µg; Fig. 2). This result indicated that homologous GDH expression effectively increased the intracellular maltose-oxidizing activity in *P. taetrolens*. The production of MBA was also compared between wild-type and recombinant *P. taetrolens* (pDSK-GDH) after 12 h of cultivation. The MBA production (g/L) of *P. taetrolens* (pDSK-GDH) was approximately 15 % higher than that of wild-type *P. taetrolens* (15.3 ± 1.03 vs. 13.3 ± 1.25 g/L; Fig. 2). Thus, the homologous expression of GDH could increase maltose-oxidizing activity and MBA production in *P. taetrolens*. We then optimized the culture conditions to improve MBA production by the recombinant *P. taetrolens* strain. In our previous study, we achieved high-level production of LBA using the wild-type *P. taetrolens* by optimizing the culture conditions such as pH and growth temperature [20]. We could highly improve the LBA production concentration in flask and fermenter cultures by using CaCO₃ as a pH control agent. Thus, in this study, we also used CaCO₃ as a pH control agent for increasing MBA production concentration.

3.3. Effects of initial maltose concentration on MBA production

To investigate the effects of initial maltose concentration on MBA production, recombinant *P. taetrolens* cells were cultivated in NB supplemented with 50, 100, 150, 200, 300, 400, and 500 g/L of maltose) in non-baffled flasks at 25 °C. The seed culture was inoculated at an initial cell density of 0.1 at OD_{600nm}. Cell growth was inhibited as the initial maltose concentration increased to >

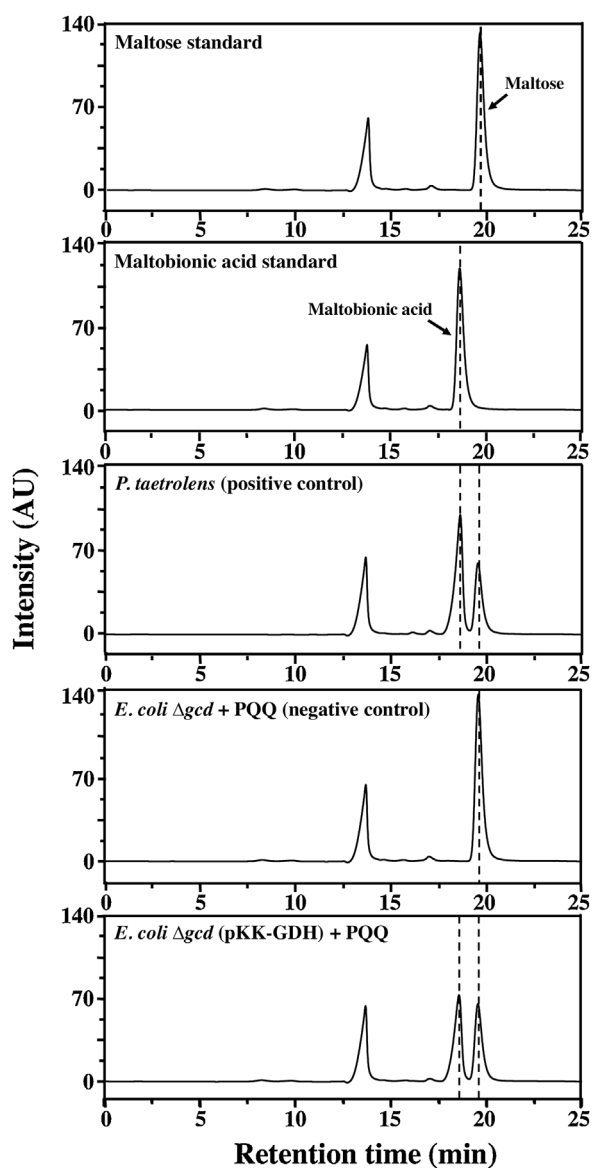


Fig. 1. Assessment of MBA production in recombinant *E. coli* by HPLC.

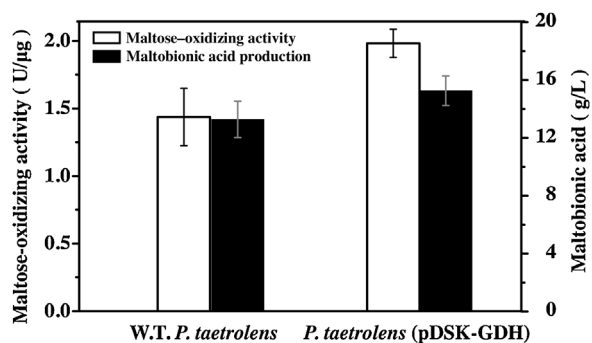


Fig. 2. Intracellular maltose-oxidizing activity and MBA production of wild-type (W.T.) and recombinant *P. taetrolens* (pDSK-GDH) in flask culture. Bars, Intracellular maltose-oxidizing activity (blank) and MBA production (filled) at 12 h of cultivation time. Error bars represent standard deviation of three independent experiments. MBA, maltobionic acid; W.T., wild-type.

200 g/L (Fig. 3A). The maltose consumption rate and MBA productivity also decreased at initial maltose concentrations > 200 g/L (Fig. 3B and 3C). The MBA productivity was maximal at 200 g/L maltose, which was approximately 211 % higher than that at 500 g/L maltose (3.33 vs. 1.07 g/L/h). These findings indicated that maltose concentrations \leq 200 g/L did not obviously affect cell growth and increased the MBA productivity of the recombinant *P. taetrolens*. Thus, 200 g/L maltose was applied for subsequent investigations.

3.4. Effects of growth temperature on MBA production

To examine the effects of growth temperature on MBA production, recombinant *P. taetrolens* strains were cultivated at 20 °C, 25 °C, 30 °C, and 35 °C in NB containing 200 g/L maltose in non-baffled flasks. The seed culture was inoculated at an initial cell density of 0.1 at OD_{600nm} . At 35 °C, the growth of *P. taetrolens* was significantly hindered (Fig. 4A) and maltose conversion into MBA was negligible (Fig. 4C). The maltose consumption rate and MBA productivity (3.33 g/L/h, respectively) were maximal at 25 °C (Fig. 4B and C). Therefore, we selected a growth temperature of 25 °C for subsequent investigations.

3.5. Effects of aeration on MBA production

To investigate the effects of aeration on MBA production, *P. taetrolens* (pDSK-GDH) was cultivated in 300-mL baffled and non-baffled flasks at 25 °C and 200 rpm in 50 mL of NB supplemented with 200 g/L maltose. The seed culture was inoculated at an initial cell density of 0.1 at OD_{600nm} . The maximum cell density at OD_{600nm} was 5.5 in both types of flasks (Fig. 5A). Although 200 g/L of maltose was fully converted into MBA, the maltose consumption rates and MBA productivity were approximately 123 % higher in the baffled, than the non-baffled flasks (7.41 vs. 3.33 g/L/h; Fig. 5B and C), showing that a high aeration rate obviously improves MBA productivity. Therefore, baffled flasks were used in subsequent investigations.

3.6. Effects of seed culture cell density on MBA production

To examine the effects of seed culture cell density on MBA production, cultured recombinant *P. taetrolens* was inoculated at densities of 0.1, 0.3, 0.5, and 1.0 at OD_{600nm} in 50 mL of NB containing 200 g/L maltose and cultivated at 25 °C. The maltose consumption rate and MBA productivity were improved as the cell density of seed culture increased (Fig. 6A, B and C). The MBA productivity was approximately 28 % higher and maximal (9.52 vs. 7.41 g/L/h), at an initial cell density of 1.0, compared with 0.1. Thus, we seeded cells at a density of 1.0 in further investigations.

3.7. Batch fermentation for MBA production in the bioreactor

We scaled up MBA production by batch fermentation using *P. taetrolens* (pDSK-GDH) in a 5-L fermenter under the culture conditions optimized in the flask culture investigation. The oxygen supply should be adequate for MBA, which is synthesized by the oxidation of maltose in *P. taetrolens*. When the level of DO was maintained at 30 % during the fermentation process, the MBA production, yield from pure maltose, and productivity were 200 g/L, 100 %, and 9.52 g/L/h, respectively (Fig. 7). These results surpassed those of previous findings of microorganisms as MBA producers [14,15].

Batch fermentation of *P. taetrolens* (pDSK-GDH) also proceeded using the substrate HMCS in a 5-L fermenter using the optimized culture conditions. The MBA production titer, yield from maltose in the HMCS, and productivity were 200 g/L, 100 %, and 6.67 g/L/h,

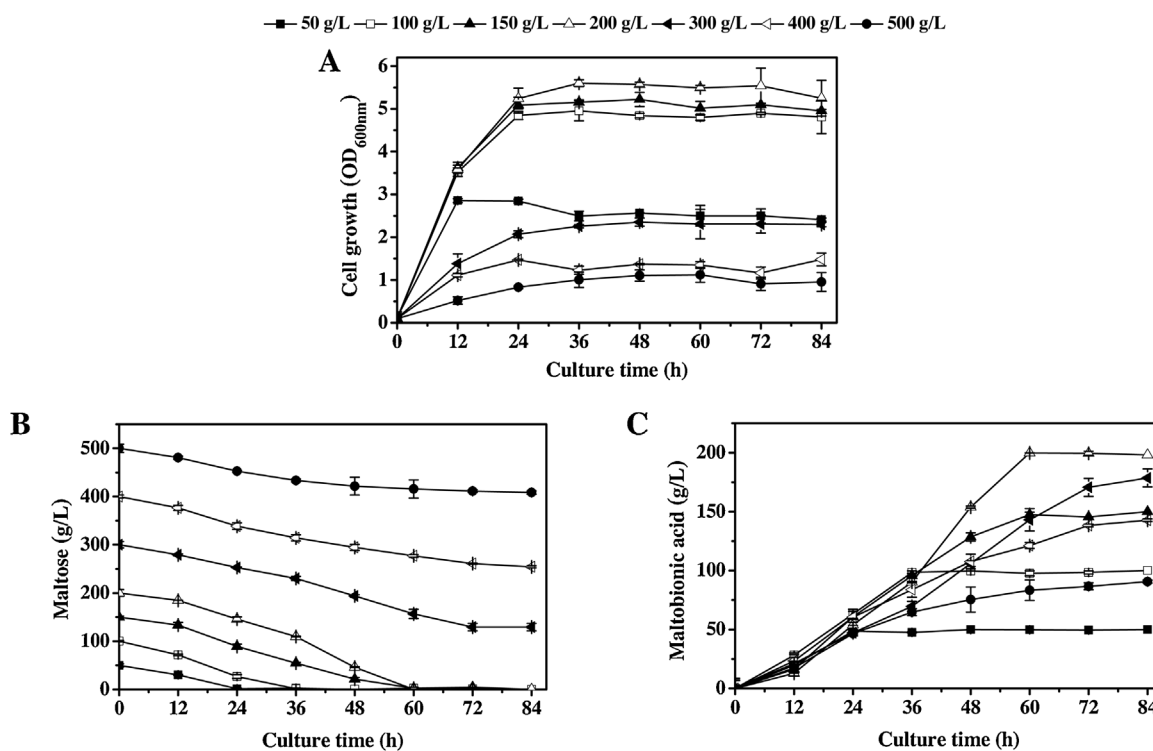


Fig. 3. Effects of initial maltose concentration on MBA production by *P. taetrolens* (pDSK-GDH). *P. taetrolens* cells (initial cell density at OD_{600nm}, 0.1) were cultivated in 300-mL baffled flasks containing 50 mL NB with 50, 100, 150, and 200 g/L of maltose and 30 g/L CaCO₃ at 200 rpm and 25 °C. Time course of cell growth (A), maltose consumption (B) and MBA production (C). Error bars, standard deviation of three independent experiments. GDH, glucose dehydrogenase; MBA, maltobionic acid; NB, nutrient broth; OD, optical density.

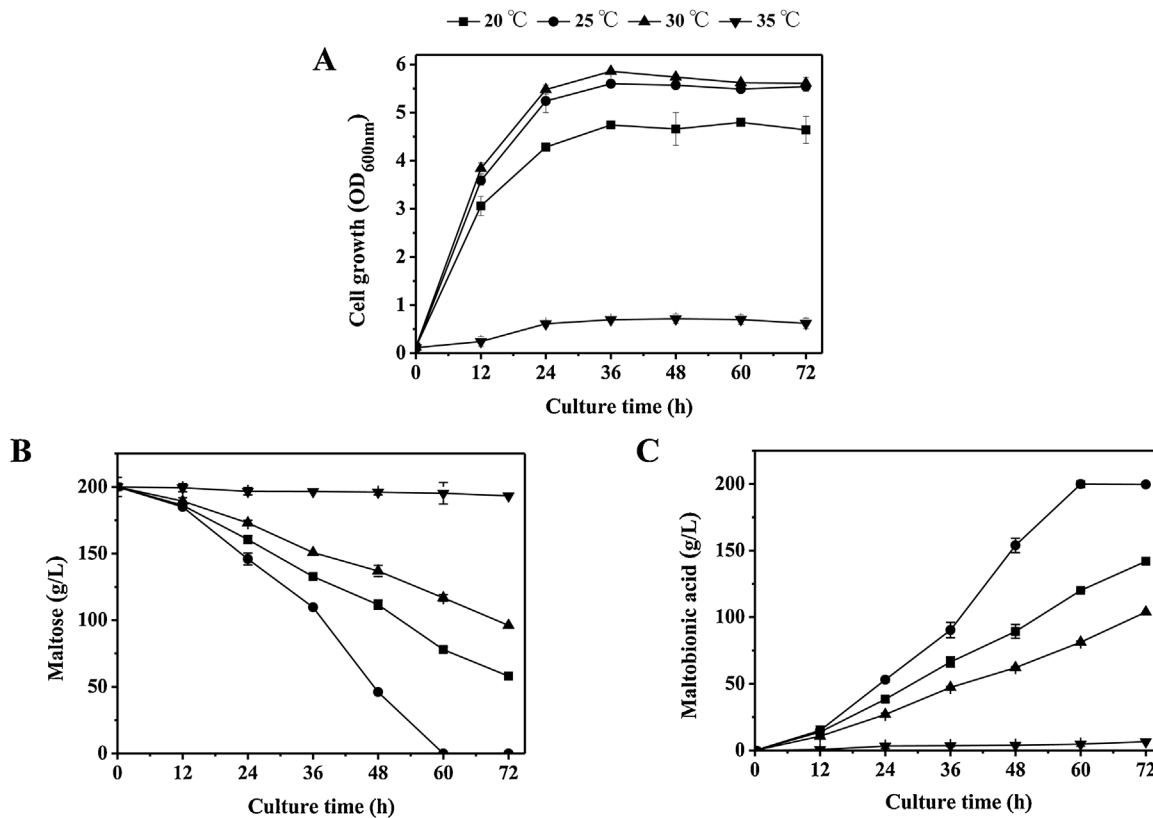


Fig. 4. Effects of temperature on MBA production by *P. taetrolens* (pDSK-GDH). *P. taetrolens* cells (initial cell density at OD_{600nm}, 0.1) were cultivated in 300-mL baffled flasks containing 50 mL of NB with 200 g/L of maltose and 30 g/L CaCO₃ at 20 °C, 25 °C, 30 °C, and 35 °C. Time course of cell growth (A), maltose consumption (B) and MBA production (C). Error bars, standard deviation of three independent experiments. GDH, glucose dehydrogenase; MBA, maltobionic acid; NB, nutrient broth; OD, optical density.

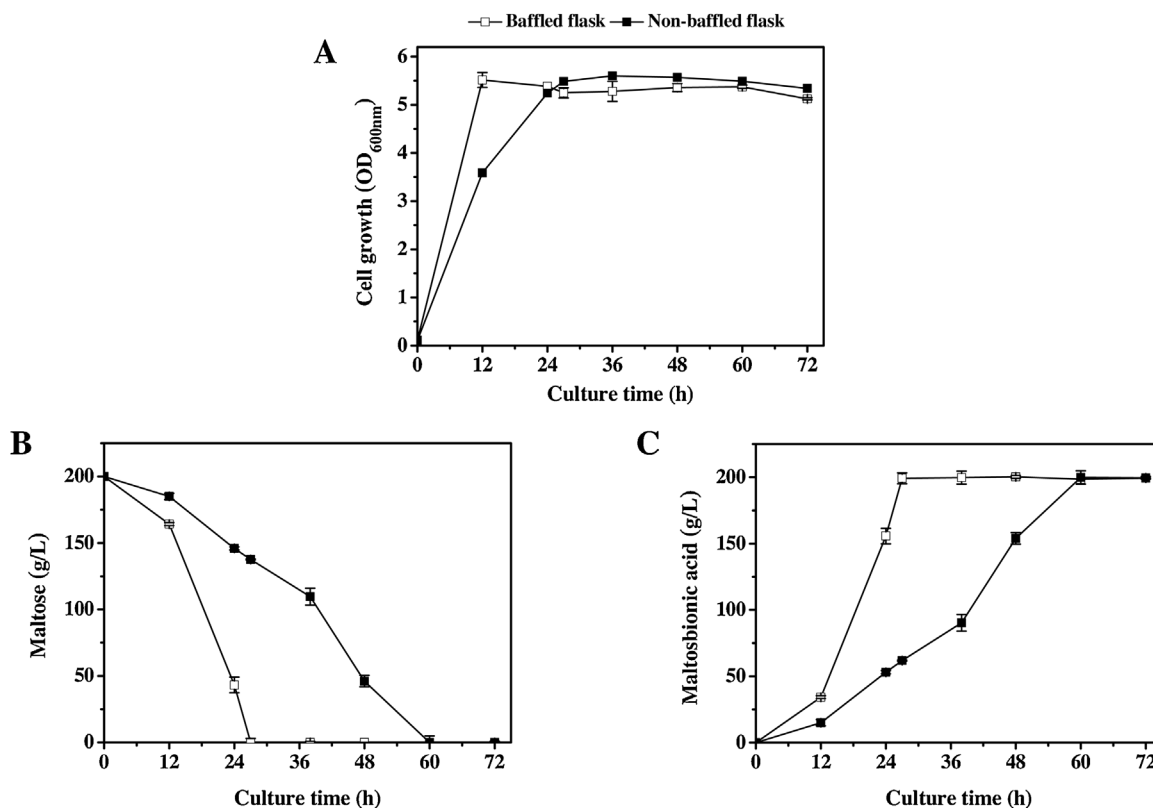


Fig. 5. Effects of aeration on MBA production in *P. taetrolens* (pDSK-GDH). *P. taetrolens* cells (initial cell density at OD_{600nm}, 0.1) were cultivated in 300-mL baffled and non-baffled flasks containing 50 mL of NB with 200 g/L of maltose and 30 g/L CaCO₃ at 200 rpm and 25 °C. Time course of cell growth (A), maltose consumption (B) and MBA production (C). Error bars, standard deviation of three independent experiments. GDH, glucose dehydrogenase; MBA, maltobionic acid; NB, nutrient broth; OD, optical density.

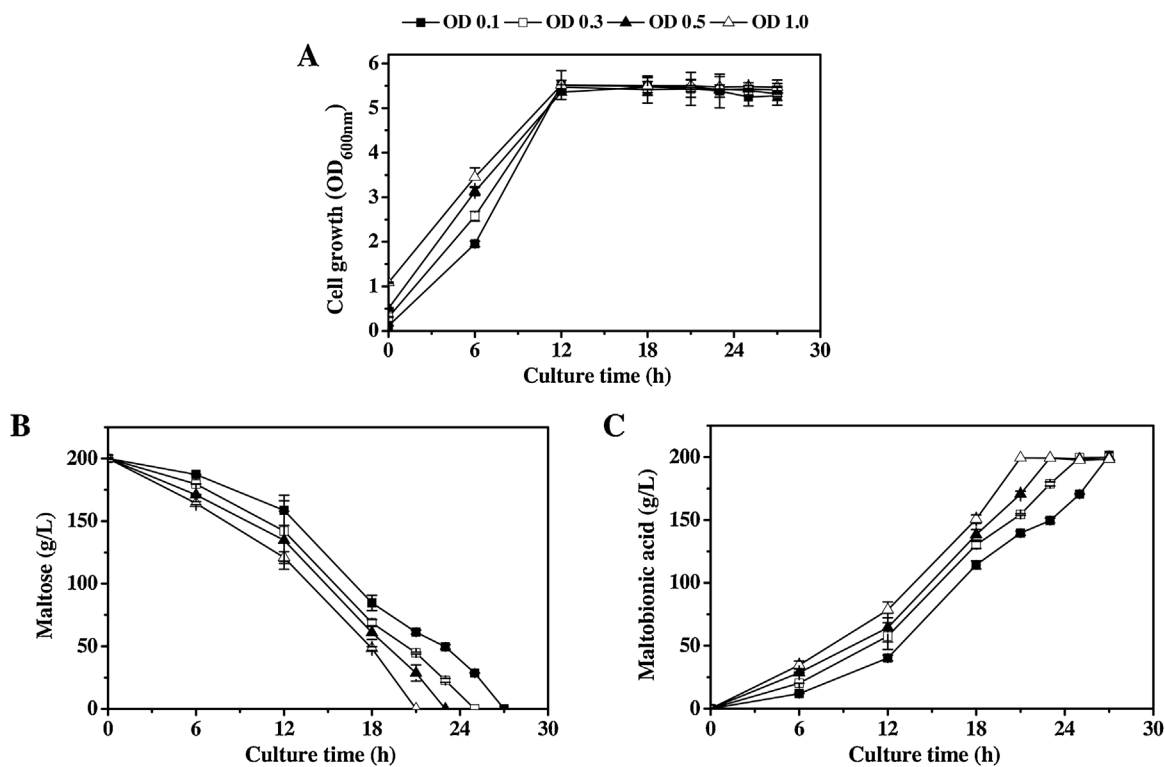


Fig. 6. Effects of density of seed culture on MBA production in *P. taetrolens* (pDSK-GDH). Cultured *P. taetrolens* cells were inoculated at densities of 0.1, 0.3, 0.5, and 1.0 (OD_{600nm}) in 50 mL of NB with 200 g/L maltose, and 30 g/L CaCO₃, and cultivated at 25 °C. Time course of cell growth (A), maltose consumption (B) and MBA production (C). Error bars standard deviation of three independent experiments. GDH, glucose dehydrogenase; MBA, maltobionic acid; NB, nutrient broth; OD, optical density.

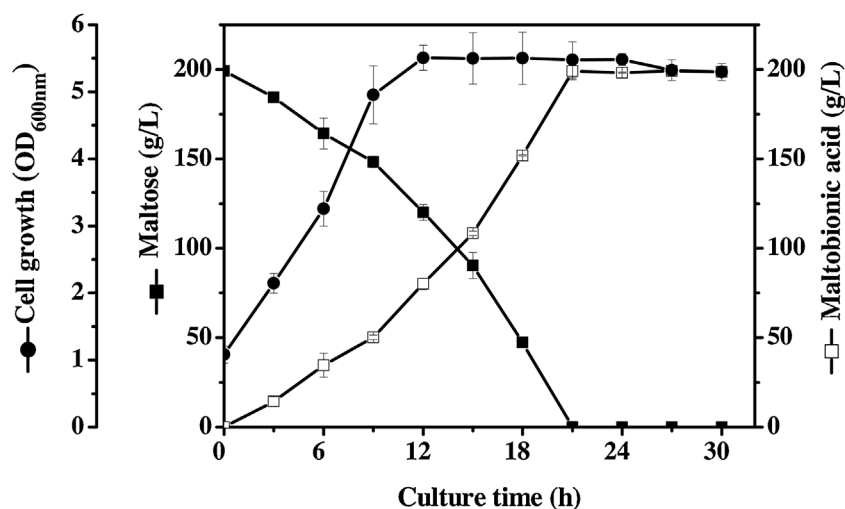


Fig. 7. Batch fermentation of pure maltose by *P. taetrolens* (pDSK-GDH) for MBA production under optimized culture conditions in 5-L jar bioreactors. *P. taetrolens* cells (the initial cell density was 1.0 at OD_{600nm}) were cultivated in 5-L bioreactors containing 2 L NB medium with 200 g/L of maltose and 30 g/L CaCO₃ at 25 °C. The error bars represent the standard deviation from three independent experiments. GDH, glucose dehydrogenase; MBA, maltobionic acid; NB, nutrient broth; OD, optical density.

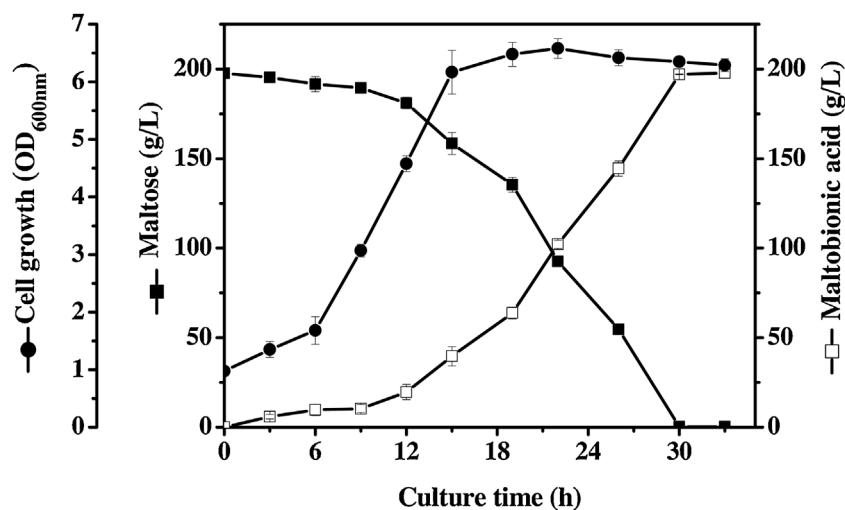


Fig. 8. Batch fermentation of *P. taetrolens* (pDSK-GDH) for MBA production using high-maltose corn syrup under optimal culture condition in 5-L jar bioreactor. *P. taetrolens* cells (initial cell density at OD_{600nm}, 0.1) were cultivated in 5-L bioreactor containing 2 L NB with 200 g/L of maltose and 30 g/L CaCO₃ at 25 °C. Error bars, standard deviation of three independent experiments. GDH, glucose dehydrogenase; MBA, maltobionic acid; NB, nutrient broth; OD, optical density.

respectively (Fig. 8). The MBA production titer and yield in batch fermentation using HMCS and pure maltose were the same, but the MBA productivity (6.67 g/L/h) was approximately 70.1 % of that (9.52 g/L/h) using pure maltose.

In an industrial point of view, the decrease in productivity is a disadvantage because it lengthens the process and thus increases the cost [25–27]. However, the bulk price of the HMCS (\$370/ton) was approximately 94.6-fold lower than that of the pure maltose (\$35,000/ton) (<https://www.alibaba.com>). This fairly low price of the HMCS, compared to that of the pure maltose, can sufficiently offset the relatively low MBA productivity and render the MBA production process more economically feasible.

4. Conclusion

We discovered that *P. taetrolens* could produce MBA from maltose and that the quinoprotein glucose dehydrogenase (GDH) of *P. taetrolens* could act as a maltose-oxidizing enzyme. The

homologous expression of GDH in *P. taetrolens* improved maltose-oxidizing activity and MBA production. Optimized culture conditions improved the MBA production by recombinant *P. taetrolens*. The MBA production, yield, and productivity of 200 g/L, 100 %, and 9.52 g/L/h, respectively, in batch fermentation using pure maltose were higher than any other reported results of MBA production, and 200 g/L, 100 % and 6.67 g/L/h, respectively for HMCS. The increased cost due to the decreased MBA productivity from HCMS was offset by the 94.6-fold lower cost of HMCS. Thus, HMCS was a better substrate for industrial MBA production than pure maltose. Therefore, recombinant *P. taetrolens* and HMCS are suitable for the commercial production of MBA based on microbial fermentation.

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CRediT authorship contribution statement

Yu-Ri Oh: Investigation, Validation, Data curation, Writing - original draft. **Young-Ah Jang:** Investigation, Data curation, Methodology. **Soon Ho Hong:** Investigation, Validation, Methodology. **Gyeong Tae Eom:** Conceptualization, Project administration, Supervision, Writing - original draft, Writing - review & editing, Funding acquisition, Resources.

Declaration of Competing Interest

The authors report no declarations of interest.

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