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DEVELOPMENT OF GLUTATHIONE PRODUCTION TECHNOLOGY BASED ON DESIGNED ACTIVE YEAST OVERPRODUCERS

The Hansenula polymorpha recombinant strain overexpressing both GSH2 gene encoding γ-glutamyl-cysteine-synthetase and MET4 gene encoding the transcription activator of genes involved in cysteine (precursor of glutathione) biosynthesis has been obtained using metabolic engineering approaches. The recombinant strain is characterized by significantly increased glutathione output as compared with in vitro wild-type strain. Conditions for efficient glutathione production by recombinant H. polymorpha strain have been optimized. A semi-industrial model for glutathione production using the designed H. polymorpha overproducer has been developed.

Keywords: glutathione, yeast, Hansenula polymorpha, and metabolic engineering.

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH) is biologically active compound of peptide nature, which plays an important role in a wide range of cellular reactions. Due to the presence of thiol groups, in the cell, glutathione acts as electron donor, provides the course of reduction reaction, and transforms into oxidized form (GSSG). In addition to the support of thiol redox status, glutathione is involved in detoxification of endogenous and exogenous metals and xenobiotics, depositing and transport of cysteine, biosynthesis of protein and DNA, transport of amino acids that are important metabolites for eukaryote cell growth, regulation of cell cycle, etc. Glutathione is a cofactor of several enzymes and major source of nitrogen and sulfur in the conditions of their exhaustion in the environment [1]. Also it plays an important role in protecting the body from bacteria, parasites, and viruses. Glutathione deficiency in the human body is associated with medical disorders caused by oxidative stress, poisoning or compromised immunity. These disorders include neurodegenerative diseases, cancer, cataracts, cirrhosis, pulmonary disease, inflammation of the gastrointestinal tract and pancreas, and hemolytic anemia.

Glutathione is widely used in medical, beauty, and food industries. Recently, the use of glutathione in manufacturing various cosmetic products has been growing rapidly. Glutathione is used as a component of emulsifiers, oily substances, and humidifiers, primarily, to enhance skin whitening effect, of sunscreens and anti-age creams insofar as GSH/GSSG ratio has been established to decrease in the human body with age [2].

Glutathione is used as an ingredient in various food products, including bakery products, alcoholic and soft drinks, breakfast cereals, cheeses, flavorings, dairy products, fats, oils, sauces, and meat. Today, the industrial demand for glutathione increases. World production of crystalline glutathione and yeast extract enriched in glu-

tathione (15%) exceeds 2500 and 500 tons annually, respectively. Accordingly, the glutathione market reaches USD 1 billion per year.

Biotechnology for glutathione production is based on using enzymatic methods or fermentation of natural or genetically modified microorganisms, such as *Saccharomyces cerevisiae*, *Candida utilis*, *Escherichia coli*, and *Lactoccocus lactis*. Among numerous microorganisms capable of accumulating significant quantities of glutathione, the most widely used are *S. cerevisiae* and *C. utilis* yeasts. Advantages of these yeasts are as follows: natural ability to accumulate high intracellular concentration of glutathione; rapid growth and high density of cell biomass. The yeasts are cultured on cheap environment, with this process being scalable [3]. One of the major drawbacks of using the yeasts for industrial production of glutathione is complex regulatory mechanisms that limit its super-synthesis.

H. polymorpha thermotolerant methylotrophic yeast with high content of glutathione and high resistance to various types of stress is considered a promising object for designing a competitive producer of tripeptide [4]. In the *H. polymorpha* methylotrophic yeast, glutathione is involved in detoxification of toxic products of methanol catabolism. For these yeast, metabolic engineering approaches have been elaborated. Recently, the sequencing of *H. polymorpha* genome has been completed and a database has been formed to create conditions for the identification and functional studies of genes involved in the regulation of the biosynthesis of glutathione, its transport into the cell, and degradation. In this regard, the methylotrophic yeast organisms are promising for designing industrial producers of this tripeptide.

Raising efficiency due to reducing the cost of production is an important factor to decrease the price of glutathione. One of the main preconditions for effective production of microbial glutathione is its high content in yeast cells and ability to accumulate microbial biomass in high concentrations.

The application of metabolic engineering methods enabled obtaining recombinant strains of *H. polymorpha* with high-performance glutathione synthesis. In particular, it has been found that the enhanced expression of both *GSH2* gene encoding γ-glutamyl-cysteinyl-glycine and *MET4* gene encoding transcriptional activator of cysteine biosynthesis genes (precursor of glutathione synthesis) stimulates glutathione synthesis in *H. polymorpha* methylotrophic yeast [5]. This paper deals with studying simultaneous amplification of *GSH2* and *MET4* gene expression in *H. polymorpha*, as well as with optimizing glutathione synthesis conditions in terms of maximizing the glutathione production.

MATERIALS AND METHODS

Strains of microorganisms and nutrient media

The researchers used the following strains of *H. polymorpha* yeast: DL-1 (*leu2*), DL-1 (Δ*ura3*,Δ*trp1::URA3, leu2::mcGSH2*$_{CBS}$*::LEU2,* Δ*trp1::TRP1*$_{PCR}$) [5]. The yeast cells were grown at t = 37 °C in the rich YPD medium (1% peptone, 1% yeast extract, 2% glucose), the minimal YNB medium (0.17% Yeast Nitrogen Base, 0.5% $(NH_4)_2SO_4$, 2 % glucose) or in the mineral medium of the following composition (g/l): $(NH_4)_2SO_4$ — 5; KH_2PO_4 — 3; $MgSO_4 \times 7H_2O$ — 0,5; glucose — 2, trace elements (mg/l): EDTA — 15; $ZnSO_4 \times 7H_2O$ — 4,5; $CoCl_2 \times$ $\times$ $6H_2O$ — 0,3; 1 mg/l $MnCl_2 \times 4H_2O$, $CuSO_4 \times$ $\times$ H_2O — 0,3; $CaCl_2 \times 2H_2O$ — 4,5; $FeSO_4 \times 7H_2O$ — 3; $NaMoO_4 \times 2H_2O$ — 0,4; H_3BO_3 — 1; KI — 0,1. For mineral medium, 1,000-time vitamin solution (biotin — 0.5; pantothenic acid — 10; nicotinic acid — 10 mg; inositol — 250; thiamin — 10; pyridoxine — 10; para-aminobenzoic acid — 2 per 10 ml (mg)) was separately prepared, sterilized by cold sterilization, and added to the medium after autoclaving. After sterilization, if necessary, tetracycline was added (20 mg/l) to the medium in a way preventing any contamination. The agar media contained bacteriological agar (2%). The bacterial strain of *Escherichia coli* DH5α (Φ80 d*lacZ*ΔM15, *recA*1, *endA*1, *gyrA*96, *thi*-1, *hsdR*17 (rK^-, mK^+), *supE*44, *relA*1, *deoR*, Δ(*lacZYA-argF*) U169) was grown at t = 37 °C, in a rich LB medium (1.5% peptone, 0.5% yeast extract, 1% NaCl; pH = 7,0).

RESEARCH METHODS

The authors used standard molecular genetic techniques [6]. *H. polymorpha* genomic DNA was separated using Wizard® Genomic DNA Purification Kit (*Promega*, Madison, WI, USA). Restriction endonucleases and ligases were used according to the manufacturer's instructions (*Fermentas*, Vilnius, Lithuania). Plasmid DNA was extracted from *E. coli* using *Wizard® Plus SV Minipreps* DNA Purification System (*Promega*, Madison, WI, USA). Polymerase chain reaction (PCR) was carried out on Gene *Amp*® PCR System 9700 (*Applied Biosystems*, Foster City, CA, USA) using *Platinum*® *Taq* DNA Polymerase (*Fermentas*) according to the manufacturer's instructions. The transformation of *H. polymorpha* was carried out by the electro-transformation technique [7]. The yeast biomass was measured by the spectrophotometric method on a *Helios* γ spectrophotometer (λ = 590 nm, cuvette 1 cm) with the dry weight calculated according to the calibration curve. The optical density of yeast cell suspension was measured at a wavelength of 663 nm, for the transformation; at 420 nm, for glutathione identification; and at 750 nm, for the protein identification.

To study the synthesis of glutathione, the yeast strains were grown under aerobic conditions in 100 ml flasks containing 30 ml YNB medium at 37 °C. The aeration conditions were reached due to stirring at a speed of 220 rpm. The initial optical density of cells inoculated into the medium was A_{600} = 0,1. Cultivation was carried out for 5 days with everyday sampling for glutathione identification. The total content of glutathione (GSH + + GSSG) was determined as described in [5].

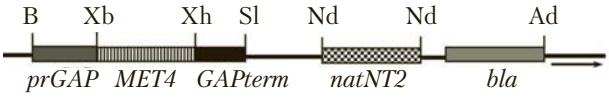
To optimize the selection and conditions glutathione supersynthesis, the yeast strains were also grown in 100 ml flasks containing 30 ml of YPD or YNB medium with 0.5% yeast extract added, at 37 °C, under aerobic conditions (220 rpm). The initial optical density of cells inoculated into the medium was A_{600} = 0,1. To study the influence of aeration on glutathione production, the strains were grown with stirring at 200 rpm and 300 rpm during cultivation in flasks containing medium in proportion 1/3 or 1/6 of their volume. The initial optical density of cells inoculated into the medium, was A_{600} = 1 or A_{600} = 4.

The capacity for growth and synthesis of glutathione was studied while growing in the bioreactor having a volume of 100 liters. The initial volume of medium was 70 liters. The medium was prepared on untreated water. The medium *pH* was additionally titrated up to 5.5. After sterilization, tetracycline (20 mg/l) was added to the medium with its sterility preserved. The starter culture of *H. polymorpha* mcGSH2/MET4 strain was grown in YNB medium with yeast extract (YNB — 1.7 g/l, $(NH4)_2SO_4$ — 5 g/l, glucose — 20 g/l, yeast extract — 0.5%) added in 2 liter Erlenmeyer flasks in an air shaker-incubator at 220 rpm and 37 °C for 24 hours to obtain optical density A_{600} = 10—12. The initial optical density of cells inoculated into the medium in the bioreactor was A_{600} = 1. The cultivation was carried out on mineral environment, with aeration (ratio of air volume to medium volume 1:1) and stirring at 200 rpm, at *pH* = 5.0—5.5.

RESULTS AND DISCUSSION

Obtaining Glutathione Strain Overproducers in H. polymorpha Yeast Using Metabolic Engineering Approaches

The synthesis of glutathione in the yeast occurs in two consecutive reactions involving γ-glutamyl-cysteine-synthetase and glutathione-synthetase. The first enzyme limits the biosynthesis due to reverse inhibition by the end product, i.e. glutathione, and prevents excessive accumulation of tripeptide in the cell [8]. The mechanisms of glutathione biosynthesis regulation in methylotrophic yeasts (particularly *H. polymorpha*) and the homeostasis of this tripeptide in the cell have remained incompletely studied in many aspects. However, it has been found that the enhanced expression of both GSH2 gene encoding *GSH2* gene encoding γ-glutamyl-cysteinyl-glycine and *MET4* gene encoding transcriptional activator of cysteine biosynthesis genes (precursor of glutathione synthesis) stimulates glutathione synthesis in



pUC19/prGAP_MET4/NTC ~ 5,9 т.п.н.

Fig. 1. Linear diagram of pUC19/prGAP_MET4/NTC plasmid: *prGAP* – promoter of glyceraldehyde-3-phosphate dehydrogenase gene; *GAPterm* – terminator of glyceraldehyde-3-phosphate dehydrogenase gene; *natNT2* – gene of resistance to nourseothricin; *MET4* – gene encoding the transcription factor involved in cysteine biosynthesis. Abbreviations of restriction sites: B, BamHI; Xb, XbaI;Xh, XhoI; Sl, SalI; Nd, NdeI; Ad, AdhI

H. polymorpha methylotrophic yeast [5, 9]. Therefore, simultaneous amplification of *GSH2* and *MET4* gene expression in *H. polymorpha,* as well as optimization of glutathione synthesis conditions in terms of maximizing the glutathione production are promising directions of research. To this end, the pUC19/prGAP_MET4/NTC plasmid was designed for recombinant *H. polymorpha* yeast strains with amplified expression of Met4 transcriptional activator (Fig. 1).

In order to search the nucleotide sequence of *H. polymorpha MET4* gene, a computer analysis was made using a respective yeast database (http://genome.jgi-psf.org/Hanpo2). Using PCR the *MET4* gene was amplified from *H. polymorpha* genomic DNA. The native promoter was replaced by a strong constitutive promoter of *H. polymorpha GAP1* gene encoding glyceraldehyde-3-phosphate dehydrogenase. At the first stage, the *GAP1* gene promoter and terminator were amplified from *H. polymorpha* genomic DNA by PCR using respective primer pairs Ko644/Ko645 and Ko646/Ko647 (Table 1).

The resulting fragments were combined by PCR using primers Ko644 and Ko647. The amplified fragment having a size of 0.8 kb was treated with restriction endonucleases BamHI and SalI. As a result, the pUC19/prGAP plasmid was designed. At the next stage, the *natNT2* nourseothricin resistance gene was amplified from plasmid pRS41N [10] by PCR using primers OK42/OK43. The resulting 1.3 kb fragment was treated with restriction endonuclease NdeI and cloned into NdeI-linearized plasmid pUC19/GAP. As a result, the designed plasmid was named pUC19/prGAP/NTC. The *MET4* gene was amplified from *H. polymorpha* genomic DNA by PCR using primers Ko655 and Ko677 and cloned into XbaI/NotI-linearized vector pUC19/prGAP/NTC. As a result, the designed plasmid was named pUC19/prGAP_MET4/NTC (Fig. 1).

The designed plasmid pUC19/prGAP_MET4/NTC was used to enhance *MET4* gene expression in the best available recombinant *H. polymorpha*,

Table 1

Sequence of Nucleotides Used in Primer Operation

Name	Nucleotide sequence 5'-3'
Ko644	CGC GGA TCC TAG ACC ACA TCC GTG CAC CAG
Ko645	GTA AAT ATG TAG ATG GAG CCG AGC CTC GAG CCC GGG GCG GCC GCT CTA GAT TTG TTT CTA TAT TAT CTT TGT ACT AAA G
Ko646	CTT TAG TAC AAA GAT AAT ATA GAA ACA AAT CTA GAG CGG CCG CCC CGG GCT CGA GGC TCG GCT CCA TCT ACA TAT TTA C
Ko647	CGC GTC GAC CTG CCA CGA GGT ACC ACA AAG
OK42	CGC CAT ATG ATA ACT TCG TAT AGC ATA CAT TAT ACG AAG TTA T CT TAA CTA TGC GGC ATC AGA G
OK43	CGC CAT ATG ATA ACT TCG TAT AAT GTA TGC TAT ACG AAG TTA TCC GAG ATT CAT CAA CTC ATT GC
Ko655	TAG TCT AGA ATG TGT GGC GCA GTA TGG C
Ko656	AAA GCG GCC GCC TAG TTT GGC TTC GGG AAA C

strains capable of ensuring glutathione supersynthesis. As initial strain the researchers used the recombinant strain with amplified *GSH2 H. polymorpha GSH2* gene expression controlled by native promoter characterized by enhanced glutathione synthesis on glucose-containing medium as compared with the wild-type strain [5].

The pUC19/prGAP_MET4/NTC vector was introduced in selected *H. polymorpha* strain by electroporation [7]. The transformants were selected on YPD nutrient-rich medium with nourseothricin (100 mg/l) added. For obtaining stable recombinant strains, the transformants were cultured under non-selective conditions with further selection of clones preserving their ability to grow on antibiotic-containing medium. In the stable transformants obtained, the plasmid integration into genome was confirmed by PCR using appropriate primer pairs Ko644/Ko677. The effectiveness of glutathione synthesis was determined for the selected stable transformants. Conditions of culturing and method for glutathione identification are described in the previous section (see Materials and Methods).

The *H. polymorpha* recombinant strain with amplified expression of *GSH2* and *MET4* (mcGSH2/MET4) genes was characterized by an increase in productive capacity of the synthesis of extracellular and intracellular glutathione during 96 hours of culturing (7.6 and 3.7 times, respectively, as compared with the *H. polymorpha* wild-type strain DL-1 (WT) and 2.4 and 1.3 times, respectively, as compared with the strain with *GSH2* (mcGSH2) gene overexpression (Table 2).

Selection and optimization of conditions for glutathione supersynthesis by the strains obtained in the laboratory

For maximizing the production of glutathione it is necessary to find an optimal combination of factors influencing its synthesis while growing the strain-producer. The best of obtained recombinant strains with amplified expression of *H. polymorpha GSH2* and *MET4* (mcGSH2/MET4) genes was used to optimize the target product output. In particular, the influence of factors that are key determinants of glutathione synthesis: composition of the medium, biomass, aeration, *pH*, time of culturing has been studied. The maximum production of glutathione was reported for culturing in minimal YNB medium, whereas the yeast extract added had a negative effect, probably, because of inhibiting the activity of enzymes involved in the biosynthesis of glutathione (Table 3).

However, the cost of minimal YNB medium is quite high, which greatly increases the cost of glutathione as final product. Therefore, it was decided to study the synthesis of glutathione while growing the strain-producer on cheaper mineral medium. The level of glutathione synthesis when cultured in the mineral medium was by 10—15% lower as compared with the YNB medium (Table 3). Important factors that influence the production of glutathione are initial biomass of strain producer, concentration of sugar in the medium, and aeration. It was established that the level of glutathione synthesis was 2 times higher during the growth of culture with a lower initial biomass

Table 2

Production Capacity of Synthesis of Intracellular and Extracellular Glutathione by *H. polymorpha* Strains on Minimum YNB Medium with Glucose (2%) During 24 and 96 Hours of Culturing in Aerobic Conditions

Strain	Glutathione			
	Intracellular, nmole/mg of protein		Extracellular, nmole/l	
	24 hours	96 hours	24 hours	96 hours
WT	160 ± 1.7	110 ± 1.2	4 ± 0.05	60 ± 0.7
mcGSH2	250 ± 2.6	350 ± 3.7	5 ± 0.05	169 ± 1.8
mcGSH2/MET4	210 ± 2.2	836 ± 8.5	5 ± 0.05	221 ± 2.3

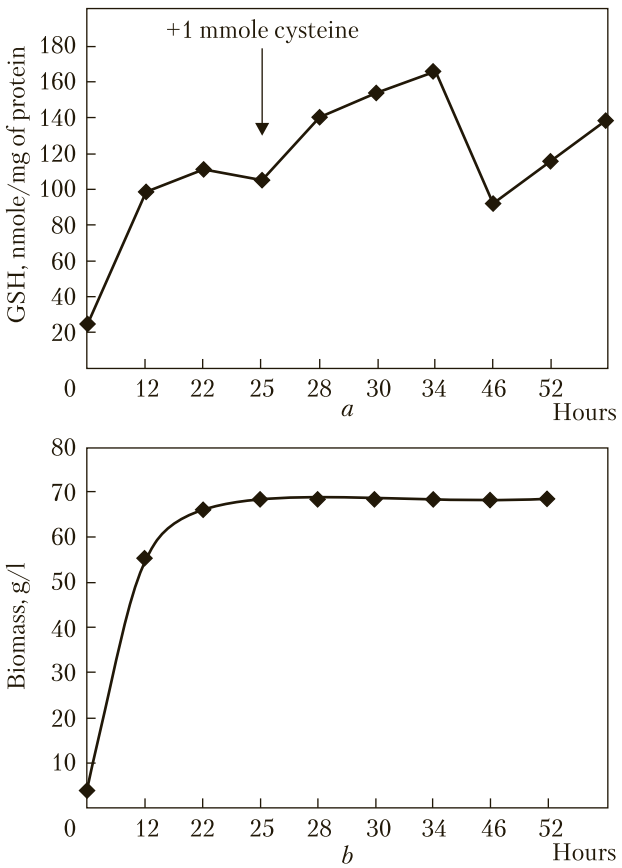


Fig. 2. Dynamics of intracellular synthesis of glutathione (*a*) and biomass (*b*) by *H. polymorpha* mcGSH2/MET4 strain during culturing in the bioreactor on mineral medium with cysteine

Table 3

Production Capacity of Synthesis of Intercellular Glutathione by Recombinant Strain *H. polymorpha* mcGSH2/MET4 During 24 and 48 Hours of Culturing in Various Media

Medium	Intracellular glutathione, nmole/mg of protein	
	24 hours	48 hours
YPD	147 ± 1.5	209 ± 2.4
YNB + 0,5 % YE	200 ± 2.3	226 ± 2.5
YNB	332 ± 3.5	389 ± 4.1
MC (2 % glucose)	323 ± 3.3	365 ± 3.8
MC (5 % glucose)	431 ± 4.5	437 ± 4.6

(A_{600} = 1), while the concentration of sugar in the medium had no significant effect on the production of glutathione (Table 3). The effect of aeration on glutathione production was studied under stirring at 200 rpm and 300 rpm, during the culturing in flasks containing the medium in proportion of 1/3 or 1/6. It has been showed that increasing aeration neither depresses glutathione synthesis nor stimulates any significant improvement.

One of the factors limiting glutathione synthesis is the presence of its predecessors, namely, sulfur-containing amino acid cysteine. The induction of glutathione synthesis after adding 1 mmol of cysteine to mineral medium containing 2% glucose has been studied. The initial optical density of cells inoculated into the medium was A_{600} = 1. It has been found that the addition of cysteine activates glutathione synthesis after 3 hours resulting in its increase 1.7 times (Table 4).

The optimum conditions for effective growth of strain-producer was used to develop interim guidelines for testing a technology of glutathione production and scaling of process in industrial environment.

Scaling of Glutathione Synthesis Process and Development of Procedure for Glutathione Production

The laboratory procedure for fermentation developed for the designed glutathione strain-producer has been adapted to industrial conditions at *Enzyme*

Table 4

Production Capacity of Synthesis of Intercellular Glutathione by Recombinant Strain *H. polymorpha* mcGSH2/MET4 in Mineral Medium Without and With Cysteine During 20 Hours of Culturing

Medium	Intercellular glutathione, nmole/mg of protein	
	20 hours	23 hours
MC (2 % glucose)	320 ± 3.3	329 ± 3.4
MC (2 % glucose) + + 1 mmole cysteine	326 ± 3.4	568 ± 5.9

JSC (Ladyzhyn, Vinnytsia Oblast). The capacity for growth and synthesis of glutathione was tested when growing in the bioreactor having a volume of 100 liters. The initial volume of mineral medium was 70 liters. The initial optical density of cells inoculated into the medium in the bioreactor was A_{600} = 1. The culturing was carried out on mineral environment under aeration (air to medium ratio is 1:1) and stirring at 200 rpm and *pH* = 5,0—5,5. While growing the culture, microscopic and microbiological control of purity was exercised; the other controlled factors were pH, temperature, sugar content in the medium, biomass, and target product. After 20 hours of culturing, cysteine at a concentration of 1 mmol/l medium was added. The fermentation lasted 58 hours. The culturing in bioreactor enabled achieving a higher output of producer biomass as compared with that in the flasks. The growth rate was also higher, with the stationary phase reached after 20 hours of incubation (Fig. 2, *b*).

The maximum glutathione production was reported after 28—34 hours of culturing and after 3—9 hours since 1mmol/l of cysteine was added. It reached 150 nmol/mg (or 1,350 mg/l) of protein (Fig. 2, *a*).

One of the best known producers of glutathione is G-14 recombinant strain of *S. cerevisiae* yeast able to synthesize about 1,620 g/l of glutathione after 52 hours of fermentation in the bioreactor having a volume of 5 liters. The optimization of biosynthesis by adding cysteine allows this strain to reach glutathione production at a rate of 2020 mg/l after 38 hours of culturing. [11] However, the volume was not expanded while culturing the strain, which complicates the estimation of glutathione production on industrial scale.

Glutathione overproducers have been designed on the basis of *H. polymorpha* yeast by co-expression of *GSH2* and *MET4* genes, with the first scaling process for the synthesis of glutathione for *H. polymorpha* yeast recombinant strains in the bioreactor having a volume of 100 liters done. The results indicate a high capacity of *H. polymorpha* yeast as glutathione producer in industrial scale, which has good prospects for further improvements using metabolic engineering methods.

CONCLUSIONS

Using metabolic engineering methods, glutathione overproducer strains of *H. polymorpha* methylotrophic yeast have been designed by amplifying expression of *GSH2* gene encoding γ-glutamylcysteine-synthetase and *MET4* gene encoding the transcriptional activator of biosynthesis genes of cysteine (precursor of glutathione synthesis).

The *H. polymorpha* recombinant strains with overexpression of *GSH2* and *MET4* genes have been showed to have a significantly improved productive capacity for glutathione synthesis as compared with the wild-type DL-1 *H. polymorpha* strain *in vitro*. Glutathione synthesis has been optimized *in vitro* using designed strains of *H. polymorpha*. In particular, the influence of key determinants of glutathione production output, i.e. composition of the medium, aeration, *pH*, and cultivation has been studied.

The maximum production of glutathione has been recorded on minimal medium, under aeration (200 rpm), after adding 1 mmol/l of cysteine.

The best glutathione producers have been tested in semi-industrial conditions, within the fermenter having a capacity of 100 liters. The maximum synthesis of glutathione has been reported after 28—34 hours of culturing and after 3—9 hours since the addition of 1 mmol/l of cysteine and amounts to 150 nmol/mg (or 1,350 mg/l) of protein.

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РОЗРОБКА ТЕХНОЛОГІЇ ОТРИМАННЯ ПРЕПАРАТІВ ГЛУТАТІОНУ НА ОСНОВІ СКОНСТРУЙОВАНИХ АКТИВНИХ СУПЕРПРОДУЦЕНТІВ ЦЬОГО ТРИПЕПТИДУ У ДРІЖДЖІВ

За допомогою метаболічної інженерії сконструйовано штам метилотрофних дріжджів *Hansenula polymorpha* з посиленою експресією генів *GSH2*, що кодує γ-глутамілцистеїнілсинтетазу, та *MET4*, що кодує транскрипційний активатор генів біосинтезу цистеїну (попередник синтезу глутатіону). Отриманий рекомбінантний штам характеризується підвищеною продуктивністю синтезу глутатіону порівняно із штамом дикого типу в лабораторних умовах. Було проведено оптимізацію синтезу глутатіону сконструйованого рекомбінантного штаму *H. polymorpha*. Розроблено напівпромислову модель технології отримання глутатіону з використанням сконструйованого дріжджового штаму-продуцента.

Ключові слова: глутатіон, дріжджі, *Hansenula polymorpha*, метаболічна інженерія.

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[2] Частное акционерное общество «Энзим», Ладыжин

РАЗРАБОТКА ТЕХНОЛОГИИ ПОЛУЧЕНИЯ ПРЕПАРАТОВ ГЛУТАТИОНА НА ОСНОВЕ СКОНСТРУИРОВАННЫХ АКТИВНЫХ СУПЕРПРОДУЦЕНТОВ ЭТОГО ТРИПЕПТИДА У ДРОЖЖЕЙ

С помощью метаболической инженерии сконструирован штамм метилотрофных дрожжей *Hansenula polymorpha* с усиленной экспрессией генов *GSH2*, кодирующего γ-глутамилцистеинсинтетазу, и *MET4*, кодирующего транскрипционный активатор генов биосинтеза цистеина (предшественник синтеза глутатиона). Полученный рекомбинантный штамм характеризовался повышенной продуктивностью синтеза глутатиона в сравнении со штаммом дикого типа в лабораторных условиях. Проведена оптимизация синтеза глутатиона сконструированного рекомбинантного штамма *H. polymorpha*. Разработана полупромышленная модель технологии получения глутатиона с использованием сконструированного дрожжевого штамма-продуцента.

Ключевые слова: глутатион, дрожжи, *Hansenula polymorpha*, метаболическая инженерия.

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