Ann Microbiol (2012) 62:1395–1402
DOI 10.1007/s13213-011-0389-0

ORIGINAL ARTICLE

Strain improvement for enhanced production of S-adenosyl-L-methionine in *Saccharomyces cerevisiae* based on ethionine-resistance and SAM synthetase activity

Xitao Cao · Minghua Yang · Yi Xia · Jie Dou · Kai Chen · Hui Wang · Tao Xi · Changlin Zhou

Received: 12 May 2011 / Accepted: 1 November 2011 / Published online: 23 December 2011

Abstract S-adenosyl-L-methionine (SAM) is an essential metabolite in all living cells, and which plays an important role in cellular functions such as methylation, sulfuration, and polyamine synthesis. The current study was carried out to obtain an industrial strain with overproduction of SAM. The wild-type strain, *Saccharomyces cerevisiae* CGMCC 1226, was subjected to successive mutagenic with ultraviolet irradiation (UV) coupled with ethionine-resistant screening procedure to achieve a rapid improvement of S-adenosyl-L-methionine production in *Saccharomyces cerevisiae*. A high SAM yield strain, designated as *Saccharomyces cerevisiae* CGMCC 2842, was successfully selected and exhibited higher SAM synthetase activity which was increased by 2.7-fold in comparison with the wild-type strain. Meanwhile, the production of SAM by *Saccharomyces cerevisiae* CGMCC 2842 in a 15-L fermentor reached 6.1 g/L after 36 h fed-batch fermentation and was increased by 4.3-fold. In addition, the ethionine-resistant genes of the mutant and wild-type strains were cloned, and analyses of nucleotide sequences suggested that the replacements of amino acid residues could be responsible for the ethionine-resistance.

Keywords S-adenosyl-L-methionine · *Saccharomyces cerevisiae* · Ethionine-resistance · SAM synthetase activity

Xitao Cao and Minghua Yang contributed equally to this work.

X. Cao · M. Yang · Y. Xia · J. Dou · K. Chen · H. Wang · T. Xi (✉) · C. Zhou (✉)
School of Life Science and Technology,
China Pharmaceutical University,
24 Tong Jia Xiang,
Nanjing 210009, People's Republic of China
e-mail: xi_tao18@sina.com
e-mail: cl_zhou@cpu.edu.cn

Introduction

S-adenosyl-L-methionine (SAM), an important metabolic intermediate, exists in all living organisms and exhibits pivotal roles in various biological reactions (Fontecave et al. 2004). In addition to serving as a major biological methyl donor, SAM is also a precursor to producing aliphatid polyamine, or glutathione (GSH), via trans-sulfuration (Cantoni 1952; Lieber and Packer 2002). It is also involved in some physiological regulation. For example, it has been successfully used in human therapy for liver disorders (Lu and Mato 2008), depression (Nguyen and Gregan 2002; Shippy et al. 2004), osteoarthritis (Bradley et al. 1994; Hosea Blewett 2008), fibromyalgia (Lieber and Packer 2002), and Alzheimer's disease (Linnebank et al. 2010; Newman 2000; Scarpa et al. 2003). Therefore, it is important to provide an efficient and low-cost method of producing SAM for industrial application.

SAM production by yeast fermentation, when compared with chemical coupling or enzymatic synthesis, is an efficient and practical approach through the extraction of yeast cells cultured in media supplemented with L-methionine (L-Met) (Schlenk et al. 1965; Shiomi et al. 1995). Mutation and screening is a practical and established method to get desirable microorganisms on an industrial scale. A range of studies have been carried out to increase intracellular SAM accumulation, including various methods of chemical and physical mutations or cultivation strategies, as well as over-expressing DNA shuffling of methionine adenosyltransferase in *Saccharomyces cerevisiae* or *Pichia pastoris* by genetic engineering (Hu et al. 2009; Shiomi et al. 1995; Zhou et al. 2008). However, these attempts are time-consuming and high cost processes, and the application of a recombinant strain has not yet been commercialized. In yeast, SAM is synthesized from methionine and ATP using

SAM synthetase (EC 2.5.1.6) as a catalyzer. This pathway was inhibited in the presence of methionine analogues, such as ethionine and selenomethionine. However, this inhibition can be counteracted through the presence of modified SAM synthetase in vivo (Barra et al. 1996). It has been reported that the SAM accumulation in a group of sake yeasts (*Saccharomyces cerevisiae*), which is generally recognized as safe (GRAS), is higher than that of other yeasts and some other microorganisms (Shiozaki et al. 1984). It is expected that the traditional mutation strategies will be in favor of a higher SAM production in *Saccharomyces cerevisiae* by using ethionine (a toxic analogue of methionine) as a selection pressure.

In the present study, we attempted to provide a simple and effective method to screen out high-yield mutants of *Saccharomyces cerevisiae* by ultraviolet (UV) mutagenesis and selection of the mutants with increased ethionine concentration. The SAM synthetase and Met synthetase activities associated with SAM accumulation in *Saccharomyces cerevisiae* were also investigated. The mechanisms of SAM accumulation and ethionine-resistance of the mutant strain were elucidated by cloning and sequencing of an ethionine-resistant gene. In addition, the SAM production of the mutant was investigated in a fed-batch fermentor.

Materials and methods

Strain

Saccharomyces cerevisiae CGMCC 1226 was used as the wild-type (WT) strain and obtained from the China General Microbiology Culture Collection Center (CGMCC, Beijing, China). The mutant strain, *Saccharomyces cerevisiae*, has been deposited at the China General Microbiology Culture Collection Center (Accession No: CGMCC 2842).

Medium

YEPD medium comprised 2.0% yeast extract, 1.0% peptone, and 2.0% glucose, and the YEPD plate medium contained 2.0% agar on the basis of the YEPD medium. The selective medium contained: 2.0% glucose, 0.3% L-isoleucine, 1.5% L-alanine, 0.2% adenine, 0.2% L-arginine, 0.3% L-lysine, 0.2% L-methionine, 0.5% L-phenylalanine, 2.0% L-threonine, 0.3% L-tyrosine, 0.4% L-histidine, 0.4% L-tryptophan, 0.2% L-leucine, and 0.4% uracil, and the following trace elements: 0.01% $CoCl_2{\cdot}6H_2O$, 0.01% $CuCl_2$, 0.01% H_3BO_3, 0.1% $Na_2MoO_4{\cdot}2H_2O$, 0.1% $ZnSO_4{\cdot}7H_2O$, 0.15% $FeSO_4{\cdot}7H_2O$, 0.18% $CaCl_2$, and 0.45% $MnSO_4{\cdot}H_2O$, pH 6.0; ethionine concentration increased from 0.4 mmol/L to 1.4mmol/L. SAM productions of the wild-type strain and the mutant strain were compared in the O-medium, which contained: 5.0% glucose, 1.0% peptone, 0.5% yeast extract, 0.4% KH_2PO_4, 0.2% K_2HPO_4, 0.05% $MgSO_4{\cdot}7H_2O$, and 0.15% methionine, pH 6.0. The fermentation medium contained: 4.0% glucose, 0.03% (v/v) H_3PO_4 (85% stock), 0.5% $(NH_4)_2SO_4$, 0.15% KCl, 0.1% $MgSO_4{\cdot}7H_2O$, 0.3% yeast extract, 0.002% $ZnSO_4$, 0.03% (v/v) maize slurry, 0.01% $CaCl_2$, 0.1% NaCl, and 0.3% Met.

Mutagenesis and mutants selection

Saccharomyces cerevisiae CGMCC 1226 grown in YPD medium at 30°C about 16 h (late-exponential-phase), was harvested by centrifugation (4,000 *g*, 10 min) and washed twice with sterilized distilled water. The washed cells were diluted with sterilized distilled water to a density of 10^5 cells/mL. The diluted cells (0.5 mL) was poured into a sterilized plate together with a magnetic stirrer, and kept at 15 cm away from the UV light (15 W) and exposed to UV irradiation for 70–90 s. The treated and untreated strains were diluted with sterile water and 0.2 mL suspension was spread onto the selection plates in the dark, and then incubated at 30°C for 48 h. The colonies, which appeared in the selection plate, were transferred into 250-mL flasks with 50 mL of YPD medium and incubated at 30°C, 250 rpm for 16 h. After incubation, a 2.5-mL portion of the YPD culture was added to a 250-mL flask containing 50 mL of O-medium and cultured on a rotary shaker at 250 rpm and 30°C for 36 h. SAM production of each colony was analyzed by HPLC described below. The mutant with the highest SAM production was selected as the starting strain for the next round of UV irradiation. The mutation procedure was repeated for seven rounds, and the concentration of ethionine increased from 0.4 mmol/L to 1.4 mmol/L.

The effect of ethionine on cells growth and SAM synthetase/Met synthetase activitities of the wild type and mutant stains

To determine the effect of ethionine on cell growth and SAM synthetase/Met synthetase activity, *Saccharomyces cerevisiae* strains were cultivated in 250-mL flasks containing 50 mL YPD medium. Strains were pre-cultured aerobically in YPD medium at 30°C for 16 h. The initial inoculums (10^5 CFU/mL) were inoculated in 250-mL flasks containing 50 mL YPD medium with different concentrations of ethionine. Samples were taken at indicated time points, and the cell growth, SAM synthetase, and Met synthetase were analyzed. All experiments by flask cultivation were carried out with three trails. The error bar was obtained using the standard deviation function (Office 2003, Microsoft) and shows the deviation along a curve.

PCR amplification, molecular cloning and sequence analysis of ethionine-resistant gene

DNA from *Saccharomyces cerevisiae* CGMCC 1226 and its mutant derivative was extracted as described by Cheng and Jiang (2006) and used as a template for PCR (Polymerase Chain Reaction) amplification. The ethionine-resistant gene was amplified using primers Eth-F (5'CCGGAATT CATGTCTAAACAATTTAGTCATACCACC3') and Eth-R (5'CCAAAGCTTTTATGATGTATGGGTCTCAGCATTGC3'). PCR amplification program was 95°C for 5 min, followed by 30 cycles of 94°C for 1 min, 57.5°C for 90 s, 72°C for 90 s and 72°C for 10 min. The amplified PCR product 1.8 kb was purified according to the TIANgel Midi Purification Kit (TIANGEN, Beijing, China) and ligated with the pMD18-T vector (Takara, Dalian, China). In order to detect the strain sequences, several clones were randomly selected for DNA sequencing. Similar gene sequences were conducted in the NCBI database by BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The deduced amino acids sequences were aligned using the Clustal W tools (http://www.ebi.ac.uk/tools/clustalw2/).

Fed-batch Fermentation of mutant strain

Fed-batch fermentation was conducted in a Biostat C-10 fermentor (B. Braun Biotech, Germany) with 10 L of fermentation medium. Inoculum (500 mL) was transferred from a 24-h pre-culture flask to the reactor. The fermentation parameters were pH of 5.0±0.1 (adjusted with ammonium hydroxide), 30°C, 400 rpm, and dissolved oxygen (DO) level was maintained at 25% saturation by air feeding. The fed-batch process was as follows: 320 g/L glucose was fed into the fermentor at 5 mL/min for 30 min when the cell density reached 6.6 g/L DCW. In the second phase, the feed rate was adjusted to 10 mL/min for 30 min after the cell density reached 11.1 g/L DCW (about 27 h). Samples were taken at different times for SAM production and biomass determination.

Analytical methods

OD and dry weight determinations

The OD value of sample was detected at 600 nm in duplicate using a Jinghua UV/Vis spectrophotometer (Shanghai, China). Dry cell weight (DCW) was determined using 0.45-μm size nitrocellulose filters according to the method described by Dynesen et al. (1998).

Analysis of SAM and Met yield

The yield of SAM and Met were determined by mixing 1.0 mL of fermentation broth with 2.0 mL of 1.5 mol/L perchloric acid and storing at 40°C for 0.5 h. The supernatant was quantified after centrifugation of the mixture (8,000 *g*, 10 min). The SAM was analyzed by HPLC (He et al. 2006). For the Met analysis, the mobile phase was 10% (v/v) methanol and monitored at 210 nm, while the other conditions were the same as the SAM assay. Peak area analysis was performed based on the standard calibration curves of SAM and Met (Sigma, USA).

SAM synthetase and Met synthetase activities

The SAM synthetase and Met synthetase activities were determined according to the established procedure (Shiozaki et al. 1984). One unit of enzyme activity was defined as the formation of 1 μmol of SAM in 1 min in the standard assay mixture. The protein concentration was measured by the Lowry method.

Results

Screening the mutants of *Saccharomyces cerevisiae* to enhance SAM production

Seven rounds of UV irradiation were carried out according to the selection procedure, and the results showed that the SAM accumulation increased gradually (Fig. 1). The SAM production of selected strains increased significantly from 1.7 g/L to 4.2 g/L by gradually increasing the ethionine concentration from 0.4 mmol/L to 1.4 mmol/L, and an increase of 3-fold compared to the wild-type strain CGMCC 1226 (SAM 1.4 g/L). However, the positive mutant rate decreased as the mutation cycles increased. A 13.2% positive mutation rate was observed in the 1st cycle, but dropped to 0.2% in the 7th cycle, which is a 65-fold decrease.

Hereditary stability of *Saccharomyces cerevisiae* CGMCC 2842

The stability of SAM production of *Saccharomyces cerevisiae* CGMCC 2842 was evaluated through transfer generation. The result showed that the production of SAM remained almost constant (about 4.0 g/L) after 50 generations, suggesting that the high accumulation mutant strain was stable.

The effect of ethionine on cell growth of the wild-type and mutant stains

To evaluate the effect of ethionine-resistant properties of the wild-type and mutant strains, the cell growth of wild-type and mutant type strains without or with ethionine in a 250-mL flask containing 50 mL YPD were investigated. The

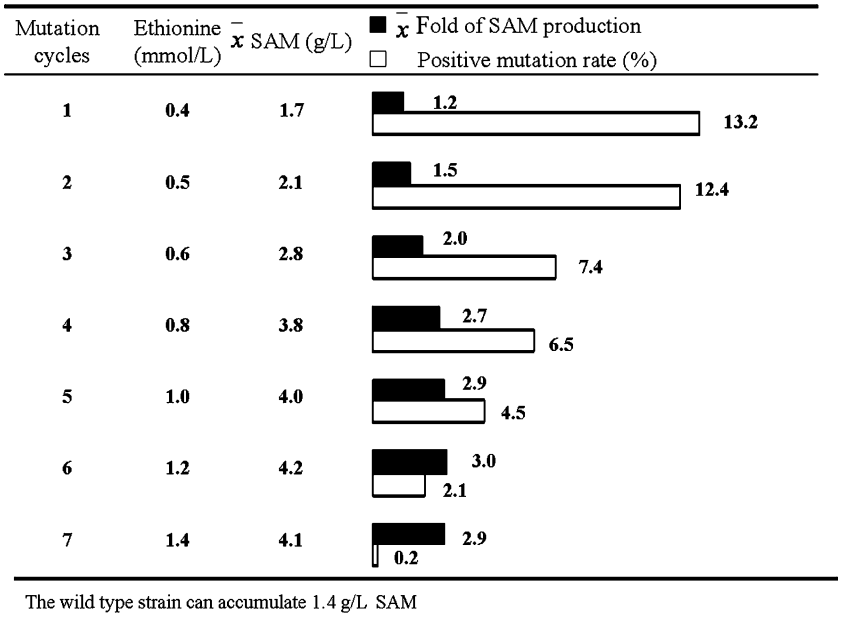

Fig. 1 The results of UV-mutagenesis combined with ethionine-resistance screening

results showed that the wild-type and mutant strains have similar growth rates when growing in a medium without ethionine (Fig. 2). At 1.5 mmol/L of ethionine, cell growth of the wild-type was significantly inhibited while the mutant strain was just partly inhibited in the medium containing 1.5 mmol/L of ethionine. These results indicated that the mutant strain had greater ethionine-tolerance than that of wild-type strain in cell growth.

The effect of ethionine on SAM synthetase/Met synthetase activity of the wild-type and mutant strains

Since ethionine is an analog of methionine, the phenotype of higher SAM accumulation might connect with the key enzymes in the SAM biosynthesis pathway. To further identify the relationship between ethionine-resistance and cellular SAM levels in detail, SAM synthetase and Met synthetase activities in a medium with or without different concentrations of ethionine were determined. Figure 3a shows that the mutant strain had 2.7-fold of SAM synthetase activity (4.9 U/mg) compared with the wild-type strain (1.8 U/mg). When ethionine was added to the medium (Fig. 3b), the SAM synthetase activity of the wild-type strain was inhibited at 0.5 mmol/L ethionine and was completely inhibited at 2.5 mmol/L. However, in contrast with the wild-type strain, the inhibitory effect of mutant strain occurred at 2 mmol/L and was completely inhibited at 4.0 mmol/L ethionine. Compared with the wild-type, the higher SAM synthetase of the mutant was proved to increase the tolerance of ethionine of yeast.

This case was not observed for the Met synthetase in either the mutant or wild-type strains (Fig. 3c). At 9 h, the activities of the two strains were 4.3 U/mg and 3.8 U/mg, respectively. During the next few hours, the activities of the both strains declined at the same rate. When ethionine was added to the medium (Fig. 3d), the enzyme activities of the wild-type and mutant strains were inhibited at 4.5 mmol/L, and their activities declined at the same rate. This experiment suggested that ethionine had no effect on Met synthetase activity.

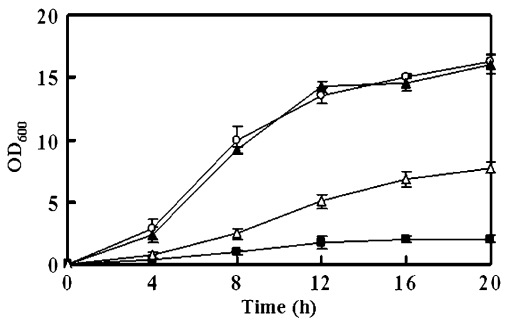

Fig. 2 The cell growth of wild-type (WT) and mutant type (MT) strains without or with ethionine in 50 mL YPD medium at 30°C and shaken in 250-mL flasks. (○ WT strain without ethionine; ▲ MT strain without ethionine; ■ WT strain with 1.5 mmol/L ethionine; △ MT strain with 1.5 mmol/L ethionine)

DNA sequence comparisons

PCR was used to amplify the ethionine-resistant gene in the wild-type and the mutant strains. Using genomics DNA as template, the genes were cloned and sequenced. The sequencing showed that the ethionine-resistant gene (1.8 kb) belonged to *YHR032W* (GenBank access number NM_001179162.1) after BLAST in the NCBI database. The nucleotide of both

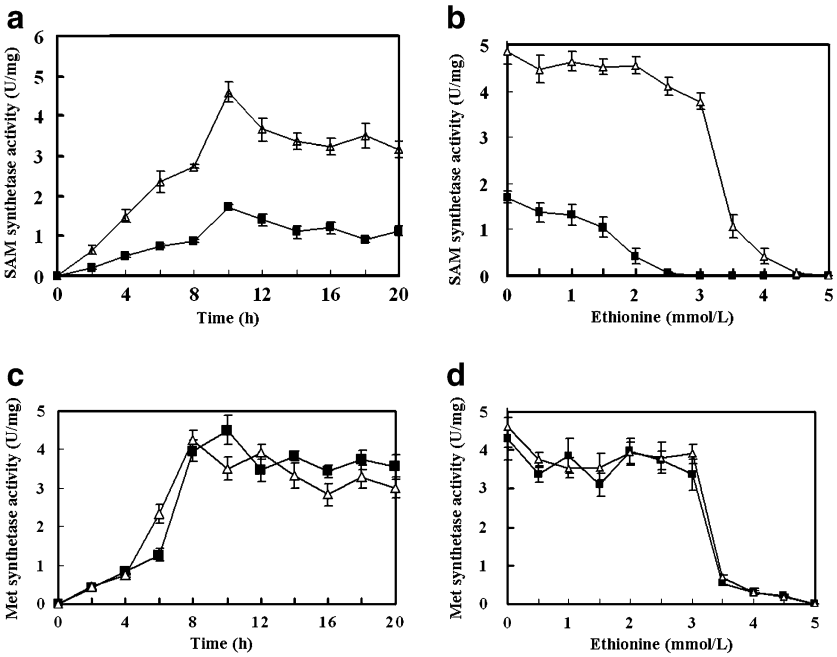

Fig. 3 The effect of ethionine on SAM synthetase and Met synthetase activity. SAM synthetase activity and Met synthetase activity were detected after different concentration of ethionine was added into reaction mixture. **a** SAM synthetase activity. **b** The effect of ethionine on SAM synthetase activity. **c** Met synthetase activity. **d** The effect of ethionine on Met synthetase activity. (△ mutant strain; ■ wild-type strain)

strains have been submitted to the NCBI database (GenBank access number: wild-type strain HQ877922, mutant strain HQ877923). The amino acid sequences of the ethionine-resistant gene were aligned showing (Fig. 4) substitution of four amino acids A194S, A211V, G227D and D448N. These mutation points by UV treatment probably played an important role in SAM accumulation, ethionine-resistance and higher activity of SAM synthetase.

Fed-batch Fermentation of mutant strain

The performance of the mutant and wild-type strains was measured with a glucose feed in a scale-up culture of 10 L. As shown in Fig. 5, there was a slight difference in the growth of the mutant and wild-type strains during all courses of the cultivation experiments. On the other hand, SAM production rate of the mutant strain was obviously higher than that of the wild-type strain. The SAM yield of the mutant strain reached 6.1 g/L after 36 h of cultivation, which was 4.3-fold higher than the wild-type strain (SAM 1.4 g/L).

During the fed-batch fermentation, it was observed that SAM accumulation of the mutant strain increased to 5.5 g/L of fermentation broth and DCW reached 8.0 g/L 1 h after feeding 40 g/L of glucose (SAM at 0.69 g/g DCW). In the next 4 h, cell density and SAM accumulation were maintained at the same level. The DCW reached 14.8 g/L and SAM production was the highest (6.1 g/L) 1 h after the other 80 g/L of glucose was fed (SAM at 0.41 g/g DCW). The result indicated that the mutant strain can produce more SAM than the wild-type strain.

Discussion

UV irradiation, EMS, ^{60}Co-γ ray and LiCl have been used to generate new phenotypes successfully for many years (Huang et al. 2010; Sridhar et al. 2002). Ethionine is an analog of methionine in protein synthesis (Maw 1966) acted as a competitive analog of methionine in protein synthesis (Maw 1966) and repressed the formation of sulfur-containing amino acids (Schlenk and Zydek 1967). Shiomi et al. (1995) reported an attempt to insert an ethionine-resistant gene into chromosomes of yeast to enhance SAM production by a yeast transposon Ty element, and the production of SAM was 0.50 g/g DCW. Li et al. (2007) isolated *Candida* sp. mutant by UV irradiation or NTG treatment using ethionine- and nystatin-resistance, which can only accumulate SAM at 0.1121 g/g DCW. The production of SAM by these strains was lower than that of the mutant of *Saccharomyces cerevisiae* CGMCC 2842 (SAM at 0.68 g/g DCW). A recombinant strain ES1 was also developed through over-expression of both ethionine-resistance-conferring gene and *SAM2* in *Saccharomyces cerevisiae sake* kyokai No. 6, and can accumulate SAM of about half of its dry cell weight in the intracellular region (Lee et al. 2010).

The mutant strain possessing higher SAM synthetase activity was slightly different from the report described by Barra et al. (1996) and Li et al. (2007), who found that the SAM synthetase did not significantly affect the amount of the ethionine-resistant mutants compared with the wild-type, which may be because of the different genetic background of the strains. In *Saccharomyces cerevisiae*, there are two SAM synthase genes, *SAM1* and *SAM2*, which are

Fig. 4 Alignment of the deduced ethionine-resistant amino acid sequences of mutant and wild-type strains. Sequences were aligned using the ClustalW tools http://www.ebi.ac.uk/Tools/clustralw2/. The replacements of amino acids are emphasized in *bold* with *gray shading*. (*MT* mutant type strain; *WT* wild-type strain)

regulated by L-Met in different ways (Thomas and Surdin-kejian 1991). The gene *SAM1* is repressed by excess L-Met, while *SAM2* is not. Considering these data, we cloned the *SAM1* and *SAM2* genes of the wild-type and mutant strains, but no point mutants were observed (data not shown). Malkowski et al. (2007) reported a mutant strain of *Saccharomyces cerevisiae* with deletion of both *SAM* genes exhibited significantly enhanced resistance to selenomethionine and inferred that selenomethionine-resistance of the *sam1*△*sam2*△ double mutant is primarily due to blocking conversion of selemethionine to a toxic compound (Se-SAM), but not due to methionine consumed in the SAM synthesis. It can trigger an increase in the intracellular methionine concentration of the mutant strain. The result is very similar to our finding that no change of Met synthetase activity was observed because selemethionine is also an analog of methionine. It was not known whether there was a DNA fragment responsible for selenomethionine-resistance or if it belonged to the same gene responsible for ethionine-resistance.

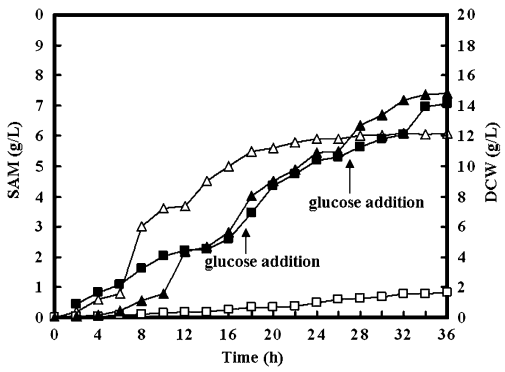

Fig. 5 Time courses of SAM fed-batch fermentation in a 15-L fermentor with wild-type (WT) or mutant (MT) strain in fermentation medium. (△ the SAM production of MT strain; □ the SAM production of WT strain; ▲ the DCW of MT strain; ■ the DCW of WT strain). *Arrows* indicate the time of glucose addition

Shiomi et al. (1991, 1995) cloned a DNA fragment that responsible for the ethionine-resistance in *Saccharomyces cerevisiae*, which was different from those of yeast *Saccharomyces cerevisiae SAM1* and *SAM2*, and the accumulation of SAM was enhanced. Jeong-Nam et al. (2004) constructed a plasmid that contained ethionine-resistance-conferring gene expression cassette and transformed it to industrial *Saccharomyces cerevisiae*, which exhibited strong resistance to DL-ethionine compared with nontranformants. However, the mechanisms of SAM accumulation enhanced by an ethionine-resistant mutant have not been offered. Multiple amino acids of an ethionine-resistant gene between the mutant and the wild-type strain were aligned, and few amino acid substitutions were observed in the sequences, suggesting that the mutant strain with higher SAM synthetase activity could survive high concentrations of ethionine, which might be due to point mutants of *YHR032W*. The product of *YHR032W* was a putative substrate (protein) of the cAMP-dependent protein kinase (PKA) (Budovskaya et al. 2005). Meanwhile, the PKA controls the transcription factor Opi, which is a negative regulator of expression of phospholipid-synthesizing, and also regulates the *SAM2* transcription via inositol and choline (IC) binding to a

conserved *cis*-acting upstream activating sequence, designated the IC-responsive element, also known as UAS_{INO}, in the upstream of the *SAM2* coding region (Santiago and Mamoun 2003; Sreenivas and Carman 2003). We propose that the phenotype of the mutant strain, the ethionine-resistant phenotype, SAM levels, and SAM activities, may be due to the point mutants of the ethionine-resistant gene.

Although the isolation of a mutant strain using ethionine-resistance with higher SAM synthetase activity can improve SAM production, it is conceivable that the strain has only a limited industrial application. Therefore, further work will be needed to develop a prospective industrial strain by using of the knowledge of metabolic regulation or genetic modifications to manipulate SAM metabolism in *Saccharomyces cerevisiae*.

Acknowledgements This study was sponsored by Natural Science Foundation of Jiangsu Province (SBK200921231), the Industrialization of Scientific Research Promotion Projects of Colleges and Universities in Jiangsu Province (JH10-12), the Qing Lan Project (2008) from Jiangsu Province and the "111 Project" from the Ministry of Education of China and the State Administration of Foreign Expert Affairs of China (No. 111-2-07).

References

Barra JL, Mautino MR, Rosa AL (1996) A dominant negative of effect of eth-1r, a mutant allele of the *Neurospora crassa* S-adenosylmethionine synthetase-encoding gene conferring resistance to the methionine toxic analogue ehtionine. Genetics 144:1455–1462

Bradley JD, Flusser D, Katz BP, Schumacher HR Jr, Brandt KD, Chambers MA, Zonay LJ (1994) A randomized, double blind, placebo controlled trial of intravenous loading with S-adenosylmethionine (SAM) followed by oral SAM therapy in patients with knee osteoarthritis. J Rheumatol 21:905–911

Budovskaya YV, Stephan JS, Deminoff SJ, Herman PK (2005) An evolutionary proteomics approach identifies substrates of the cAMP-dependent protein kinase. Proc Natl Acad Sci USA 102:13933–13938

Cantoni GL (1952) The nature of the active methyl donor formed enzymatically from L-methionine and adenosinetriphosphate. J Am Chem Soc 74:2942–2943

Cheng HR, Jiang N (2006) Extremely rapid extraction of DNA from bacteria and yeasts. Biotechnol Lett 28:55–59

Dynesen J, Smits HP, Olsson L, Nielsen J (1998) Carbon catabolite repression of invertase during batch cultivations of *Saccharomyces cerevisiae*: the role of glucose, fructose, and mannose. Appl Microbiol Biotechnol 50:579–582

Fontecave M, Atta M, Mulliez E (2004) S-adenosyl-L-methionine: nothing goes to waste. Trends Biochem Sci 29:243–249

He J, Deng J, Zheng Y, Gu J (2006) A synergistic effect on the production of S-adenosyl-l-methionine in *Pichia pastoris* by knocking in of S-adenosyl-l-methionine synthase and knocking out of cystathionine-synthase. J Biotechnol 126:519–527

Hosea Blewett HJ (2008) Exploring the mechanisms behind. S-adenosylmethionine (SAMe) in the treatment of osteoarthritis. Crit Rev Food Sci Nutr 48:458–463

Hu H, Qian JC, Chu J, Wang Y, Zhuang Y, Zhang S (2009) DNA shuffling of methionine adenosyltransferase gene leads to improved S-adenosyl-L-methionine production in *Pichia pastoris*. J Biotechnol 141:97–103

Huang L, Wei PL, Zang R, Xu ZN, Cen PL (2010) High-throughput screening of high-yield colonies of Rhizopus oryzae for enhanced production of fumaric acid. Ann Microbiol 60:287–292

Jeong-Nam P, Lee KH, Ko KH, Seo KH, Chin JE, Lee HB, Bai S (2004) Expression of ethionine resistance conferring gene in an industrial strain of *Saccharomyces cerevisiae*. Korean J Microbiol Biotech 32:356–361

Lee SW, Park BS, Choi ES, Oh MK (2010) Overexpression of ethionine resistance gene for maximized production of S-adenosylmethionine in *Saccharomyces cerevisiae sake* kyokai No. 6. Korean J Chem Eng 27:587–589

Li W, Ye S, Luo KJ, Ge FL, Du LJ, Wu K, Ding CW (2007) Isolation and characterisation of Candida sp. mutants enriched in S-adenosylmethionine (SAM). Ann Microbiol 57:383–387

Lieber CS, Packer L (2002) S-Adenosy-l-methionine: molecular, biological, and clinical aspects-an introduction. Am J Clin Nutr 76 (Suppl):1148S–1150S

Linnebank M, Popp J, Smulders Y, Smith D, Semmler A, Farkas M, Kulic L, Cvetanovska G, Blom H, Stoffel-Wagner B, Kölsch H, Weller M, Jessen F (2010) S-Adenosylmethionine Is Decreased in the Cerebrospinal Fluid of Patients with Alzheimer's Disease. Neurodegenerative Dis 7:373–378

Lu SC, Mato JM (2008) S-Adenosy-l-methionine in cell growth, apoptosis and liver cancer. J Gastroenterol Hepatol 23 (Suppl):73S–77S

Malkowski MG, Quartley E, Friedman AE, Babulski J, Kon Y, Wolfley J, Said M, Luft JR, Phizicky EM, DeTitta GT, Grayhack EJ (2007) Blocking S-adenosylmethionine synthesis in yeast allows selenomethionine incorporation and multiavelength anomalous dispersion phasing. Proc Natl Acad Sci USA 104:6678–6683

Maw GA (1966) Incorporation and distribution of ethionine-sulfur in the protein of ethionine-sensitive and ethionine-resistant yeasts. Arch Biochem Biophys 115:291–301

Newman PE (2000) Alzheimer's disease revisited. Med Hypotheses 54:774–776

Nguyen M, Gregan A (2002) S-adenosyl-l-methionine and depression. Aust Fam Physician 31:339–343

Santiago TC, Mamoun CB (2003) Genome expression analysis in yeast reveals novel transcriptional regulation by Inositol and Choline and New Regulatory Functions for Opi1p, Ino2p, and Ino4p. J Biol Chem 278:38723–38730

Scarpa S, Fuso A, D'Anselmi F, Cavallaro RA (2003) Presenilin 1 gene silencing by S-adenosylmethionine: a treatment for Alzheimer disease? FEBS Lett 541:145–148

Schlenk F, Zydek CR (1967) The radiopurity of S-adenosylmethionine and S-adenosylethionine preparations. J Labelled Compounds 3:137–143

Schlenk F, Zydek CR, Ehninger DJ, Dainko JL (1965) The production of S-adenosyl-L-methionine and S-adenosyl-L-ethionine by yeast. Enzymologia 29:283–298

Shiomi N, Fukuda H, Fukuda Y, Murata K, Kimura A (1991) Nucleotide sequence and characterization of a gene conferring resisitance to ethionine in yeast *Saccharomyces cerevisiae*. J Ferment Bioeng 4:211–215

Shiomi N, Fukuda H, Murata K, Kimura A (1995) Improvement of S-adenosylmethionine production by integration of the ethionine-resistance gene into chromosomes of the yeast *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 42:730–733

Shiozaki S, Shimizu S, Yamada H (1984) Unusual intracellular accumulation of S-adenosyl-L-methionine by microorganisms. Agric Biol Chem 48:2293–2300

Shippy RA, Mendez D, Jones K, Cergnul I, Karpiak SE (2004) S-adenosy-l-methioine (SAM-e) for the treatment of depression in people living with HIV/AIDS. BMC Psychiatry 4:38

Sreenivas A, Carman GM (2003) Phosphorylation of the yeast phospholipid synthesis regulatory protein Opi1p by protein kinase A. J Biol Chem 278:20673–20680

Sridhar M, KiranSree N, Venkateswa RL (2002) Effect of UV radiation on thermotolerance, ethanol tolerance and osmotolerance of *Saccharomyces cerevisiae* VS1 and VS3 strains. Bioresour Technol 83:199–202

Thomas D, Surdin-kejian Y (1991) The synthesis of the two S-adenosyl-L-methionine synthetase is differently regulated in *Sacchromyces cerevisiae*. Mol Gen Genet 226:224–232

Zhou J, Chu J, Wang YH, Zhang SL, Zhuang YP, Yuan ZY (2008) Purification and properties of *Saccharomyces cerevisiae* S-adenosylmethionine synthetase expressed in recombinant *Pichia pastoris*. World J Microbiol Biotechnol 24:789–796