ORIGINAL SCIENTIFIC PAPER

S-adenosyl-L-methionine production by an ergosterol-deficient mutant of *Scheffersomyces stipitis*

Stela Križanović, Jasna Mrvčić, Marina Stanić, Maja Cepanec, Damir Stanzer*

Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

Summary

S-adenosyl-L-methionine (SAM, SAMe; AdoMet) is an important methyl donor in many reactions, and recently has attracted much attention due to its expanding application in pharmacy. Efficient ways of its production have been widely researched. In this study it was demonstrated that erg6 mutant of yeast Scheffersomyces stipitis (former Pichia stipitis), obtained by UV mutagenesis and selected by resistance to nystatin, could be used for production of SAM. The mutant strain showed higher ability to accumulate SAM than its parental strain. The experiments demonstrated different dependence of erg6 mutant strain on several cultivation conditions in comparison to parental strain, its higher sensibility to oxidative stress, as well as strong dependence of SAM accumulation on the cell growth phase and supplementation of growth media with methionine..

Key words: *S-adenosyl-L-methionine (SAM, SAMe, AdoMet), Scheffersomyces stipitis (former Pichia stipitis), nystatin*

Sažetak

S-adenozil-L-metionin (SAM, SAMe, AdoMet) važan je donor metilne skupine u mnogim reakcijama. U posljednje vrijeme privlači puno pažnje zbog širenja njegove primjene u farmaceutskoj industriji, te se intenzivno istražuju učinkoviti načini njegove proizvodnje. U ovom istraživanju pokazano je da se erg6 mutanti kvasca Scheffersomyces stipitis (prije: Pichia stipitis) dobiveni UV mutagenezom i selekcijom s pomoću rezistencije na nistatin mogu koristiti za proizvodnju SAM. Mutant je pokazao veću sposobnost nakupljanja SAM od divljeg tipa. Pokusi su pokazali različitu ovisnost erg6 mutanta o nekoliko uvjeta kultivacije u odnosu na divlji tip, njegovu veću osjetljivost na oksidativni stres, kao i jaku ovisnost akumulacije SAM o fazi staničnog rasta i obogaćivanju hranjive podloge metioninom.

Ključne riječi: *S-adenozil-L-metionin (SAM, SAMe, AdoMet), Scheffersomyces stipitis (prije: Pichia stipitis), nistatin*

1. Introduction

S-adenosyl-L-methionine (SAM, SAMe, AdoMet) is an important metabolic intermediate that participates in many essential biochemical reactions in all organisms. Except being involved in trans-sulphuration and synthesis of polyamines, SAM is the major donor of methyl group in transmethylation of lipids, nucleic acids, proteins and polysaccharides (Tabor and Tabor, 1984; Chawla et al., 1990; Bottiglieri, 2002). There is a great interest for clinical use of SAM. It is widely researched and used as a therapeutic means to treat various diseases such as depression (Mischoulon and Fava, 2002; Criconia et al., 1994), liver diseases (Mato et al., 2002; Martinez-Chantar et al., 2002), Alzheimer's disease (Newman, 2000), osteoathritis (Di Padova, 1987), Parkinson's disease (Carieri et al, 1990) etc. Diverse functions of SAM hold promise for treatment of many other diseases (Chiang et al., 1996). Commercially, SAM is prepared by isolation from yeast cells grown in media rich in L-methionine (Shiomi et al., 1995). Yeasts *Saccharomyces cerevisiae* (Shiomi et al., 1990, Zhang et al., 2005, Wang et al., 2009, Yin et Wang, 2011), *Saccharomyces sake* (Shiozaki et al., 1989; Shobayashi et al., 2006; Choi et al., 2009) and *Candida sp*. (Holcomb et Shapiro, 1975; Li et al., 2007) are the most researched yeasts for production of SAM. Besides these yeasts, there is a possibility of using spent brewer's yeast (*Saccharomyces uvarum*) for production of SAM in medium with addition of L-methionineL-methionine (Liu et al., 2004). The production of SAM by *Kluyveromyces lactis* and *Kluyveromyces marxianus* in whey with addition of L-methionine has also been investigated (Mincheva et al., 2002). In the last few years, an application of recombinant *P. pastoris* in production of SAM has been studied (Zhang et al., 2008; Hu et al., 2009; Yu et al., 2012). However, currently employed ways of production of SAM are rather expensive and new production methods are still being researched.

Among the other pathways, SAM participates in the synthesis of ergosterol. In *S. cerevisiae*, SAM is a donor of the methyl group in transmethylation of zymosterol to fecosterol by methyltransferase enzyme, encoded by *ERG6* (Daum et al., 1998). Nystatin, as antifungal agent that interacts with ergosterol in cell membrane and increases its permeability in this way, could be used for selection of SAM accumulating *S. cerevisiae* and *Candida sp.* mutant strains (Shobayashi et al., 2006; Li et al., 2007). The nystatin-resistant *S. cerevisiae erg6* mutants accumulate more SAM, because SAM is not consumed in this part of ergosterol biosynthesis pathway. However, there was no report on further development of SAM production by these mutant strains.

In this study, nystatin-resistant *Scheffersomyces stipitis* (former *Pichia stipitis)* mutant strain M12 was used for production of SAM. The dependence of the SAM accumulation on production parameters, as well as operational potential of this ergosterol-deficient mutant were investigated.

*Corresponding author: dstanzer@pbf.hr

2. MATERIALS AND METHODS

2.1. Strains and cultivation media

S. stipitis CBS 5776 wild type was obtained from DSMZ (German Collection of Microorganisms and Cell Cultures). The strain M12 was derived by UV mutagenesis of the *S. stipitis* wild type, and it was isolated and selected as nystatin-resistant mutant.

The procedure of mutagenesis will be described in the following text.

S. stipitis wild type cells were cultivated overnight at 30°C in 500 mL Erlenmayer flasks containing 50 mL of YPD medium (1% yeast extract, 2% peptone, 2% glucose) with shaking at 130 rpm (Braun Certomat-IS). One mililitre from overnight yeast suspension was placed in 9 ml of sterile water and homogenized. The suspension was put into a plastic Petri plate and irradiated by ultraviolet light (UV) for 120 seconds. After UV irradiation, cells were spread on solid YPD medium at density of 10^6 cells/mL, and incubated for 48 h at 30˚C. The grown colonies were transferred using replica techniques to a solid YPD medium containing 15 µg/mL of nystatin. The plates were incubated at 30˚C for 10 days in darkness. Colonies grown on the selection medium were isolated as nystatin-resistant mutants. Mutant M12 was derived by UV irradiation of 160J/m^2 and isolated from YPD medium with 15 µg/mL of nystatin.

The wild type was maintained by monthly transfer on YPD agar and stored at 4˚C. Mutant strain was maintained by monthly transfer on YPD agar with 10 µg/mL nystatin and stored at 4˚C.

YPD medium was used for yeast cultivation. Solid YPD medium containing 2,5% agar and 15 µg/ml nystatin (Fisher Scientific, China) was used as a selection medium for the nystatin–resistant mutants. In order to compare SAM production of the wild type and mutant strain, O-medium (5% D-glucose, 1% peptone, 0.5% yeast extract, 0.4% KH_2PO_4, 0.2% K_2HPO_4, 0.05% $MgSO_4 \cdot 7\ H_2O$) with addition of L-, D-, and D,L-methionine was used (Shiozaki et al., 1984).

2.2. Fermentation studies and analytical procedures

For the production of SAM, strains were cultivated in 500 mL Erlenmayer flasks containing 100 mL of O-medium. Cultivations were conducted without and with various forms (L-, D-, and D,L-) and concentrations (1.5; 3.0; 4.5; 6.0 and 7.5 g/L) of methionine, for 48 h at 30˚C with shaking at 130 rpm. Variations of other parameters (e.g temperature, shaking, cultivation time) were introduced for some cultivations. All experiments were repeated three times. SAM was quantified by HPLC method of Valko et al., (1993). The cells were collected and washed twice with sterilised distilled water. Cell lysis was done by the addition of 0.25 mL of ethyl acetate and 1 mL of 1M H_2SO_4 in 2 ml of yeast suspension, shaken on a vortex mixer for 30 minutes. Distilled water was added to lysed samples to a total of 10 mL, mixed in a vortex mixer for 4 min and centrifuged at 4000 × g for 10 min. The amount of SAM in the extracted supernatant was analysed using ChromSep HPLC Column SS (250 x 4.6 mm); guard column IonoSpher 5C with Varian Prostar 230 pumping system and using UV lamp at 260 nm. The column eluate (flow rate 1 mL/min) was composed of ammonium formate buffer (0.25 mol/L, pH = 4.0 ± 0,1) / methanol (90:10; v:v). The quantity of SAM was calculated from the peak area based on a standard calibration curve. SAM standard was purchased from Sigma Aldrich.

The increase in growth of the strains was measured and expressed in gramm of absolutely dry cell weight per liter. Dry cell weight was measured by centrifuging a 5 mL sample, then re-suspending the pellet in 5 mL of distilled water and centrifuging again, drying the pellet in a pre-weight tube to constant mass at 104˚C.

The glucose content of the medium was measured following the procedure described by Trinder (1969).

3. RESULTS AND DISCUSSION

3.1 Cell growth

Cell growths of *Scheffersomyces stipitis* strains CBS 5776 (parental strain) and M12 (*erg6* mutant) in glucose media during 54 hours were compared (Fig 1.).

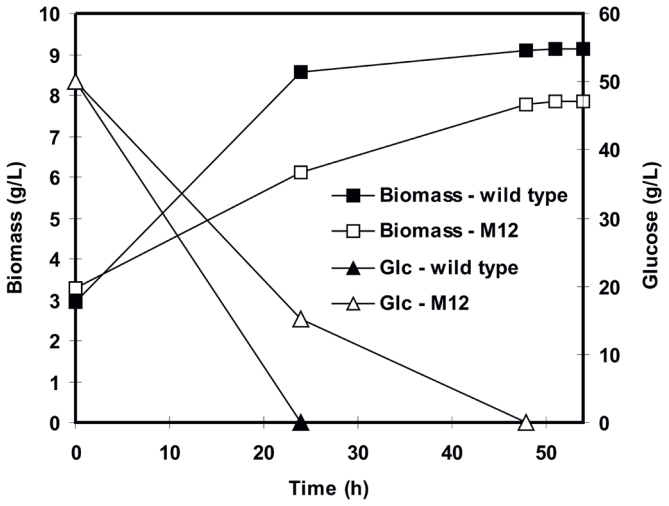

Figure 1. *Biomass growth and glucose consumption during 54 hours of cultivation of wild type S. stipitis and mutant strain M12 in O-media containing 1.5 g/L D,L -methionine.*

The *M12* strain grew much slower compared to congenic *ERG6* wild-type strain. The *M12* strain needed longer time than a wild-type to exhaust glucose from media and to enter the stationary growth phase. Thus, the M12 biomass yield was lower. Ergosterol is involved in cell cycle initiation in *S. cerevisiae* (Gaber et al., 1989) and it could be expected to play similar role in *S. stipitis*. Mutants *erg6* showed slower rates of metabolic activity (Higgins et al., 2003) and decreased growth rates (Palermo et al., 1997; Welihinda et al., 1994). Furthermore, *erg6* mutation in *S. cerevisiae* leads to altered membrane permeability and to increased uptake of various substances influencing cell state and growth (Shobayashi et al., 2006). Although we cannot simply apply previous fungal ergosterol findings with *S. cerevisiae* to *S. stipitis*, it is reasonable to assume that reduced ergosterol content of M12, which could be a consequence of *erg6* mutation, may be a reason for the observed defect in growth. Lower biomass yield could be

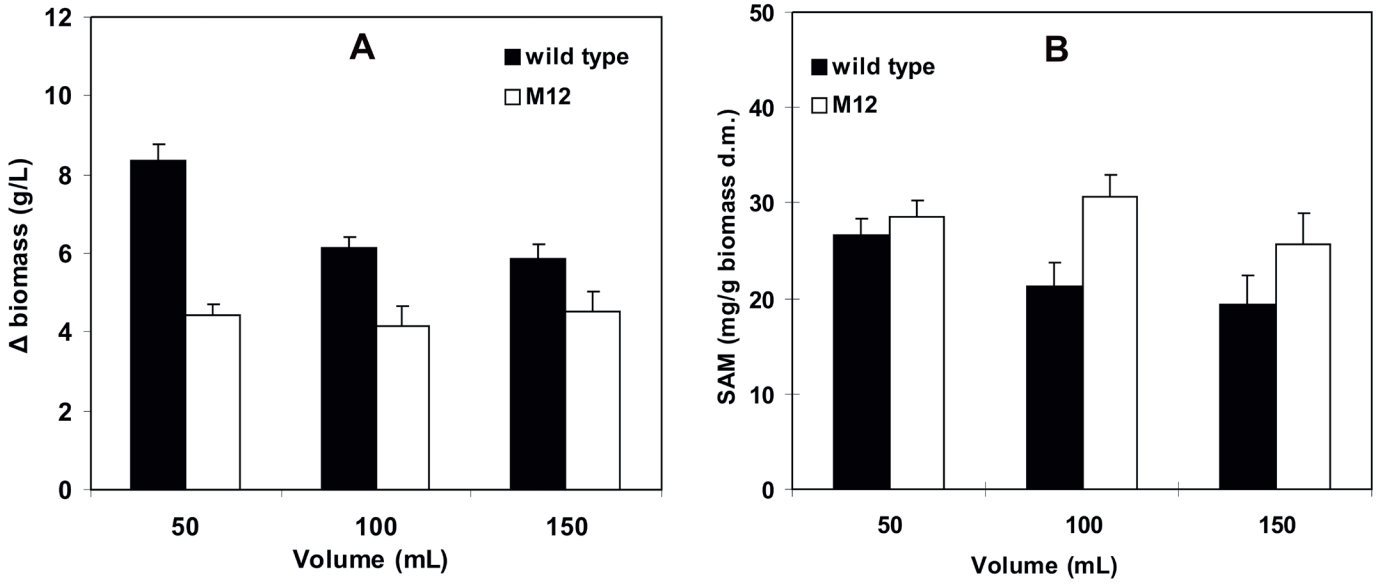

Figure 2. *Biomass growth (A) and accumulation of SAM (B) of wild type and mutant strain M12 after 48 hours of cultivation in different volume of shake flask culture. Results are mean values of three cultivations with SD.*

explained by different ability to utilize and tolerate ethanol formed during flask cultivation. The lack of ability to grow on ethanol was reported for *Saccharomyces* ergosterol-deficient mutants (Shobayashi et al, 2006). Since wild type of *S. stipitis* can grow on ethanol, the difference in final biomass yield could be explained by the use for ethanol formed during the cultivation in the case of wild-type strain. Negative influence of ethanol on cell growth may be avoided by application of fed-batch strategy in large scale process

3.2. Effects of aeration, pH, and temperature on SAM production

Fermentations in this study were carried out as shaken cultures in volume of 100 mL at the temperature of 30°C and pH = 5. The effects of aeration, pH, and temperature on biomass growth and SAM production were examined with wild strain and mutant strain M12 by variating the basic cultivation parameters (Figures 2-4).

To establish the effect of aeration on SAM production, the variation of culture volume in Erlenmeyer flask was examined. High DO value was favorable for the production of ATP, a substrate for biosynthesis of SAM, since most of ATP is produced through oxidative phosphorylation.

As expected, higher aeration effect at lower volume caused both higher yeast growth and higher SAM production of wild strain (Fig 2.), but the mutant strain did not follow this pattern. The M12 cell yield was similar in three quite different cultivations regardless of aeration effect. It is possible that the absence of higher yields at higher aeration effects was caused by oxidative stress in these conditions due to the lack of ergosterol, which is, as a part of the cell membrane, essential for protecting cells from oxidative stress (Higgins et al., 2003).

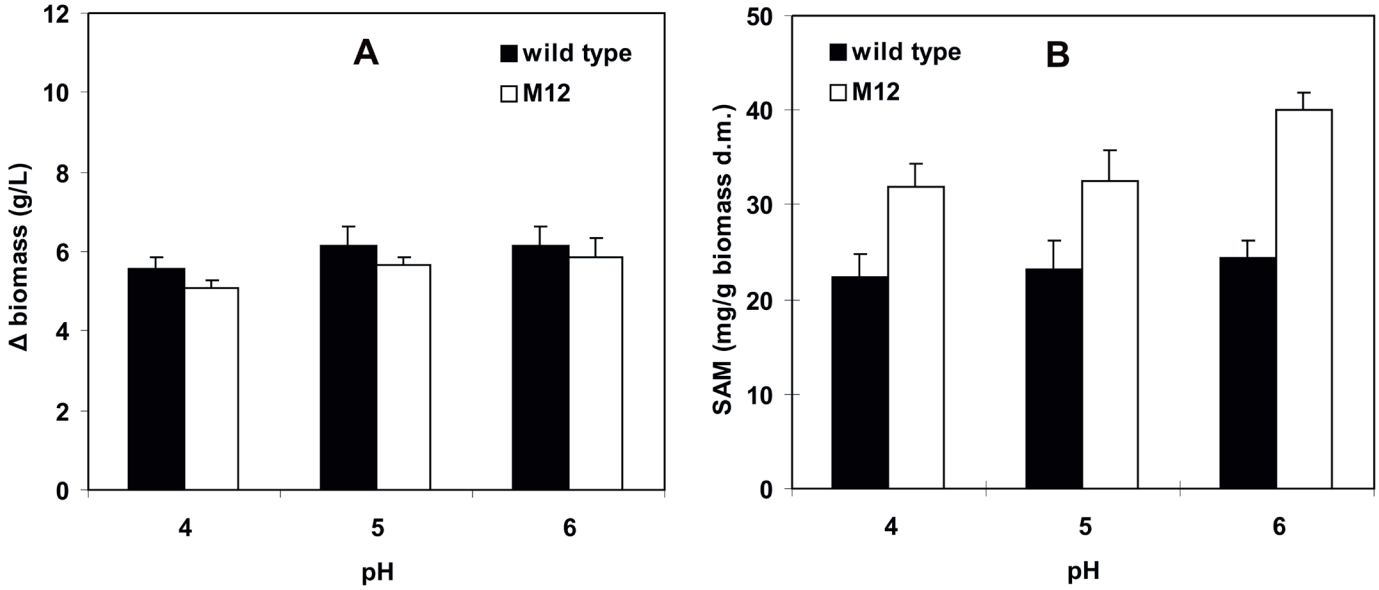

Figure 3. *Biomass growth (A) and accumulation of SAM (B) of wild type and mutant strain M12 after 48 hours of cultivation at different pH values of O-media. Results are mean values of three cultivations with SD.*

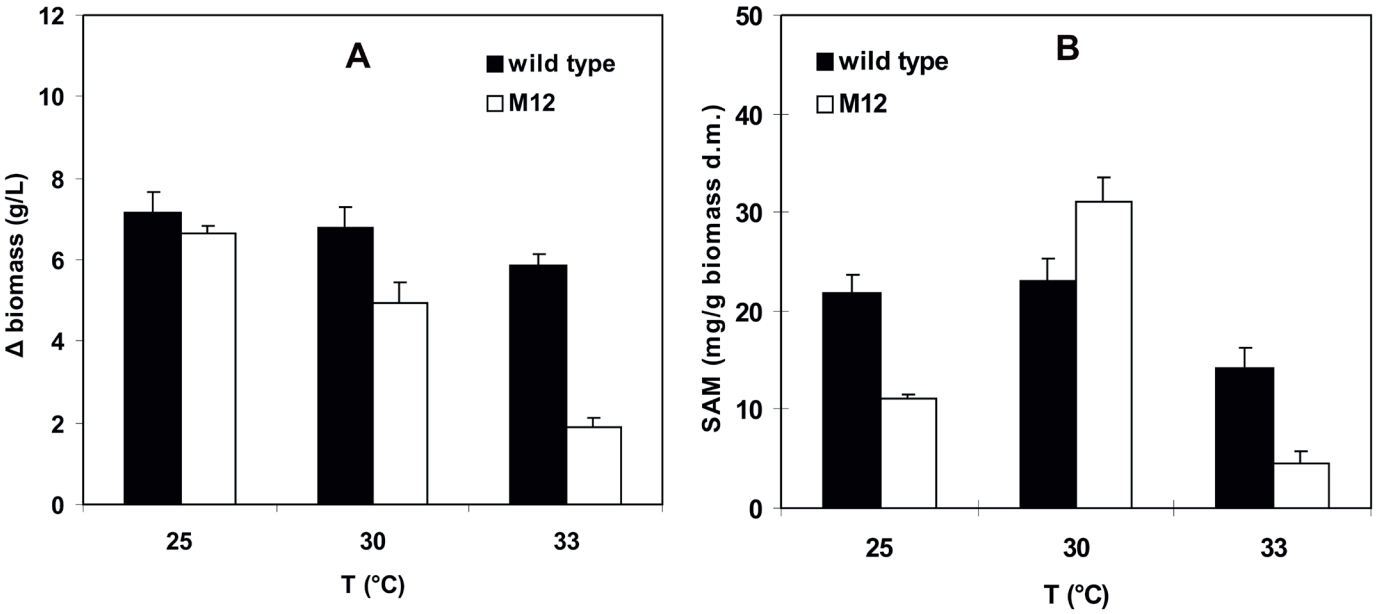

Figure 4. *Biomass growth (A) and accumulation of SAM (B) of wild type and mutant strain M12 after 48 hours of cultivation in O-media at different temperatures. Results are mean values of three cultivations with SD.*

On the other hand, mutant strain showed ability of higher accumulation of SAM than wild strain, the aeration effect being of no account. This fact supports the hypothesis that reduction of ergosterol synthesis leads to higher level of SAM.

The effect of culture pH is presented by Figure 3. There was no significant difference in biomass yield between cultivations performed at different pH-values, in both strains. However, it seems that higher pH allows mutant M12 to accumulate higher concentration of SAM.

Temperature (Fig 4.) affected the production of SAM in M12 more significantly than either aeration or pH.

Contrary to the wild strain, whose growth was affected by temperature variation between 25-33°C only to lower extent, mutant strain showed a clear drop of biomass yield with the increase of temperature to 33°C. This temperature also caused dramatically lower accumulation of SAM. Lower accumulation could be related to slow biomass growth and to the fact that stationary phase was not reached by the end of fermentation at this temperature. It is expected for ergosterol mutant strain to accumulate SAM in stationary phase of growth (Shiozaki et al., 1984). Lower accumulation of SAM at 25°C could be due to the lower activity of sterol-methyltransferase, an enzyme with temperature optimum between 30-34°C in case of *S. cerevisiae* (Thompson and Parks, 1974). It is obvious that relatively small changes of temperature could lead to important differences in production and accumulation of SAM. Additionally, temperature optimum for growth and optimum for SAM accumulation by mutant strain could differ.

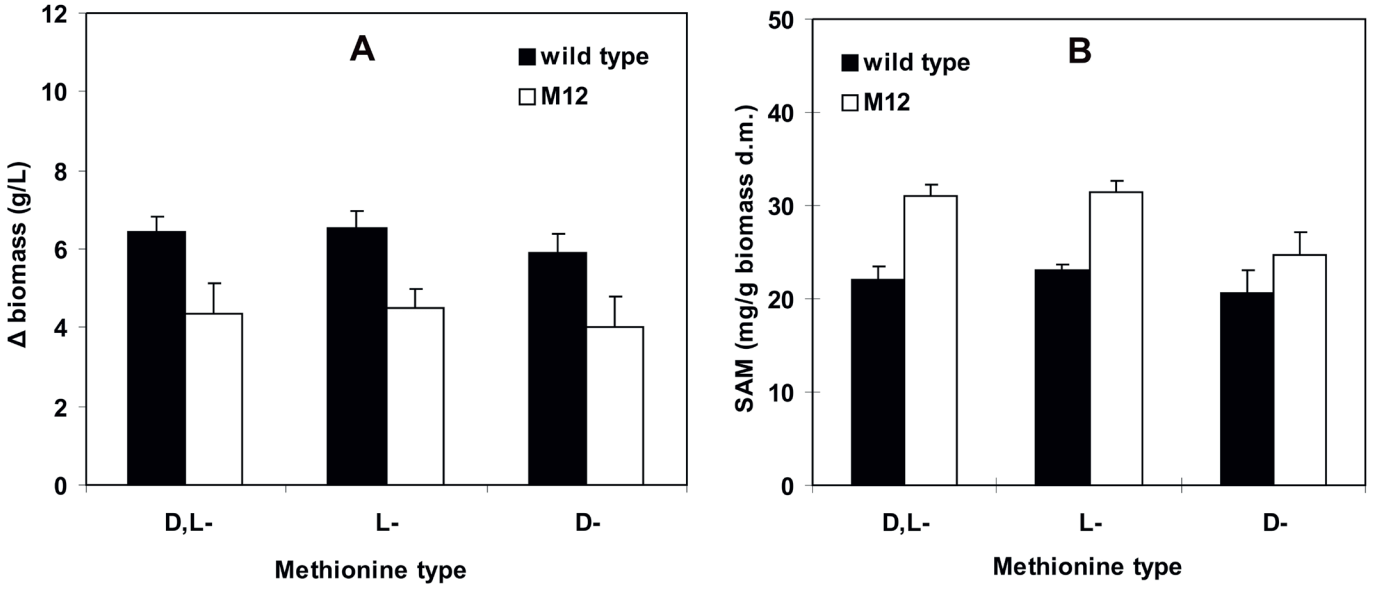

Figure 5. *Biomass growth (A) and accumulation of SAM (B) of wild type and mutant strain M12 after 48 hours of cultivation in O-media with 1,5 g/L D,L-; D- and L-methionine added to the media. Results are mean values of three cultivations with SD.*

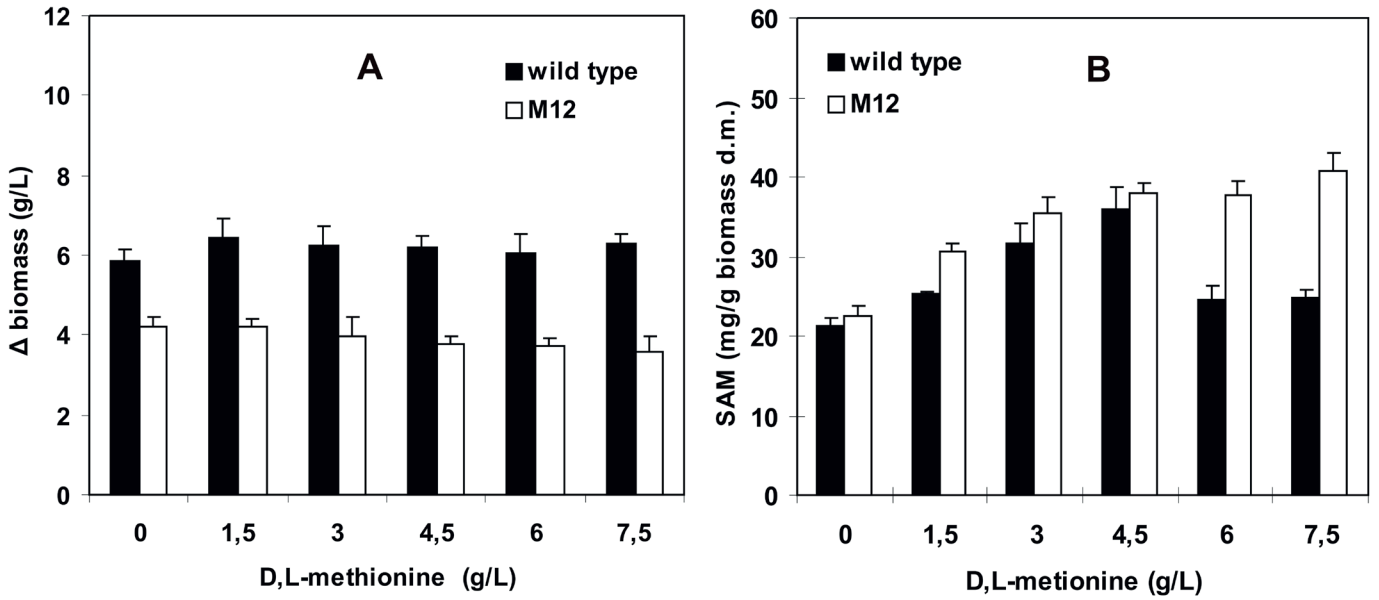

Figure 6. *Biomass growth and accumulation of SAM of wild type and mutant strain M12 after 48 hours of cultivation in O-media with 0.0; 1.5; 3.0; 4.5; 6.0 and 7.5 g/L D,L-methionine added to the media. Results are mean values of three cultivations with SD.*

3.3. Effect of medium composition on SAM production

L-methionine is an essential amino acid required for protein synthesis, but also, together with ATP, it participates in formation of SAM (*S*-adenosyl-L-methionine). As a first step in exploring possible effect of medium composition on SAM accumulation both in wild strain and M12 mutant, cultivations were conducted with three different forms of methionine added to the media (Fig. 5). It turned out that only marginal difference between effects of D- and L- form of methionine added in media existed in these cultivation conditions, while addition of D,L- isomer caused the same growth and SAM accumulation as L- form. For this reason, cheaper D,L –form was chosen for improvement of SAM production. This economical benefit has little consequence when studying pure cultures in laboratory scale, but could be very significant on industrial scale.

Concentration of methionine added in media is one of important parameters in SAM biosynthesis. It was established that L-methionine in media promote SAM production, but also that excess of L-methionine suppresses cell growth and SAM accumulation (Jakubowski and Goldman, 1993; Thomas and Surdin-Kerjan, 1991). The optimums of L-methionine added in media reported in papers related to SAM production, dependent on type of media, microorganism species/strain, type of process and other conditions, ranged from 0.45 g/L with *Saccharomyces sake* (Shiozaki et al., 1989) to 4 g/L with *Kluyveromyces lactis* (Mincheva and Balutsov, 2002).

The results presented here (Fig. 6) showed the optimum of 4.5 g/L of D,L-methionine in media for SAM production with wild strain *S. stipitis*, while growth was not affected by increase of D,L-methionine concentration in media. In mutant M12 the addition of different concentration of D, L-methionine caused only marginal changes in the yield of cells, and did improve the yield of SAM. The highest SAM yield (40.88 mg/g) was achieved at 7. 5 g / L of D,L-methionine. It is important to note that when there was no supplementation of D,L-methionine in medium, no difference between the strains in SAM yield was observed. These results indicates that supplementation of methionine is a prerequisite for higher production and accumulation of SAM.

3.4. Accumulation of SAM depending on growth phase

Comparison of SAM accumulation in wild strain and M12 mutant during 72 hours of cultivation without and with 1,5 g/l of D,L-methionine (Figures 7 and 8) clearly confirmed the assumption of strong dependence of the accumulation on growth phase. The wild strain reached the stationary growth phase in less than 18 hours and at this time reached the maximal content of SAM. Due to much slower growth, at that time M12 entered the exponential phase with minimal SAM content. Intensive growth is accompanied with a high energy consumption and the ATP is consumed in other reactions besides SAM production in this growth phase. Beside this, it is possible that already existent SAM is decomposed to produce energy in this phase of growth (Yin and Wang, 2011). The M12 cell concentration reached the stationary phase after 39-48 hours of flask culture, and there was a dramatic increase in SAM content after reaching this point in both, methionine unsupllemented medium and medium supplemented with 1,5 g/L D,L-methionine. In *S. cerevisiae* one of SAM synthase genes is repressed by excess L-methionine. It is interesting to note that higher D,L-methionine concentration caused lower SAM content in M12 strain during the exponential growth phase (compared to unsupplemented media), while in stationary phase, accumulation of SAM was favorable by the exogenous methionine. It could be concluded that a process with methionine supplementation at the end of exponential growth phase would be an appropriate approach in production of SAM with the M12 *erg6* mutant strain.

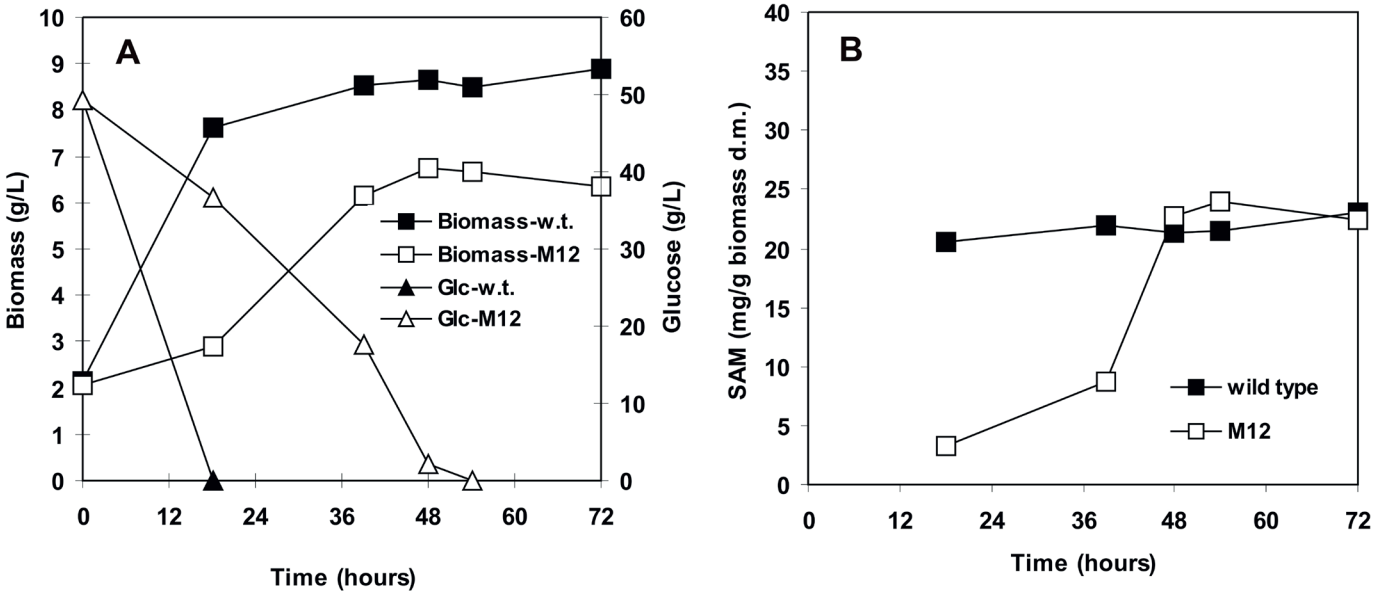

Figure 7. *Biomass growth and glucose consumption (A), and accumulation of SAM (B) of wild type S. stipitis and mutant strain M12 during 72 hours of cultivation in media without D,L –methionine supplementation.*

4. CONCLUSION

The knowledge of biosynthesis of SAM comes mostly from the studies of *S. cerevisiae*. Ergosterol has been shown to have vital functions in *Saccharomyces cerevisiae* cells affecting membrane fluidity and permeability and providing the "sparking function" that is thought to be involved in the progression through the G_1 phase of the cell cycle. The transmethylation of zymosterol to fecosterol by methyltransferase enzyme is one of the most metabolically expensive reactions in the organism's biochemistry (Parks, 1978; Parks and Casey, 1995). Since SAM is a donor of the methyl group in this reaction, our hypothesis was that the reduction of ergosterol synthesis in *Scheffersomyces stipitis* with block of this pathway in fecosterol synthesis and would lead to a higher level of SAM in a cell. We have confirmed this hypothesis and successfully made the ergosterol-deficient *S. stipitis* mutant strain that in shake flask culture showed a higher ability to accumulate SAM than parental strain, and thus showed possible ways for further development of large scale production system. Experiments for optimization of cultural conditions confirmed assumptions that fed-batch process and methionine supplementation at the end of exponential growth phase would be the proper way to develop the production of SAM using the M12 *erg6* mutant strain. During this initial phase of the study, a considerable increase of SAM content in the biomass of yeast *S. stipitis* was achieved. Mutant M12 achieved the highest content of 40.88 mg SAM/g of biomass, which is about 2 times higher than that of SAM obtained in the wild type in media without methionine supplementation. Information about the process parameters

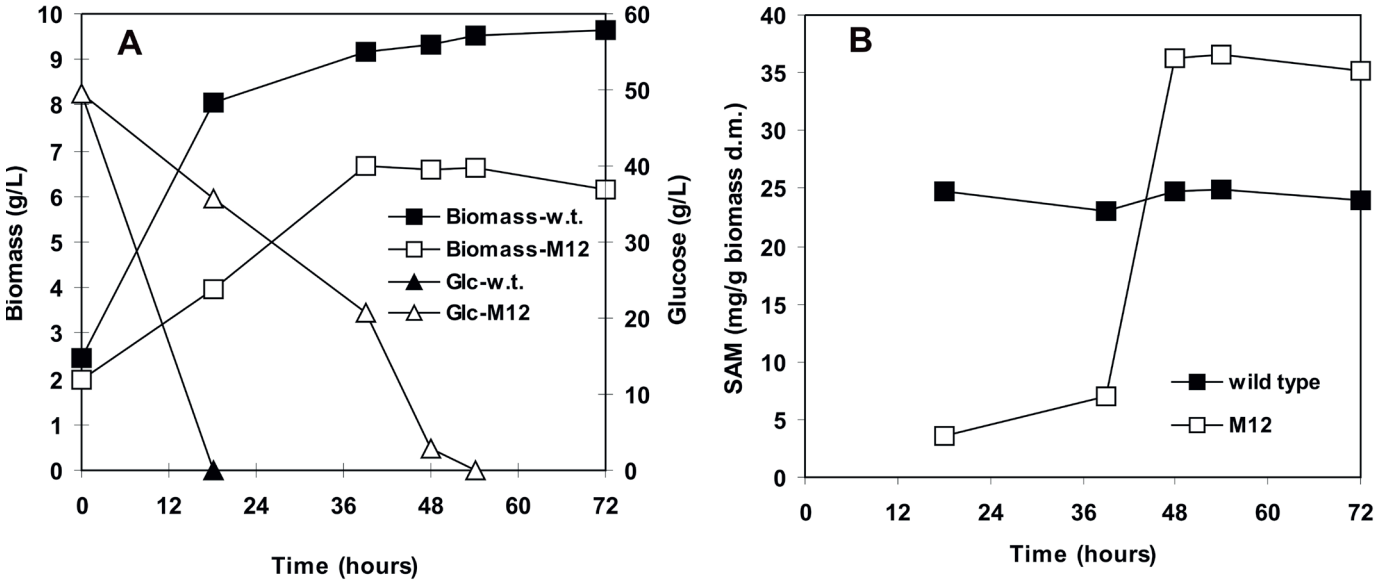

Figure 8. *Biomass growth and glucose consumption (A), and accumulation of SAM (B) of wild type S. stipitis and mutant strain M12 during 72 hours of cultivation in media with 1.5 g/L D,L –methionine added to media.*

that enhance both SAM production and its accumulation in the mutant M12, obtained in this study, provide an excellent foundation for further research and yield increase. The combination of these parameters, as well as the questions of oxygen supply that also appeared in our research, should be further investigated in a larger scale.

Aknowledgement

This project was funded by the Ministry of Science, Education and Sports of the Republic of Croatia.

5. REFERENCES

Bottiglieri T. (2002) S-Adenosyl-L-methionine (SAMe): from the bench to the bedside: molecular basis of a pleiotrophic molecule. *American Journal of Clinical Nutrition*, 71 614-620.

Carrieri P.B., Indaco A., Gentile S., Troisi, E., Campanella, G. (1990) *S*-adenosyl-L-methionine treatment of depression in patients with Parkinson's disease. A double-blind, crossover study versus placebo. *Current Therapeutic Research*, 48 154-160.

Chawla R.K., Bonkovsky H.L., Galambos J.T. (1990) Biochemistry and pharmacology of S-adenosyl-L-methionine and rationale for its use in liver disease. *Drugs*, 40 98-110.

Chiang P.K., Gordon R.K., Tal J., Zeng G.C., Doctor B.P., Pardhasaradhi K., McCann P.P. (1996) S-Adenosylmethionine and methylation. *The Journal of the Federation of American Societies for Experimental Biology*, 10 471-480.

Choi E.S., Park B.S., Lee S.W., Oh M.K. (2009) Increased production of *S*-adenosyl-L-methionine in recombinant *Saccharomyces cerevisiae* sake K6. *Korean Journal of Chemical Engineering* 26 (1) 156–159.

Criconia A.M., Araquistain J.M., Darrina N., Navajas F., Bordino M (1994). Results of treatment with S-adenosyl-L-methionine in patients with major depression and internal illnesses. *Current Therapeutic Research*, 55 666-674.

Daum G., Lees N.D., Bard M., Dickson R. (1998) Biochemistry, cell biology and molecular biology of lipids of *Saccharomices cerevisiae*. *Yeast*, 14 (16) 1471-1510.

Di Padova C. (1987) *S*-adenosyl-L-methionine in the treatment of osteoarthritis. Review of the clinical studies. *American Journal of Medicine*, 83 (5A) 60-65.

Gaber R.F., Copple D.M., Kennedy B.K., Vidal M., Bard M. (1989) The yeast gene *ERG 6* is required for normal membrane functions but is not essential for biosynthesis of the cell-cycle-sparking sterol. *Molecular and Cellular Biology*, 9 3447–3456.

Higgins V.J., Beckhouse A.G., Oliver A.D., Rogers P.J., Dawes I.W. (2003) Yeast genome-wide expression analysis identifies a strong ergosterol and oxidative stress response during the initial stages of an industrial lager fermentation. *Applied and Environmental Microbiology*, 69 (8) 4777-4787.

Holcomb, E.R., Shapiro S.K. (1975) Assay and regulation of *S*-adenosyl-L-methionine synthetase in *Saccharomyces cerevisiae* and *Candida utilis*. *Journal of Bacteriology*, 121 267-271.

Hu H., Qian J.C., Chu J., Wang Y.H., Zhuang Y.P., Zhang S.L. (2009) Optimization of L-methionine feeding strategy for improving *S*-adenosyl-L-methionine production by methionine adenosyltransferase overexpressed *Pichia pastoris*. *Applied Microbiology and Biotechnology,* 83 1105–1114.

Jakubowski H., Goldman E. (1993) Methionine-mediated lethality in yeast cells at elevated temperature. *Journal of Bacteriology*, 175 5469-5476.

Li W., Ye S., Luo K., Ge F., Du L., Wu K., Ding C. (2007) Isolation and characterisation of *Candida sp.* mutants enriched in *S*-adenosyl-L-methionine (SAM). *Annals of Microbiology*, 57(3) 383-387.

Liu H., Lin J.P., Cen P.L., Pan Y.J. (2004) Coproduction of *S*-adenosyl-L-methionine and glutathione from spent brewer's yeast cells. *Process Biochemistry,* 39 1993–1997.

Martínez-Chantar M.L., García-Trevijano E.R., Latasa M.U., Pérez-Mato I., Sánchez del Pino M. MAvila M.A. (2002) Importance of a deficiency in S-adenosyl-L-methionine synthesis in the pathogenesis of liver injury. *The American Journal of Clinical Nutrition*, 76 (5) 1177-1182.

Mato J.M., Corrales F.J., Lu S.C., Avila M.A. (2002) S-Adenosyl-L-methionine: a control switch that regulates liver function. *The Journal of the Federation of American Societies for Experimental Biology*, 16 (1) 15-26.

Mincheva K., Kamburova V., Balutzov V. (2002) Production of S-adenosyl-L-methionine by a mutant strain of *Kluyveromyces lactis*. *Biotechnology Letters***,** 24 985-988.

Mischoulon D., Fava M. (2002) Role of S-adenosyl-L-methionine in the treatment of depression: a review of the evidence. *The American Journal of Clinical Nutrition,* 76(5) 1158-1161.

Newman P. E. (2000) Alzheimer's disease revisited. *Medical Hypotheses* 54 774-746.

Palermo, L.M., Leak, F.W., Tove, S., Parks, L.W. (1997) Assessment of the essentiality of *ERG6* genes late in ergosterol biosynthesis in *Saccharomyces cerevisiae. Current Genetics, 32* 93–99.

Parks L.W., Casey W.M. (1995) Physiological implications of sterol biosynthesis in yeast**.** *Annual Review of Microbiology,* 49 95-116.

Parks L.W. (1978) Metabolism of sterols in yeast. *CRC Critical Reviews in Microbiology*, 6 (4) 301–341.

Shiomi N., Fukuda H., Fukuda Y., Murata K., Kimura A. (1990) Production of S-adenosyl-L-methionine by *Saccharomyces cerevisiae* cells carrying a gene for ethionine resistance. *Biotechnology & Bioengineering*, 35 1120-1124.

Shiomi N., Fukuda H., Murata K., Kimura A. (1995) Improvement of *S*-adenosyl-L-methionine production by integration of the ethionine-resistance gene into chromosomes of the yeast *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology*, 42 (5) 730-733.

Shiozaki S., Shimizu S., Yamada, H. (1984) Unusual intracellular accumulation of S-adenosyl-L-methionine by microorganisms. *Agricultural Biological Chemistry*, 48 2293-2300.

Shiozaki S., Shimizu S., Yamada H. (1989) Production of S-adenosyl-L-methionine by *Saccharomyces sake*: optimization of the culture conditions for the production of cells with a high S-adenosyl-L-methionine content. *Agricultural Biological Chemistry*, 53 3269-3274.

Shobayashi M., Mukai N., Iwashita K., Hiraga Y., Iefuji H. (2006) A new method for isolation of *S*-adenosyl-L-methionine (SAM)-accumulating yeast. *Applied Microbiology and Biotechnology*, 69(6) 704-710.

Tabor, C.W., Tabor H. (1984) Methionine adenosyltransferase (*S*-adenosyl-L-methionine synthetase) and *S*-adenosyl-L-methionine decarboxylase. *Advances in Enzymology and Related Areas of Molecular Biology*, 56 251-282.

Thomas D., Surdin-Kerjan Y. (1991) The synthesis of the two *S*-adenosyl-L-methionine is differently regulated in *Saccharomyces cerevisiae*. *Molecular and Geneneral Genetics*, 226 224–232.

Thompson E.D., Parks L.W. (1974) Effect of altered sterol composition on growth characteristics of *Saccharomyces cerevisiae*. *Journal of Bacteriology*, 120 779–784.

Trinder, P. (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry,* 6 24-27.

Valko, K., Hamedani, M.P., Ascah, T.L., Gibbons, W.A. (1993) Comparative study of the reversed-phase HPLC retention behaviour of S-adenosyl-L-methionine and its related metabolites on Hypersil ODS and Supelcosil LC-ABZ stationary phases. *Journal of Pharmaceutical and Biomedical Analysis*, 11 361-366.

Wang, J., Han, J., Li, X., Liu, P., Tan, T. (2009) Optimization of high-cell-density fermentation process for S-adenosyl-L-methionine production. *Chinese Journal of Bioprocess Engineering*. 25 (4) 533-536.

Welihinda A.A., Beavis A.D., Trumbly R.J. *(*1994*)*. Mutations in LIS1 (ERG6) gene confer increased sodium and lithium uptake in *Saccharomyces cerevisiae. Biochimica et Biophysica Acta*, 1193. 107–117.

Yin Y., Wang W. (2011) Optimization of overproducing S-adenosyl-L-methionine *Saccharomyces cerevisiae* S-W55 mutant utilizing unpolished rice from aging paddy by feeding L-methionine. *African Journal of Biotechnology,* 10(36) 6874-6882.

Yu P., Shen X. (2012) Enhancing the production of S-adenosyl-L-methionine in *Pichia pastoris* GS115 by metabolic engineering. *AMB Express* 2 (1) 57.

Zhang Y.Y., Li Y.G., Jin J.,Yang D., Shen G.M., (2005) Kinetics and specific growth rate control strategy of *Saccharomyces cerevisiae* HYS98 fermentation for high productivity of SAM. *Chinese Journal of Process Engineering*, 5 (3) 322-326.

Zhang J.G., Wang X.D., Su E.Z., Fang G.C., Ren Y.H., Wei D.Z. (2008) A new fermentation strategy for *S*-adenosyl-L-methionine production in recombinant *Pichia pastoris*. *Biochemical Engineering Journal*, 41 74-78.