รายงานการวิจัยฉบับสมบูรณ์
ทุนอุดหนุนการวิจัยจากเงินอุดหนุนทั่วไปจากรัฐบาล

การศึกษาทางอณูพันธุศาสตร์ของการสังเคราะห์กรดไขมันและไขมันในยีสต์ *Hansenula polymorpha*

Genetic study of fatty acid and lipid biosynthesis in *Hansenula polymorpha*

โดย

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บทคัดย่อภาษาไทย

ยีสต์กลายพันธุ์สองกลุ่มของ *Hansenula polymorpha* ถูกคัดเลือกจากความต้องการกรดไขมันในการเจริญเติบโต กลุ่มหนึ่ง คือ ยีสต์กลายพันธุ์ที่ต้องการกรดไขมันอิ่มตัวสำหรับการเจริญเติบโต (*sfa*- mutamts) อีกกลุ่มหนึ่งคือ ยีสต์กลายพันธุ์ที่ต้องการกรดไขมันไม่อิ่มตัวสำหรับการเจริญเติบโต (*mfa*- and *pfa*- mutants) ยีสต์กลายพันธุ์ในกลุ่ม *sfa*- สองตัว คือ S7 และ S16 มีองค์ประกอบของกรดไขมันที่แตกต่างจากยีสต์กลายพันธุ์ตัวอื่นอย่างชัดเจน โดยยีสต์กลายพันธุ์ S7 บกพร่องในการผลิตกรดไขมันไม่อิ่มตัว C18:2$\Delta^{9,12}$ และ C18:3$\Delta^{9,12,15}$ ขณะที่ยีสต์กลายพันธุ์ S16 มีการสะสมกรดไขมันอิ่มตัวสายกลาง คือ C12:0 และ C14:0 ในปริมาณมาก จากการวิเคราะห์เทเทรด (Tetrad analysis) พบว่า เชื้อกลายพันธุ์ S7 มีลักษณะที่ถูกกลายพันธุ์ไปสองลักษณะ (ตำแหน่ง) คือการกลายพันธุ์ที่ทำให้เกิดการบกพร่องในกระบวนการสังเคราะห์กรดไขมัน (*Hpsfa7*) และการกลายพันธุ์ที่ทำให้เกิดความบกพร่องที่การเติมพันธะคู่ที่คาร์บอนตำแหน่งที่ 12 บนสายของกรดไขมัน (*Hpdes12*) ลูกที่แยกได้จากกระบวนการไมโอซิส คือ H69-2C และ H69-2D มียีนกลายพันธุ์ *Hpdes12* มีลักษณะที่แสดงออกเป็น Sfa+ ที่ไม่ต้องการกรดไขมันในการเจริญเติบโต ชี้ให้เห็นว่าการขาด C18 polyunsaturated fatty acids ไม่ส่งผลต่อการเจริญเติบโต ส่วน H69-2A และ H69-2B มียีน *Hpsfa7* มีลักษณะที่แสดงออกเป็น Sfa- และต้องการกรดไขมันอิ่มตัวสำหรับการเจริญเติบโต อย่างไรก็ตาม มีเฉพาะ H69-2B เท่านั้นที่แสดงคุณสมบัติในการเจริญบนอาหารที่เสริมด้วยกรดไขมันชนิดต่าง ๆ ที่เหมือนกับยีสต์กลายพันธุ์ที่ใช้เป็นเซลล์แม่ (S7) ในขณะที่ H69-2A มีความแตกต่างจากการโคลนยีน Δ^{12}-desaturase ของ *H. polymorpha* SH4330 (*HpDES12*) และ S7 เมื่อเปรียบเทียบลำดับนิวคลีโอไทด์ของยีนที่โคลนได้จากยีสต์ทั้งสองสายพันธุ์ ทำให้ยืนยันได้ว่าความบกพร่องในการสร้าง C18:2$\Delta^{9,12}$ และ C18:3$\Delta^{9,12,15}$ ในยีสต์ S7 เป็นผลมาจากการกลายพันธุ์ที่ยีน Δ^{12}-desaturase นอกจากนี้ผลการโคลนยีนยังทำให้ทราบลักษณะของยีน Δ^{12}-desaturase ของ *H. polymorpha* ซึ่งพบว่า บริเวณที่ถอดรหัสให้เป็นกรดอะมิโน (open reading frame) มีความยาว 1215 เบส ถอดรหัสให้กรดอะมิโน 404 ตัว (GenBank Accession No. GU226432) โดยลำดับกรดอะมิโนนี้มีความคล้ายคลึงสูงสุดกับลำดับอะมิโนของเอ็นไซม์ Δ^{12}-desaturase ของยีสต์ *Pichia pastoris* ซึ่งมีความสัมพันธ์กับผลของการวิเคราะห์แบบไฟโลเจนิค (phylogenetic analysis) ซึ่งยีน Δ^{12}-desaturase ของ *H. polymorpha* ที่โคลนได้นี้ และยีสต์กลายพันธุ์ต่าง ๆ ที่ได้จากการศึกษานี้สามารถใช้เป็นเครื่องมือในการศึกษาความสัมพันธ์ระหว่างเมตาลอลิสมของกรดไขมันและสรีรวิทยาของสิ่งมีชีวิตยูคาริโอต

บทคัดย่อภาษาอังกฤษ (Abstract)

Two groups of *Hansenula polymorpha* mutants were screened by their fatty acid requirement, one requires saturated fatty acids for growth (*sfa*- mutamts) and the other requires unsaturated fatty acids (*mfa*- and *pfa*- mutants). Two of the *sfa*- mutants, S7 and S16, showed significantly difference in the fatty acid composition. S7 clearly defected in the production of C18:2$\Delta^{9,12}$ and C18:3$\Delta^{9,12,15}$, while S16 significantly accumulated medium-chain saturated fatty acids, C12:0 and C14:0. By tetrad analysis, the results showed that S7 had double mutation which composed of fatty acid synthesis mutation (*Hpsfa7*) and Δ^{12}-desaturase mutation (*Hpdes12*). Two meiotic segregants, H69-2C and H69-2D harboring *Hpdes12* gene, showed Sfa+ phenotype and grew on YPD without fatty acid supplementation indicating that the absence of C18 polyunsaturated fatty acids did not affect cell growth of the mutant. H69-2A and H69-2B segregants (*Hpsfa7*) showed Sfa- phenotype and required saturated fatty acids for growth. However, only the segregant H69-2B display similar properties to parental mutant (S7) on the growth on media supplemented with various fatty acids, while H69-2A was different. The comparison of the cloned Δ^{12}-desaturase gene (*HpDES12*) of *H. polymorpha* SH4330 and S7 confirmed that the defect of C18:2$\Delta^{9,12}$ และ C18:3$\Delta^{9,12,15}$ synthesis was resulted from the mutation at Δ^{12}-desaturase gene. Besides, the cloning gene also provided the characteristic of Δ^{12}-desaturase of the yeast *H. polymorpha*. An open reading frame of the full length cDNA of Δ^{12}-desaturase gene of *H. polymorpha* showed 1215 bp encoding for 404 amino acid residues (GenBank Accession No. GU226432). The deduce amino acids had the highest similarity to the Δ^{12}-desaturase of *Pichia pastoris* that corresponds to the result of phylogenetic analysis. The cloned gene and mutants obtained in this study can be used as tools for studying association between fatty acid metabolism and cell physiology of eukaryotes.

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คำอธิบายสัญญาลักษณ์และคำย่อที่ใช้ในการวิจัย (List of Abbreviations)

C14:0	myristic acid
C16:0	palmitic acid
C18:0	stearic acid
C16:1Δ^{9}	palmitoleic acid
C18:1Δ^{9}	oleic acid
C18:2$\Delta^{9,12}$; C18:2 *n*-6; LA	linoleic acid
C18:3$\Delta^{9,12,15}$; C18:3 *n*-3; ALA	α–linolenic acid
AA	arachidonic acid
DHA	docosahexaenoic acid
EMS	ethyl methanesulfonate
EPA	eicosapentaenoic acid
FA	fatty acid
GLA	γ-linolenic acid
LC-PUFAs	long chain-polyunsaturated fatty acids
MUFAs	monounsaturated fatty acids
PUFAs	polyunsaturated fatty acids
MFA	the mixture of C16:1Δ^{9}, C18:1Δ^{9}
PFA	the mixture of C18:2$\Delta^{9.12}$, C18:3$\Delta^{9,12,15}$
SFA	the mixture of C14:0, C16:0, C18:0
TAG	Triglyceride
YPD	yeast extract, peptone and dextrose medium
WT	wild type

บทนำ (Introduction)

1.1 ความสำคัญและที่มาของปัญหาที่ทำการวิจัย

There has been much interest in the beneficial impact of *n*-3 and *n*-6 polyunsaturated fatty acids (PUFAs). These fatty acids have important roles in human health and nutrition. Reliable dietary sources of PUFAs are fish oils and some plant oils. Because of insufficiency of the natural resources for the market demand, therefore, the alternative sources for production of PUFA-rich oils are required. Many attempts have paid on improvement of producer strain through engineering of metabolic pathways involved in fatty acid biosynthesis. More understanding of fatty acid metabolism offers new insights into the metabolic fluxes governing the production of valuable fatty acids. The concept of obtaining them from microorganisms in sustainable quantities is attractive. It will be express the genes in specific manner and in a lipid background rich in the fatty acid substrates. With advance in genetic manipulation, fermentation, and ability to synthesize both *n*-3 and *n*-6 PUFAs, *Hansenula polymorpha* is of considerable interest as an excellent model for study of fatty acid and lipid metabolisms in eukaryotes, which prompts it to be a candidate for improvement of edible microbial oils and single cell oils. To understand the fatty acid and lipid synthesis in *H. polymorpha*, in this research project, a number of mutants defecting in fatty acid synthesis and having apparently altered phenotypes including growth, fatty acid composition were screened and selected. The mutants were used as tools for searching gene(s)/enzyme(s) involved in fatty acid and lipid synthesis. The valuable information obtained can be used to reconstruct the biosynthetic pathway of fatty acid that lead to more understanding in fatty acid and lipid metabolisms of *H. polymorpha*. In addition to the basic viewpoint, the knowledge gained in this research is considerably useful for rationale design in improvement of microbial oils that has a significant important in pharmaceutical and biotechnological applications.

1.2 วัตถุประสงค์ของโครงการวิจัย

The goal of this research project is to understand fatty acid and lipid synthesis in yeast *H. polymorpha* that would provide information for more rationale design in manipulating its fatty acid composition and content. This study also provides an insight into production of single cell oils and edible microbial oils having valuable PUFAs with pharmaceutical benefit by using *H. polymorpha* as an alternative host.

1.3 ขอบเขตของโครงการวิจัย

1.3.1 To generate a class of *H. polymorpha* mutants with altered phenotypes of fatty acid using chemical mutagenesis

1.3.2 To characterize the mutants by physiological and genetic studies

1.3.3 To clone lipogenic gene(s) from the mutant(s) of interest

1.4 ทฤษฎี สมมุติฐาน (ถ้ามี) และกรอบแนวความคิดของโครงการวิจัย

There has been a great emphasis placed upon the beneficial of essential fatty acids consumption. Polyunsaturated fatty acids (PUFAs) in *n*-3 and *n*-6 series are important for normal development and function of all organisms and long chain-polyunsaturated fatty acids (LC-PUFAs) containing 18-22 carbons with two or more double bonds, have been known to play important roles in human health. Apart from a structural role of cellular membranes, these PUFAs classes also serve as precursors for synthesizing eicosanoids, including prostaglandins, leukotrienes and thromboxanes, which have important biological function in various biological systems such as cardiovascular, respiratory and immune systems. The *n*-3 eicosanoids are either weak agonists or more usually, strong antagonists of the potent *n*-6 metabolites (Simopoulos 2002; Funk 2001; Gill and Valivety 1997). Humans are incapable of synthesizing essential fatty acids, linoleic acid (LA, C18:2 *n*-6) and α–linolenic acid (ALA, C18:3 *n*-3), due to lack of the Δ^{12}– and Δ^{15}–desaturases. However, human can further metabolize these two fatty acids (LA and ALA) obtained from the diet from LC-PUFAs through a series of desaturation and elongation steps. As the demand of these beneficial PUFAs has drastically increased in recent years and the limitation of marine fish oils and some of seed oils, which represent the natural reserves of *n*-3 and *n*-6 LC-PUFAs, respectively, alternative sources are urgently required. The concept of obtaining them from oilseed plants and microorganisms in commercial and sustainable quantities is particularly attractive. It will be necessary to express the genes in question in a specific manner and in a lipid background rich in the fatty acid substrates. Recent attempts to reconstitute the desaturation pathway in yeast and plants met with limited success. Their relatively yield of LC-PUFAs can be explained by availability of the substrate pool (Lopez and Garcia 2000). A central question remains as to the mechanism(s) by which unnatural fatty acids are accumulated in triglyceride (TAG). With advance in multidisciplinary research emerged, the fatty acid metabolism has been studied extensively for full understanding and consequently for effective manipulation of

composition and content of the desired fatty acids. Selection and chemical mutagenesis as well as construction of transgenic organisms would be potential strategies for genetic modifications for oil quality and production.

The variable existence of biosynthetic rotes of such PUFAs has been found in the microbial kingdom (Mukherjee 1999). The amazing diversity of fatty acid profile in yeasts and fungi provides a means for study of the possibility of manipulation of cellular fatty acids. The methylotrophic yeast *H. polymorpha* is a non-conventional yeast and has been recognized as a model system for investigation of several biological processes such as methanol metabolism, peroxisome biogenesis and for producing several useful bioproducts (Veale et al. 1992; de Roubin et al. 1991; Gellissen et al. 1991; Escalante et al. 1990) and is regarded as GRAS organism (generally recognized as safe). The genetic transformation system and high-cell-density fermentation of yeast has been developed (Gillissen and Hollenberg 1997). *H. polymorpha* is, unlike *Saccharomyces cereviase*, able to synthesize both *n*-6 and *n*-3 PUFAs including LA and ALA, in addition to monounsaturated fatty acids (MUFAs) (Anamnart et al. 1998) that is similar to higher plants. Moreover, thermotolerance of *H. polymorpha* is a favorable characteristic for industrial application, especially in tropic countries. By these advances, *H. polymorpha* is of considerable interest as an excellent model for study of fatty acid metabolism in eukaryotes, which prompts it to be a candidate for improvement of edible microbial oils and single cell oils by manipulating fatty acid composition. This yeast has been proven to offer potential to be an alternative host as a delivery vehicle for important LC-PUFAs.

Although several genes including Δ^9–desaturase (Lu et al. 2000), two elongases and fatty acid synthase (Prasitchoke et al. 2008; Prasitchoke et al. 2007a; Prasitchoke et al. 2007b; Kaneko et al. 2003) have been cloned recently from *H. polymorpha*, the PUFA synthetic pathway of this yeast is poorly defined and the enzymes and substrates involved are uncharacterized. Furthermore, other relevant genes encoding lipid biosynthetic enzymes, β-oxidation and fatty acid activation and transport have not been identified in *H. polymorpha*. In this research project, a number of mutants defecting in fatty acid synthesis and having apparently altered phenotypes including growth, fatty acid composition were screened and selected. The mutants of interest were further characterized by physiological and genetic studies. The mutants were used as tools for searching gene(s)/enzyme(s) involved in fatty acid synthesis. The valuable information obtained would be used to develop the biosynthetic

pathway of fatty acid and lead to more understanding in fatty acid metabolism of *H. polymorpha*. In addition to the basic viewpoint, the knowledge gained in this research is considerably useful for modifying oils having potentially valuable fatty acid, γ-linolenic acid (GLA), arachidonic acid (AA), eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) with pharmaceutical and biotechnological applications through metabolic pathway engineering of this alternative microorganism.

1.5 วิธีการดำเนินการวิจัยโดยสรุป

1.5.1 Chemical mutation treatment

1.5.2 Screening and isolation of *H. polymorpha* mutants

1.5.3 Characterization of the mutants

1.5.4 Physiological study of the mutants

A) Growth determination

B) Analysis of fatty acid composition

1.5.5 Genetic study of the mutants

A) Complementation study

B) Cloning of novel gene(s) involved in fatty acid synthesis

1.6 ประโยชน์ที่คาดว่าจะได้รับ

1.6.1 The mutant as useful tools for studying fatty acid and lipid metabolism in higher eukaryotes

1.6.2 Gene(s) / enzyme(s) involved in fatty acid and lipid metabolism in eukaryotes

1.6.3 A mutant strain(s) with application in fatty acid production

เนื้อเรื่อง (Main body)

2.1 วิธีดำเนินการวิจัย (Materials & Method)

2.1.1 Organisms

The auxotrophic strains *ura3-1* (SH 4330) and *ade1-1* (SH4331) of *H. polymorpha* were derived from CBS 1976 (NCYC 495), a gift from Prof. J.A.K.W. Kiel (University of Groningen, The Netherlands) and Prof. Satoshi Harashima (Osaka University, Japan). The *ura3-1* strain was used as parental or wild type strain (WT) for generating and screening mutant strains.

2.1.2 Media and cultivations

Standard yeast genetics methods were subjected for mating, sporulation and tetrad analysis (Gleeson and Sudbery 1988; Sherman et al. 1986). The yeast cells were grown either in complete medium (YPD) containing 1% yeast extract, 2% Bacto peptone and 2% glucose or in minimal medium (synthetic dextrose) containing 0.67% yeast nitrogen base without amino acids and 2% glucose. To screen the fatty acid auxotrophic mutants, C14:0, C16:0, C18:0, C16:1Δ^{9}, C18:1Δ^{9}, C18:2$\Delta^{9,12}$ or C18:3$\Delta^{9,12,15}$ emulsifying in 1% Triton X-100 were supplemented in the media at the concentration of 0.5 to 1.5 mM. The transformation and genetic manipulation for *Escherichia coli* was described previously (Sambrook et al. 1989).

2.1.3 Mutagenic treatment, screening and isolation of *H. polymorpha* mutants

Mutagenesis of *H. polymorpha* was performed by treatment with ethyl methanesulfonate (EMS) as previously described (Anamnart et al. 1998). Depending on a desired phenotype, mutagenized cells were plated onto either YPD supplemented with a mixture of saturated fatty acids (SFA; 0.5 mM each of C14:0, C16:0 and C18:0) or a mixture of monounsaturated fatty acids (MFA; 0.5 mM each of C16:1Δ^{9} and C18:1Δ^{9}) or a mixture of polyunsaturated fatty acids (PFA; 0.5 mM each of C18:2$\Delta^{9,12}$ and C18:3$\Delta^{9,12,15}$) and incubated at 20 ^{O}C or 30^{O}C until colonies appeared. The master plates were replica-plated onto YPD plates for screening the fatty acid auxotrophic mutants. Based on the requirement of a specific fatty acid for the growth and the changes in gross morphology, determination of mutant growth on the media containing the individual fatty acids was further done for mutant grouping. The secondary screening of the mutants will be performed using analyses of fatty acid composition.

2.1.4 Lipid extraction and fatty acid analyses

Lipids were extracted from cell homogenates as previously described (Anamnart et al. 1998) or direct-transmethylation (Lepage and Roy 1984). Cellular fatty acid composition was analyzed by Agilent 6890N Series GC, using a Hewlett-Packard HP-INOWAX column (30 m x 0.32 mm x 0.25 μm in film thickness) with a temperature gradient (10^{O}C/min from 150^{O}C to 180^{O}C, 5^{O}C/min from 180^{O}C to

200^{O}C, 0.5^{O}C/min from 200^{O}C to 205^{O}C, 5^{O}C/min from 205^{O}C to 250^{O}C and 5 min at 250^{O}C). Fatty acids were identified by comparison their retention times with those of commercially available methyl ester standards.

2.1.5 Oligonucleotides and genetic manipulations

Tetrad analyses of *H. polymorpha* were kindly done by Assoc. Prof. Yoshinobu Kaneko, performed as described (Gleeson and Sudbery 1988). Total RNA of *H. polymorpha* SH4330 was extracted by TRI reagent (Molecular Research Center) and used as template for first strand cDNA synthesis by reverse transcription-polymerase chain reaction (RT-PCR) using Superscript III reverse transcriptase (Invitrogen) and oligo−dT as primer. Based on available sequence information, the Δ^{12}-desaturase cDNA of *H. polymorpha* (*HpDES12*) was amplified by using two degenerate primers (KL112-FD12 and KL113-RD12) designed from the conserved histidine domains (AHECGHQ and THVLHH) of Δ^{12}-desaturases of yeast and fungi. The 3' end of the Δ^{12}-desaturase cDNA was amplified by 3'-RACE method, using primer#7 and the synthesized first stand cDNA as template. For amplifying the partial Δ^{12}-desaturase cDNA, the nested PCR was done in which primer KL112-FD12 as sense primer and primer #8 as antisense primer were used for the 1st PCR following by 2nd PCR (KL114-3RACE and and primer #8 were used as sense and antisense primers, respectively). The 5' end of the Δ^{12}-desaturase cDNA was amplified by 5'-RACE system for rapid amplification of cDNA ends version 2.0 (Invitrogen). Using total RNA of *H. polymorpha* SH4330 as template, the first strand cDNA was amplified by using KL113-RD12 as primer and then RNA was removed by RNaseH treatment. To get dC-tailed first strand cDNA, dCTP was added to the first strand cDNA by enzyme terminal deoxynucleotidyl transferase (TdT). The dC-tailed first strand cDNA was used as template for nested PCR; 1st PCR was performed using AAP and KL113-RD12 as sense and antisense primers, respectively following by 2nd PCR (AAP and KL115-5RACE were used as sense and antisense primers). Finally, the complete open reading frame (ORF) of *HpDES12* was amplified by using KL116-FD12 and KL117-RD12 primers. The oligonucleotides used in this study are shown in Table 1.

2.1.6 Analysis of the structural gene

Analysis of the amino acid sequences was performed using GENETYXE-WIN Version 3.1. Hydropathy profiles were analyzed using a Kyte-Doolittle scale (Kyte and Doolittle 1982). Phylogenetic tree was generated using MAFFT program.

Table 1 Oligonucleotides used in this study

Oligonucleotides	Sequence
KL112-FD12	5′-GCN CAY GAR TGY GGN CAY CA-3′
KL113-RD12	5′ TGR TGN ARN ACR TGN GT-3′
primer #7	5′-GAG GAC TCG AGC TCA AGC TTT TTT TTT TTT TTT TT-3′
primer #8	5′-GAG GAC TCG AGC TCA AGC-3′
KL114-3RACE	5′-CAA CAC ACC GAT GTC TCG C-3′
AAP (Abridged anchor primer)	5′-GGG CCA CGC GTC GAC TAG TAC GGG IIG GGI IGG GII G-3′
KL115-5RACE	5′-CTT GTG AGG TGT CCG GTG-3′
KL116-FD12	5′-CCC AAG CTT ATG TCG ACC ACT GTG ACA C -3′
KL117-RD12	5′-TCC CCC GGG TCA AGA TTT TGG AGC CAC C -3′

2.2 ผลการวิจัย (Results)

2.2.1 Isolation and characterization of fatty acid auxotrophic mutants

Cells of *H. polymorpha* SH4330 (*ura3-1*) were subjected to EMS mutagenesis. To identify mutants with incapability of saturated fatty acid synthesis (*sfa*- mutants), the mutagen-treated cells were spread onto YPD agar supplemented with SFA. Colonies were then replicated onto the YPD medium. Therefore, cells defective in fatty acid synthesis, especially SFA synthesis, would fail to grow on YPD without SFA supplementation. From comparison of >20,000 colonies between the master and replica plates based on the growth ability, 30 presumptive mutants were identified and designated as S1 to S30 After subculturing for 10 generations, only 24 mutants demonstrated their stable inability to proliferate on YPD agar without addition of exogenous SFA. In the same manner, *mfa*- and *pfa*- mutants were identified by their inability to proliferate on YPD agar without addition of exogenous MFA and PFA, respectively. After subculturing for 10 generations, only 2 *mfa*- and 8 *pfa*- mutants displayed their stable inability to proliferate on YPD agar without addition of exogenous MFA and PFA, respectively.

The *sfa*- mutants were further characterized on agar plates of YPD supplemented with either individual saturated fatty acids or the mixture of MFA or PFA (Table 2). Most of the *sfa*- mutants could grow on YPD supplemented with C14:0 as well as the mixture of SFA and could not grow on YPD supplemented with C16:0 or C18:0. The mutants S1, S2, S3, S4, S7, S15 and S29 could slightly grow on the media supplemented with C16:0. Obviously, only S1 was able to slightly grow on YPD with the mixture of MFA or PFA. Like in the *Saccharomyces cerevisiae* mutant defective in saturated fatty acid biosynthesis, the growth-promoting effect of long-chain fatty acids for the *fas*- mutant distinctly decreases from myristate to stearate (Schweizer and Bolling 1970). The *mfa*- mutants were characterized on YPD agar plates supplemented with either individual monounsaturated fatty acids or the mixture of SFA or PFA (Table 3). M84 and M85 showed the same phenotype that they could grow on YPD supplemented with C16:1Δ^{9} as well as the mixture of MFA but could not grow on YPD supplemented with SFA. They had a slow growth on YPD supplemented with C18:1Δ^{9} or the mixture of PFA. All of the *pfa*- mutants could grow on YPD supplemented with either the mixture of MFA or PFA or individual unsaturated fatty acids, C16:1Δ^{9}, C18:1Δ^{9}, C18:2$\Delta^{9,12}$. It is noteworthy that, C18:3$\Delta^{9,12,15}$ inhibited the growth of both wild type and *pfa*- mutants. This may a result of the toxic effect of the products caused by autooxidation of polyunsaturated fatty acids in cellular lipids (Bilinski et al. 1989).

Table 2 Growth phenotype of *sfa*- mutants grown on YPD with or without FA supplementation at 30^{O}C, for 3-4 days

Strains	YPD	YPD supplemented with FA					
		SFA	MFA	PFA	C14:0	C16:0	C18:0
S1	-	++	+	+	++	+	-
S2	-	++	-	-	++	±	-
S3	-	++	-	-	++	+	-
S4	-	++	-	-	++	+	-
S5	-	+	-	-	++	-	-
S6	-	++	-	-	++	-	-
S7	-	++	-	-	++	+	-
S9	-	++	-	-	++	-	-
S11	-	++	-	-	++	-	-
S12	-	++	-	-	++	-	-
S14	-	++	±	±	++	-	-
S15	-	++	-	-	++	+	-
S16	-	++	-	-	++	-	-
S17	-	++	-	-	++	-	-
S18	-	++	-	-	++	-	-
S19	-	++	-	-	++	-	-
S20	-	++	-	-	++	-	-
S22	-	++	-	-	++	-	-
S23	-	++	-	-	++	-	-
S24	-	++	-	-	++	-	-
S27	-	++	-	-	++	-	-
S28	-	++	-	-	++	-	-
S29	-	++	-	-	++	±	-
S30	-	+	-	-	++	-	-
SH4330	+++	+++	+++	+++	+++	+++	+++

Note: +++ means fast growth, ++ means growth, + means slow growth, ± means poor growth, - means no growth

Table 3 Growth phenotype of *mfa*- mutants grown on YPD with or without FA supplementation at 30^{O}C, for 3-4 days

Strains	YPD	YPD supplemented with FA				
		SFA	MFA	PFA	C16:1Δ^{9}	C18:1Δ^{9}
M84	-	-	++	+	++	+
M85	-	-	++	+	++	+
SH4330	+++	+++	+++	+++	+++	+++

Note: +++ means fast growth, ++ means growth, + means slow growth, - means no growth

Table 4 Growth phenotype of *pfa*- mutants grown on YPD with or without FA supplementation at 20^{O}C for 7 days

Strains	YPD	YPD supplemented with FA						
		SFA	MFA	PFA	C16:1$^{\Delta_9}$	C18:1$^{\Delta_9}$	C18:2$^{\Delta_{9,12}}$	C18:3$^{\Delta_{9,12,15}}$
P36	-	-	++	++	++	++	+	-
P37	-	-	++	++	++	++	++	-
P49	-	-	+	++	+	++	+	-
P52	-	-	+	++	+	++	+	-
P55	-	-	++	++	++	++	++	-
P60	-	-	+	+	+	+	+	-
P62	-	-	+	++	$\pm$	+	+	-
P63	-	-	+	++	+	+	+	$\pm$
SH4330	+++	+++	+++	+++	+++	+++	+++	+

Note: +++ means fast growth, ++ means growth, + means slow growth, $\pm$ means poor growth, - means no growth

2.2.2 Fatty acid compositions of *H. polymorpha* fatty acid auxotrophic mutants

Fatty acid compositions of the WT and the *sfa*- mutant strains cultivated in YPD supplemented with C14:0 or SFA are presented in Table 5. As observed in *H. polymorpha* strains examined, fatty acids supplied to yeast cells in the growth media were found to be preferentially incorporated into the cells during the cultivation and become a major fraction of the cellular fatty acids (Anamnart et al. 1998; Rakpuang 2009) in contrast with the cultures supplemented with exogenous C14:0.

In this study, the exogenous C14:0 was not highly accumulated in cells of the both WT and the *sfa*- mutants. These may a cause of the cell response to maintain proper C14:0 homeostasis, which is a critical mechanism of the cells (Orme et al. 1972).

Myristate is essential for eukaryotic cells, not only to provide a concentrated engery source and to serve as an important building block for membrane structure, but also to mediate post-translation modification of proteins (Cross 1987, Towler et al. 1987). A deficit in the myristoyl-CoA pool leads to arrest of cell growth in yeast (Duronio et al. 1991). In contrast, excess intracellular accumulation of the free-fatty acid can be toxic (Oshiro et al. 2003; Tong et al. 2006). Prasitchoke and coworkers (2008) found that C14:0, but not other fatty acids, caused severe growth retardation in *Hpelo1*Δ and *Hpelo2*Δ mutants of yeast *H. polymorpha*, defective in elongation of very long-chain fatty acids. Thus, it is important to understand how cells regulate the C14:0 pool, and that of other fatty acids. From the results of fatty acid analysis, two *sfa*- mutants S7 and S16 showed significant difference in the fatty acid composition. It is clear that S7 defected in the production of PUFAs as the absence of C18:2$\Delta^{9,12}$ and C18:3$\Delta^{9,12,15}$ in its fatty acid profile. We simply assumed that S7 may have a defect of Δ^{12}-desaturase activity. However, S7 required the saturated fatty acids for growth. This might be a possibility that S7 had double mutation, while S16 significantly accumulated medium-chain saturated fatty acids, C12:0 and C14:0. Myristoyl-CoA pool in S16 mutant could be changed by the dynamic interplay between non-Fas acyl chain elongation systems and degradative pathways.

Fatty acid compositions of the WT and the *mfa*- and *pfa*- mutant strains cultivated in YPD supplemented with C16:1Δ^{9} are presented in Table 6. We designed to use C16:1Δ^{9} as exogenous uasaturated fatty acid for supplementing growth of the both *mfa*- and *pfa*- mutants for fatty acid analysis because of *H. polymorpha* WT usually contains very small amount of C16:1Δ^{9} (Anamnart et al. 1998) and the both *mfa*- and *pfa*- mutants could grow on the media supplemented with either C16:1Δ^{9} or C18:1Δ^{9}. The changes in fatty acid profile occurred when the culture was exposed to supplemented fatty acid implies that there was a modification of the activity of enzymes involved in fatty acid synthesis, elongation, desaturation, and β-oxidation. The data obtained from the analysis of the cell culture of *mfa*-, *pfa*- mutants and WT that grown in the presence of C16:1Δ^{9} showed a high level of unknown fatty acid (supposed to be 16:1$\Delta^{9,12}$). These data evidently showed that C16:1Δ^{9} and its derivatives were incorporated into lipid-containing cell constituents instead of the C18 unsaturated fatty acids.

Table 5 Fatty acid composition (% of total fatty acids) of wild type and *sfa*- mutant of *H. polymorpha*

Strain	C12:0	C14:0	C16:0	C16:1$^{\Delta 9}$	C16?	C17:0	C18:0	C18:1$^{\Delta 9}$	C18:1$^{\Delta 11}$	C18:2$^{\Delta 9,12}$	C18:3$^{\Delta 9,12,15}$
S1[a]	0	1.32	10.68	2.26	2.24	3.06	4.07	18.10	1.97	42.84	8.51
S2[a]	0	1.09	13.96	2.62	3.57	1.54	4.89	9.56	3.50	40.16	11.19
S3[a]	0.16	1.39	7.58	2.34	3.34	1.60	4.78	11.22	2.68	45.93	12.78
S4[a]	0	3.60	14.20	3.05	3.60	1.12	4.89	8.49	2.28	41.87	14.78
S5[a]	0	2.16	12.65	2.14	1.59	0	5.68	15.97	2.75	39.67	14.77
S6[a]	0	1.81	12.39	2.82	3.88	1.11	5.00	8.81	3.83	39.98	12.15
S7[a]	-	1.37	32.04	6.08	0	-	12.97	34.33	0	0	0
S7[b]	-	1.17	24.08	2.79	0	-	14.48	42.34	0	0	0
S9[a]	0.19	1.35	13.57	3.45	3.86	1.28	4.76	9.96	3.52	40.83	10.79
S11[a]	0	1.57	13.46	3.02	4.32	1.50	6.37	9.23	3.58	40.90	11.59
S12[a]	0	0.68	8.85	1.07	1.93	1.76	6.99	9.96	3.53	41.05	15.10
S15[b]	-	0.92	19.84	1.18	0	-	11.49	10.56	0	34.20	8.28
S16[a]	30.75	8.78	10.20	1.29	2.03	0	3.17	5.16	0	20.90	7.32
S17[a]	0	2.36	15.84	2.57	2.56	1.21	7.94	11.31	1.97	37.26	13.30
S18[a]	0	1.16	11.60	2.39	3.23	1.36	5.02	10.01	3.55	38.92	15.26
S19[a]	0.20	1.52	16.12	1.97	3.26	1.34	9.74	8.28	2.97	31.53	13.98
S20[a]	0	1.33	13.13	1.73	2.61	0	4.71	8.37	2.96	41.85	17.91
S22[a]	0	2.35	14.65	2.77	3.61	0	4.96	8.49	2.45	38.60	15.53
S23[a]	0.19	1.38	13.38	2.49	2.94	1.35	7.41	8.75	3.38	35.87	14.49
S24[a]	0	1.48	12.61	3.26	4.33	0	4.62	7.95	3.85	42.64	17.48
S27[a]	0	6.04	17.45	3.91	4.26	1.29	7.18	8.95	0	35.34	13.29
S28[a]	0	1.20	11.80	2.26	3.36	1.71	6.19	10.57	3.36	40.79	11.55
S29[a]	0	1.95	11.35	3.16	3.83	1.16	3.85	10.60	3.81	44.14	11.03
S30[a]	0.19	2.44	22.31	1.98	2.55	0.91	7.04	14.20	2.88	34.09	9.65
WT[a]	-	2.93	26.04	4.40	0	-	8.09	23.70	0	22.24	5.31
WT[b]	-	1.32	23.81	2.61	0.56	-	5.38	31.73	0	18.23	2.04

[a]Fatty acid composition in cells grown on YPD+C14:0 (0.5 mM) at 30^{O}C, 150 rpm for 48 h.

[b]Fatty acid composition in cells grown on YPD+SFA (0.5 mM each of C14:0, C16:0, C18:0) at 30^{O}C, 150 rpm for 24 h and then transfer to YPD to prolonged cultivation for 24 h.

Table 6 Fatty acid composition (% of total fatty acids) of wild type *H. polymorpha* and *mfa-* and *pfa-* mutants

Strain	C12:0	C14:0	C16:0	C16:1$^{\Delta_9}$	C16?	C17:0	C18:0	C18:1$^{\Delta_9}$	C18:1$^{\Delta_{11}}$	C18:2$^{\Delta_{9,12}}$	C18:3$^{\Delta_{9,12,15}}$
M84[c]	0	0.83	30.58	24.77	9.89	0.48	30.38	0	1.13	0	0
M85[c]	0.14	0.93	29.97	22.84	9.63	0.52	31.34	1.92	1.07	0	0
P36[c]	0.12	1.18	25.69	19.52	10.92	0	24.44	4.77	2.56	6.15	0
P37[c]	0.14	1.10	18.18	33.19	11.80	0.64	12.50	5.74	9.14	4.94	0
P52[c]	0.14	0.84	24.56	31.16	13.99	0	20.53	1.38	1.85	1.39	0
P52[d]	0.10	0.69	21.17	1.47	0.52	1.08	4.81	23.38	0	38.37	6.19
P55[c]	0	0.72	15.89	52.48	5.56	1.41	10.87	1.50	10.09	0	0
P60[c]	0	1.02	25.56	31.56	18.39	0	19.74	0	0	0	0
P63[c]	0	0.61	20.57	37.84	15.04	0.53	19.17	0	2.49	0	0
WT[c]	-	0.45	16.38	32.63	3.70	-	7.36	7.93	9.17	7.00	0.85

[c]Fatty acid composition in cells grown in YPD+C16:1Δ^9 (0.5 mM) at 30^{O}C, 150 rpm for 48 h.

[d]Fatty acid composition in cells grown in YPD+C18:1Δ^9 (0.5 mM) at 30^{O}C, 150 rpm for 48 h.

C16? was supposed to be C16:2$\Delta^{9,12}$ because it has been reported in the previous study in a similar fashion (Lu et al. 2000; Anamnart et al. 1998).

2.2.3 Genetic analysis of the saturated fatty acid auxotrophic mutant (S7)

We further investigated S7 mutant by constructing diploid hybrid (H69) between S7 *sfa-* auxotrophic mutants (*ura3-1*) and WT strains (*ade1-1*). It was observed that the diploid was prototrophic for SFA, indicating the recessiveness of mutation in the mutant. Tetrad analysis of the hybrid diploid demonstrated 2 Sfa+ : 2 Sfa- segregation in l0 tetrads, indicating that single nuclear mutation conferred Sfa- phenotype on the mutant (Table 7). However, from the result of fatty acid composition S7 was defective in the production of C18:2$\Delta^{9,12}$ and C18:3$\Delta^{9,12,15}$ (Table 5). We assumed that if S7 had two lesions or double mutation (*Hpsfa7*, *Hpdes12*), we could able to separate the two mutants by tetrad analysis. For this purpose, we picked up the meiotic segregants of two asci to analyse fatty acid composition. The results (Table 8) showed that S7 had double mutation (*Hpsfa7*, *Hpdes12*) and we could separate *Hpsfa7* (in H69-2A and H69-2B) and *Hpdes12* (in H69-2C and H69-2D) from each other. Both H69-2C and H69-2D showed Sfa+ phenotype and grew on YPD without fatty acid supplementation indicating that the lesion at Δ^{12}-desaturation (*Hpdes12*) of the mutant did not affect cell growth (Table 7).

Table 7 Phenotype of the meiotic segregants from three asci of diploid hybrid (H69) between S7 saturated fatty acid auxotrophic mutants (*ura3-1*) and *H. polymopha* WT strains (*ade1-1*)

	YPD+C14:0	YPD	Drop Out medium (amino acid/base) + C14:0				
			Ade-	Leu-	Ura-	Ade-Leu-Ura-	Synthetic Complete
H69-1A	+	-	+	+	-	-	+
H69-1B	+	-	-	+	-	-	+
H69-1C	+	+	+	+	+	+	+
H69-1D	+	+	-	+	+	-	+
H69-2A	+	-	+	+	-	-	+
H69-2B	+	-	-	+	-	-	+
H69-2C	+	+	-	+	+	-	+
H69-2D	+	+	+	+	+	+	+
H69-3A	+	-	+	+	-	-	+
H69-3B	+	-	-	+	-	-	+
H69-3C	+	+	+	+	+	+	+
H69-3D	+	+	-	+	+	-	+
H69	+	+	+	+	+	+	+
SH4331	+	+	-	+	+	-	+
S7	+	-	+	+	-	-	+

Note: + means Growth; - mean No growth

Table 8 Fatty composition of the meiotic segregants of H69 grown in the YPD medium supplemented with C14:0 at 30°C

Segregant	Fatty acid composition (% in TFA)						
	C14:0	C16:0	C16:1$^{\Delta_9}$	C18:0	C18:1$^{\Delta_9}$	C18:2$^{\Delta_{9,12}}$	C18:3$^{\Delta_{9,12,15}}$
H69-1A (*ura3-1*, *Hpsfa7*, *Hpdes12*)	15.4	18.9	20.4	6.2	39.1	0.0	0.0
H69-1B (*ade1-1, ura3-1*, *Hpsfa7*, *Hpdes12*)	5.8	15.6	18.4	7.6	52.6	0.0	0.0
H69-1C (Prototroph)	6.4	18.6	16.8	8.2	24.3	20.2	5.4
H69-1D (*ade1-1*)	5.2	19.4	13.2	7.8	24.9	24.6	4.9
H69-2A (*ura3-1*, *Hpsfa7*)	7.8	17.4	12.0	5.0	27.7	23.9	6.3
H69-2B (*ade1-1, ura3-1*, *Hpsfa7*)	18.6	19.4	12.2	3.1	11.7	23.3	11.6
H69-2C (*ade1-1, Hpdes12*)	6.0	22.4	16.1	5.5	50.0	0.0	0.0
H69-2D (*Hpdes12*)	2.7	14.7	16.8	3.7	62.1	0.0	0.0

2.2.4 Characterization of *Hpsfa7* mutation

To clarify the phenotype of *Hpsfa7*, we conducted the comparison of S7 parental strain (*Hpsfa7*, *Hpdes12*) and its segregants, H69-2A and H69-2B (*Hpsfa7*), by growing them on the media that supplemented with various fatty acids (Figures 1-3). Results showed that the segregant H69-2B displayed similar properties to parental mutant (S7) on media supplemented with various fatty acids in contrast to H69-2A. At 30^{O}C, the mutant S7 and its segregant H69-B could slightly grow on YPD supplemented with C16:0 but H69-2A could not. However, the segregant H69-2A could be rescued by exogenous C16:0 at a higher temperature, 42^{O}C (Figure 2).

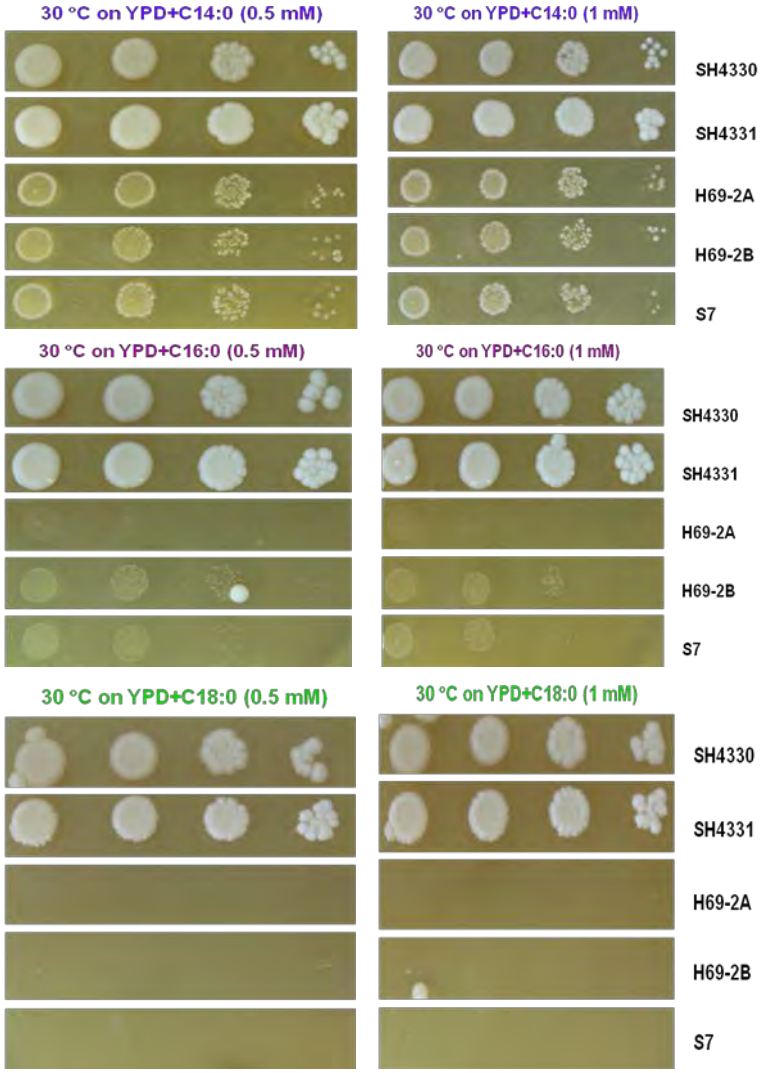

Figure 1 Serial dilution of *H. polymorpha* SH4330 (WT), SH4331 (WT), S7 (*Hpsfa7*, *Hpdes12*), H69-2A (*Hpsfa7*) and H69-2B (*Hpsfa7*) on the YPD media supplemented with C14:0 (0.5 mM, 1.0 mM), C16:0 (0.5 mM, 1.0 mM), C18:0 (0.5 mM, 1.0 mM)

In *S. cerevisiae*, treatment of strains containing *nmt1-181* with cerulenin, the antibiotic which inhibits fatty acid synthetase activity, causes growth arrest at 24, 30 and 36^{O}C. This arrest can be reversed by adding exogenous myristate but not palmitate to the medium, suggesting that metabolic interconversion of C16:0 to C14:0 is not sufficient to restore myristoyl-CoA pools to a level that permits growth of this mutant (Duronio et al. 1991). The *S. cerevisiae nmt1-181* mutant without cerulenin was able to grow in YPD supplemented with 1mM palmitate at 24^{O}C but was not able to grow at 30 and 36^{O}C (Duronio et al. 1992).

Moreover, at 30^{O}C the combination of C16:0, C18:0 and C18:2 could rescue the lethality of H69-2A, while mixture of C16:0+C18:0 or only C18:2 could not support the growth.

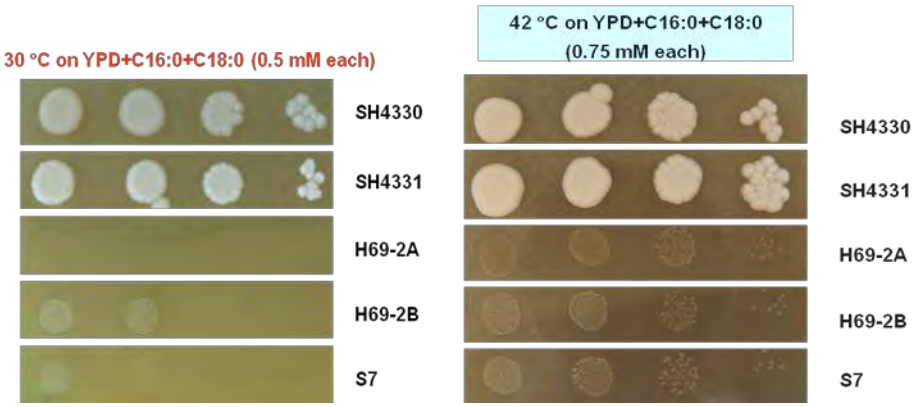

Figure 2 Effect of temperature on the growth of H69-2A segregant. Serial dilution of *H. polymorpha* SH4330 (WT), SH4331 (WT), S7 (*Hpsfa7*, *Hpdes12*), H69-2A (*Hpsfa7*) and H69-2B (*Hpsfa7*) on the YPD media supplemented with C16:0 and C18:0 (0.5 mM each) incubated at 30^{O}C and 42^{O}C.

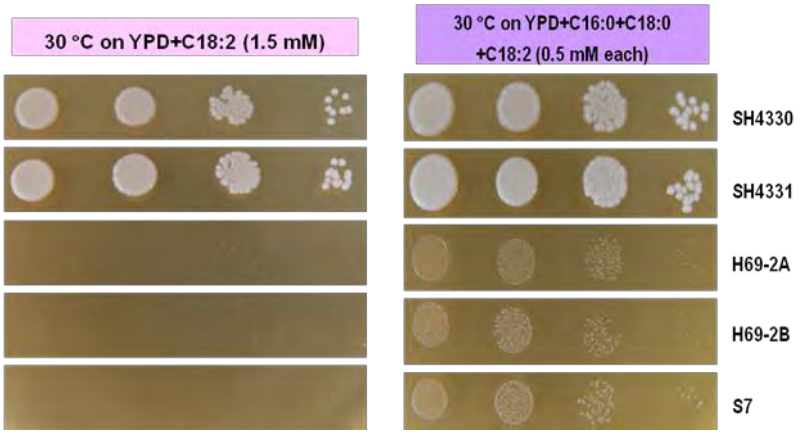

Figure 3 Supplementaton of a mixture of C16:0, C18:0 and C18:2 rescued the lethality of H69-2A segregant at 30^{O}C. Serial dilution of *H. polymorpha* SH4330 (WT), SH4331 (WT), S7 (*Hpsfa7*, *Hpdes12*), H69-2A and H69-2B (*Hpsfa7*) on YPD media supplemented with C18:2 (1.5 mM) or the mixture of C16:0, 18:0 and C18:2 (0.5 mM each).

2.2.5 Cloning and sequencing of the *HpDES12* of *H. polmorpha* SH4330 and *Hpdes12* of S7

Based on available sequence information, the Δ^{12}-desaturase cDNA was cloned from *H. polymorpha* SH4330 by RT-PCR using two degenerate primers designed from the conserved histidine domains (AHECGHQ and THVLHH) of Δ^{12}-desaturases of yeast and fungi (Passorn et al. 1999). The 5' and 3' ends of the Δ^{12}-desaturase cDNA were amplified by RACE method (as described in materials and methods 2.1.5). Sequence analysis of the full length cDNA of *H. polymorpha* showed an open reading frame of 1215 bp encoding for 404 amino acid residues (GenBank Accession No. GU226432) (Figure 4).

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ATGTCGACCACTGTGACACAACGCCGTTTTGGTGACAAGTCTACCAAAGGGCTGCGCGCT   60
 M  S  T  T  V  T  Q  R  R  F  G  D  K  S  T  K  G  L  R  A
ATAGACACCGAGGGCAACGAGTTCCAAGTGCCAGACTACTCCATTAAGGAGATTTTGGAC  120
 I  D  T  E  G  N  E  F  Q  V  P  D  Y  S  I  K  E  I  L  D
AGTATCCCTAAAGAGTGTTTCGAACGCAGACTGACCACGTCCTTTTATTACGTTTTCAGA  180
 S  I  P  K  E  C  F  E  R  R  L  T  T  S  F  Y  Y  V  F  R
GATATTGCCGTTTGTCTCGCCATGGGCTGGGTGGCCAATACATACATTCCGCAGGTTCCC  240
 D  I  A  V  C  L  A  M  G  W  V  A  N  T  Y  I  P  Q  V  P
TGGGCAGCCTTGAGAGGCGCCTTGTGGCTTGGTTACGCGATCCTGCTGTCGCTGCCTTAT  300
 W  A  A  L  R  G  A  L  W  L  G  Y  A  I  L  L  S  L  P  Y
ACTGGACTATGGGTTTTGGGACACGAGTGCGGCCACCAGGCATTTTCGGACTACGGCTGG  360
 T  G  L  W  V  L  G  H  E  C  G  H  Q  A  F  S  D  Y  G  W
CTTAACGACACTGTGGGATGGATCATCCACTCCTACTTGTTCGTTCCGTACTTCTCCTGG  420
 L  N  D  T  V  G  W  I  I  H  S  Y  L  F  V  P  Y  F  S  W
AAATACAGCCACGGCAAGCACCACAAGGCCACCGGACACCTCACAAGGGATATGGTTTTC  480
 K  Y  S  H  G  K  H  H  K  A  T  G  H  L  T  R  D  M  V  F
GTTCCTAAGACTGTTGAGGAGTTCAAGAAAGAGCGGGCAGGGGATTCGCGTGTGAAGCTC  540
 V  P  K  T  V  E  E  F  K  K  E  R  A  G  D  S  R  V  K  L
AGTGAGCTTTCTGAGGACACTCCAATCCAGACCCTGACGTCGTTGCTAATGCAGCAGTTT  600
 S  E  L  S  E  D  T  P  I  Q  T  L  T  S  L  L  M  Q  Q  F
GGAGGCTGGTGGTGGTATCTGTTGACCAACGTCACTGGCCAAAAATACCCAGACCACAAC  660
 G  G  W  W  W  Y  L  L  T  N  V  T  G  Q  K  Y  P  D  H  N
AAGTTTGCCGTGTCGCACTTCAACCCTGCGTCGCCGATCTTTGAGAAGCGCGATTACTGG  720
 K  F  A  V  S  H  F  N  P  A  S  P  I  F  E  K  R  D  Y  W
TACGTCGTGCTCTCCGACATCGGCGTGCTGGCGCAGTCGTTTGTGGTGTACCAGTGGTGC  780
 Y  V  V  L  S  D  I  G  V  L  A  Q  S  F  V  V  Y  Q  W  C
AAGAGCTTTGGAGGCTTCCACTGCTTCATCAACTGGTTTCTGCCATACGTTTTCACCAAC  840
 K  S  F  G  G  F  H  C  F  I  N  W  F  L  P  Y  V  F  T  N
CATTGGCTTGTGTTCATCACGTATCTGCAACACACCGATGTCTCGCTGCCTCACTACGAC  900
 H  W  L  V  F  I  T  Y  L  Q  H  T  D  V  S  L  P  H  Y  D
AACAACGAATGGACATTTGCTAGAGGCGCCGCCGCCACGATCGATAGAGAGTTTGGCTTC  960
 N  N  E  W  T  F  A  R  G  A  A  A  T  I  D  R  E  F  G  F
GTTGGCTGGTTTTTTTTCCACGATATTATAGAGACCCACGTTCTGCACCACTACGTTTCC  1020
 V  G  W  F  F  F  H  D  I  I  E  T  H  V  L  H  H  Y  V  S
CGTATTCCGTTCTACAACGCTAGGCCGGCCACTGAGGGCATCAAGAAGGCCATGGGCATC  1080
 R  I  P  F  Y  N  A  R  P  A  T  E  G  I  K  K  A  M  G  I
CATTACCGCCACAGCGACGAGTCCATGTGGTACACTCTGTGGAAATCCGCCAAGGCGTGC  1140
 H  Y  R  H  S  D  E  S  M  W  Y  T  L  W  K  S  A  K  A  C
CAGTTCGTGGAGGGTGATAACGGTGTTAGGATGTTCCGCAATATAAACGGTGTGGGGGTG  1200
 Q  F  V  E  G  D  N  G  V  R  M  F  R  N  I  N  G  V  G  V
GCTCCAAAATCTTGA                                               1215
 A  P  K  S  *
```

Figure 4 The nucleotide sequence of *HpDES12* of *H. polymorpha* SH4330 and its deduced amino acid sequence.

The deduced amino acid sequences exhibited homology with the Δ^{12}-desaturases of other organisms including yeast, fungi and plants. Moreover, three histidine-rich motifs with eight invariant histidine residues are presented in the hydrophilic parts (Figure 5) that have been proposed to be essential for catalytic function of the enzyme (Passorn et al. 1999).

Figure 5 Alignment of the amino acid sequence of Δ^{12}-desaturase of *H. polymorpha* SH4330, *Pichia pastoris*, *Mucor circinelloides* and *Mortierella alpina.* Histidine-rich motifs are shown on black background.

However, it had the highest similarity to the Δ^{12}-desaturase of yeast that corresponds to the result of phylogenetic analysis (Figure 6). The conserved characteristics of membrane-bound desaturases were found in the *H. polymorpha* gene (Lost and Murata 1998). Two long hydrophobic regions, which are membrane spanning domains, were observed (Figure 7).

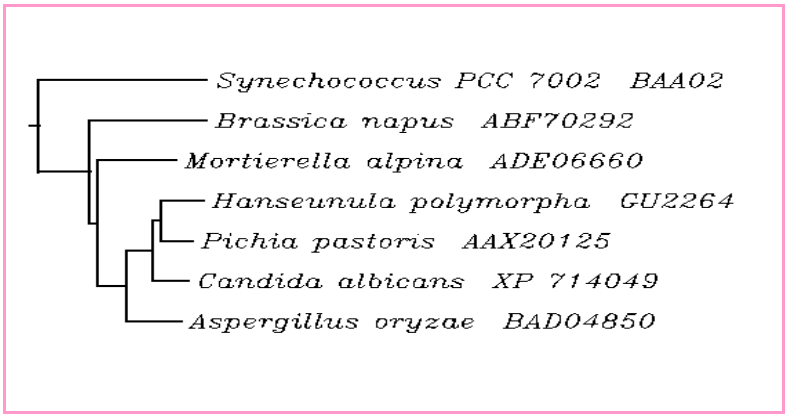

Figure 6 Phylogenic relationships of Δ^{12}-desaturases of various organisms.

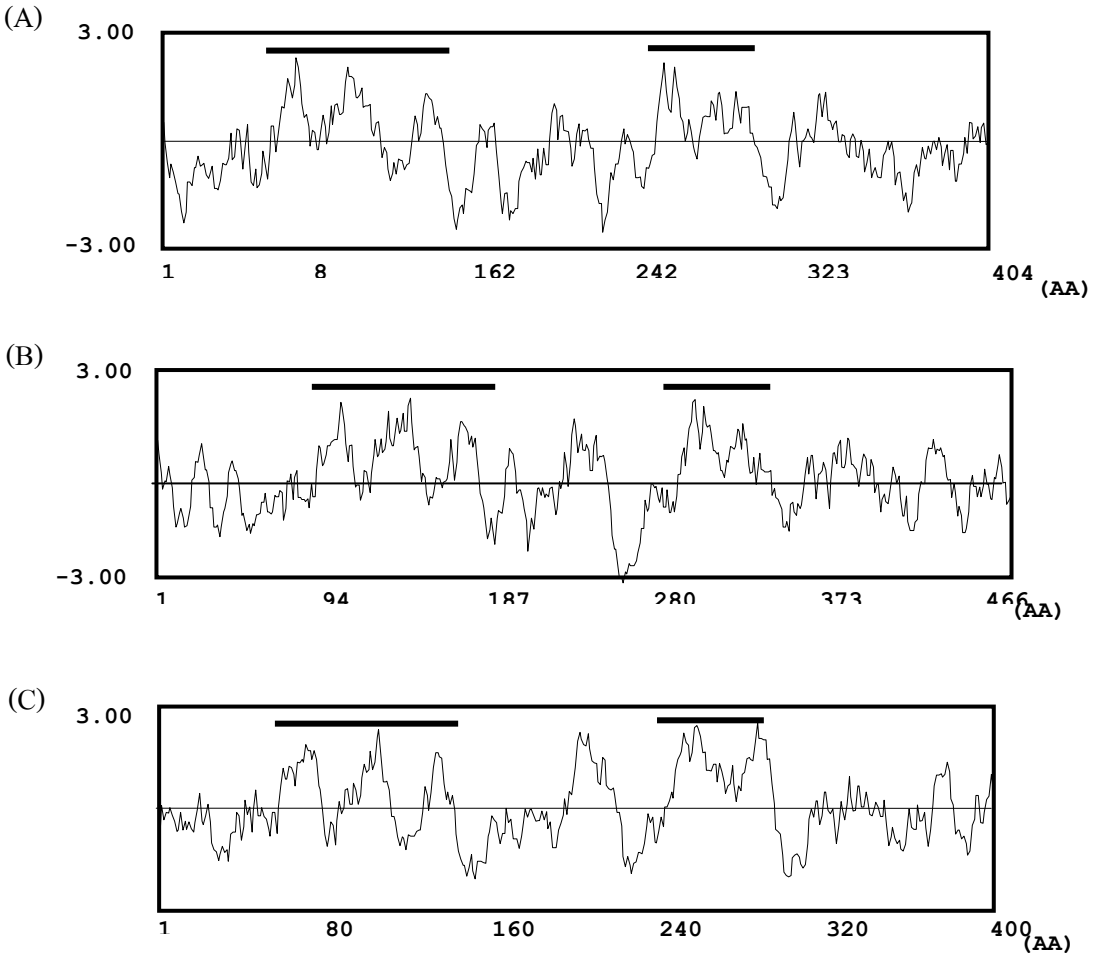

Figure 7 Hydropathy analysis of Δ^{12}-desaturases of *H. polymorpha* SH4330 (A), *Aspergillus oryzae* (B) and *Mortierella alpina* (C) by using Kyte-Doolittle scale. X-axis is the number of amino acid. Bars indicate hydrophobic domains.

Hpdes12 was also cloned from cDNA of *H. polymorpha* S7 mutant. We found that the nucleotide sequence of *Hpdes12* at nt +245 relative to translation start site (ATG) was changed from G to A leading to missense mutation that tryptophan was changed to stop codon (TGG to TGA) (data not shown).

อภิปราย/วิจารณ์ (Discussion)

Sfa- phenotype of the *H. polymorpha* saturated fatty acid auxotrophic (*sfa*-) mutants can be fully suppressed by supplementing media with myristate (C14:0), but not longer chain saturated fatty acids (C16:0, C18:0) that was closely similar to *fas-15* and *nmt1-181* phenotypes of *S. cerevisiae* defective in the function of fatty acid synthetase complex and N-myristoyltransferase (NMT), respectively (Johnson et al. 1994; Duronioet al. 1992, Duronioet al. 1991; Schweizer and Bolling 1980). At least two pathways are available for generating myristoyl-CoA, de novo synthesis by fatty acid synthetase complex (FAS) and activation of exogenous myristate by acyl-CoA synthetase. The de novo pathway for production of long chain acyl-CoAs in *S. cerevisiae* requires acetyl-CoA carboxylase (ACC), which produces malonyl-CoA for utilization by FAS (Mishina et al., 1980). FAS produces palmitoyl-CoA and stearoyl-CoA as its principal products (Lynen, 1980). Myristoyl-CoA is a minor product representing 3-5% of the total acyl-CoAs produced by FAS in vitro and in vivo (Singh et al., 1985). Considering that C14:0 is an essential and multi-functional fatty acid in a variety of organisms although it comprises a small fraction of cellular fatty acids (1-5% fatty acids). These may a cause of the cell response to maintain proper C14:0 homeostasis that is a critical mechanism of the cells (Orme et al. 1972). As though, the exogenous C14:0 was not highly accumulated in cells of the both *H. polymorpha* WT and the *sfa*- mutants, except S16 mutant. In *S. cerevisiae*, the *FAA1* (fatty acid activation) gene is required for the utilization of exogenous myristate by NMT and for the synthesis of several phospholipid species. Supplementation of cellular myristoylCoA pools through activation of imported myristate is predominantly a function of Faa1p, although Faa4p contributes to this process (Johnson et al. 1994). The *H. polymorpha* S16 mutant may be mutated in the gene that responsible for targeting imported fatty acids to peroxisomal β-oxidation pathways, consequently, it accumulated medium-chain saturated fatty acids, C12:0 and C14:0. For the lesion at Δ^{12}–desaturation (*Hpdes12*) of the segregants of *H. polymorpha* S7 mutant having Sfa+ phenotype, the sequence analysis of its cloned Δ^{12}-desaturase gene confirms that the defect of PUFA synthesis was a result of missense mutation at the coding sequence of Δ^{12}-desaturase gene. However, the lack of intracellular PUFAs of the mutant did not affect cell growth. The question is how C18:2$\Delta^{9,12}$ and C18:3$\Delta^{9,12,15}$ the products of Δ^{12}-desaturase have any important function in cells. As the result of fatty acid supplementation, the combination of C16:0, C18:0 and C18:2 could promote the growth of S7 better than that supplemented with a mixture of saturated fatty acids (C16:0+C18:0), particularly at 30°C as shown in Figures 2 and 3. These results indicate the C18 PUFAs might play a role in adaptation of cell to environmental stress, such as oxidative stress response and proteasomal activity similar to the previous

study in the PUFA producing strain of *S. cerevisiae* carrying the *M. rouxii* Δ^{12} and Δ^{6}-desaturase genes (Ruenwai et al. 2011). Therefore, these mutants obtained are useful for further study of role of specific fatty acids on cell function of eukaryotes.

สรุปและเสนอแนะ (Summary)

Fatty acids are essential compounds in the cell. The bulk of cellular fatty acids that serve structural and biological functions contain acyl chain of 14-18 carbon atoms in length. Almost all subcellular organelles are involved in fatty acid metabolism; thus, maintenance of fatty acid homeostasis requires regulation at multiple levels. In this study, a number of *H. polymorpha* mutants defecting in fatty acid synthesis and having apparently altered phenotypes including growth, fatty acid composition was screened and selected. Based on the growth phenotype, the mutants were classified into two groups. One requires saturated fatty acids for growth (*sfa*- mutamts) and the other requires unsaturated fatty acids (*mfa*- and *pfa*- mutants). From the results of fatty acid analysis, two *sfa*- mutants S7 and S16 showed significant difference in the fatty acid composition compared with the wild type. The S7, which is a *sfa*- mutamt, was unable to produce C18:2$\Delta^{9,12}$ and C18:3$\Delta^{9,12,15}$ that was caused from the missence mutation at Δ^{12}-desaturase gene based on comparison between the nucleotide sequence of the cloned genes of the wild type and the mutant strains. This result indicates that it had double mutation when combined with the results of tetrad analysis and growth phenotype. The another *sfa*- mutant S16 significantly accumulated medium-chain saturated fatty acids, C12:0 and C14:0. Myristoyl-CoA pool in S16 mutant might be changed by the dynamic interplay between non-Fas acyl chain elongation systems and degradative pathways.

Further investigation was implemented using the mutants as tool for dissecting the role of fatty acid on cell response at particular conditions. Tetrad analysis provided us the *Hpsfa7* (H69-2A and H69-2B) and *Hpdes12* (H69-2C and H69-2D) segregants. Although H69-2C and H69-2D having Sfa+ phenotype was not able to synthesize C18 PUFAs, they grew normally on YPD without requirement of exogenous fatty acid indicating that the lesion at Δ^{12}-desaturase (*Hpdes12*) or in turn the absence of C18 PUFAs of the mutant did not affect cell growth. We found a difference between H69-2A and H69-2B on their growth at 30^{O}C. Possibly, H69-2A segregant had a mutation at other lesion in addition to the defect of fatty acid synthesis. In addition to the mutants characterized in this study, other several mutants are of interest based on their fatty acid and growth phenotypes, which should be further characterized. Not only fatty acid composition, the lipid composition and content of the mutants should be analyzed. These mutants are useful tools for addressing the association between fatty acid metabolism, cell physiology and function that may enhance cell properties as a host system for producing valuable metabolites.

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6. สาขาวิชาที่มีความชำนาญพิเศษ
   6.1 Yeast Genetics
   6.2 Fatty acid biosynthesis in yeasts

7. ประสบการณ์ที่เกี่ยวข้องกับการบริหารงานวิจัยทั้งภายในและภายนอกประเทศ
   7.1 ผู้อำนวยการแผนงานวิจัย – ชื่อแผนงานวิจัย

   7.2 หัวหน้าโครงการวิจัย:

   7.2.1 การวิเคราะห์สูตรโครงสร้างของกรดไขมันไม่อิ่มตัวชนิดใหม่จากเชื้อกลายพันธุ์ของยีสต์ แฮนเซนนูล่า โพลีมอรฟ่า (ทุนนักวิจัยใหม่ สวทช. ปี 2542-2544)

   7.2.2 การพัฒนาการผลิตน้ำมันจากเมล็ดชาน้ำมัน *Camellia oleifera*. (ทุนหน่วยวิจัย-พัฒนาอุตสาหกรรม จากสถาบันเทคโนโลยีชีวภาพและวิศวกรรมพันธุศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปี 2550)

   7.2.3 การสกัดน้ำมันจากเมล็ดแมงลักและคุณสมบัติการพองตัวของสารเมือกจากกากที่ เหลือ (ทุนรัชดาภิเษก จุฬาลงกรณ์มหาวิทยาลัย ปี 2550)

   7.2.4 การศึกษาแบบแผนกรดไขมันของเพรียงทรายจากฟาร์มเลี้ยงและจากธรรมชาติ (ทุนสำนักงานคณะกรรมการวิจัยแห่งชาติ (วช.) ปี 2551)

7.2.5 การศึกษาทางอณูพันธุศาสตร์ของการสังเคราะห์กรดไขมันและไขมันในยีสต์ *Hansenula polymorpha* (เงินทุนอุดหนุนงบประมาณแผ่นดินประจำปี 2551 – 2553)

7.3 งานวิจัยที่ทำเสร็จแล้ว

7.3.1 **Anamnart, S**., Tomita, T., Fukui, F., Fujimori, K., Harashima, S., Yamada, Y. and Oshima, Y. (1997) The *P*-OLE1 gene of *Pichia angusta* encodes a Δ9-fatty acid desaturase and complements the *ole1* mutation of *Saccharomyces cerevisiae*. *Gene* 184, 299-306.

7.3.2 **Anamnart, S**., Tolstorukov, II., Kaneko, Y. and Harashima, S. (1998**)** Fatty acid desaturation in methylotrophic yeast *Hansenula polymorpha* strain CBS 1976 and unsaturated fatty acid auxotrophic mutants. *J. Ferment. Bioeng*. 85, 476-482.

7.3.3 Fujimori, K., **Anamnart, S**., Nakagawa, Y., Sugioka, S., Oshima, Y., Yamada, Y. and Harashima, S. (1997**)** Isolation and characterization of mutations affecting expression of the Δ9- fatty acid desaturase gene,*OLE1*, in *Saccharomyces cerevisiae*. *FEBS Lett*. 413, 226-230.

7.3.4 Lu, S.F., Tolstorukov, I.I., **Anamnart S**., Kaneko, Y., Harashima, S. (2000) Cloning, sequencing, and functional analysis of *H-OLE1* gene encoding delta9-fatty acid desaturase in *Hansenula polymorpha*. *Appl Microbiol Biotechnol*. 54(4):499-509.

7.3.5 Matsuura, H., Okamoto, S., **Anamnart, S**., Wang, Q., Zhou, ZY., Nihira, T., Yamada, Y., Kuzuyama, T., Seto, H., Nakayama, J., Suzuki, A., Nagasawa, H., and Sakuda, S. (2003) Nucleotide sequences of genes encoding allosamidin-sensitive and -insensitive chitinases produced by allosamidin-producing Streptomyces**.** *Biosci Biotechnol Biochem*. 67(9), 2002-2005.

7.3.6 Wongsumpanchai, W., **Anamnart, S**., Laoteng, K. and Petsom, A. (2004) Elongation of C16:0 to C18:0 fatty acids in methylotrophic yeast *Hansenula polymorpha* CBS 1976 and fatty acid auxotrophic mutants**.** *FEMS Microbiology Letters* 237:213-218.

7.3.7 ศจี น้อยตั้ง, **ศรินทิพ สุกใส** และอมร เพชรสม (2548) การศึกษาคุณค่าทางอาหารของเมล็ดเกาลัดน่าน และศึกษาคุณสมบัติของแป้งจากเมล็ดเกาลัดน่าน. การประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย (วทท.) ครั้งที่ 31: 274.

7.3.8 สมคิด บุมี, บุญศรี จงรุ่งเรืองจิตต์ และ **ศรินทิพ สุกใส** (2549) การศึกษากรดไขมันไม่อิ่มตัวจากเชื้อราที่แยกจากปลาทะเล**.** *วารสารวิทยาศาสตร์ในพระบรมราชูปถัมภ์* ปีที่ 60 ฉบับที่ 1 หน้า 16-25.

7.3.9 **ศรินทิพ สุกใส** และศจี น้อยตั้ง (2550) รายงานการวิจัยเรื่องการพัฒนาผลิตภัณฑ์เสริมเส้นใยจากเมล็ดแมงลัก**.** ทุนหน่วยวิจัย-พัฒนาอุตสาหกรรม จากสถาบันเทคโนโลยีชีวภาพและวิศวกรรมพันธุศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปีงบประมาณ 2550.

7.3.10 **ศรินทิพ สุกใส**, ศจี น้อยตั้ง และปาริชาติ ลบแย้ม (2550) รายงานการวิจัยเรื่องการพัฒนาการผลิตน้ำมันจากเมล็ดชาน้ำมัน *Camellia oleifera*. ทุนหน่วยวิจัย-พัฒนาอุตสาหกรรม จากสถาบันเทคโนโลยีชีวภาพและวิศวกรรมพันธุศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปีงบประมาณ 2550.

7.3.11 ศจี น้อยตั้ง, **ศรินทิพ สุกใส** และอมร เพชรสม (2551) การสกัดน้ำมันจากเมล็ดแมงลักและคุณสมบัติการพองตัวของสารเมือกจากกากที่เหลือ. การประชุมวิชาการประจำปีการแพทย์แผนไทย การแพทย์พื้นบ้าน การแพทย์ทางเลือกแห่งชาติ ครั้งที่ 5:114.

7.3.12 **ศรินทิพ สุกใส**, ศจีน้อยตั้ง, วีระเดช สุขเอียด และอมร เพชรสม (2552) คุณสมบัติการพองตัวและอุ้มน้ำของสารเมือกจากผงเมล็ดแมงลักหลังสกัดน้ำมัน. *วารสารวิทยาศาสตร์เกษตร* ปีที่ 40 ฉบับที่ 2 หน้า 219-228.

7.3.13 Noitang, S., **Sooksai, S.A.**, Foophow, T. and Petsom, A. (2009) Proximate Analysis and Physico-Chemical Properties of Flour from the Seed of the China Chestnut, *Sterculia monosperma* Ventenat. *Pakistan Journal of Biological Sciences* 12(19): 1314-1319.

7.4 งานวิจัยที่กำลังทำอยู่

7.4.1 การศึกษาชนิดและปริมาณของกรดไขมันจากพืชน้ำมันชนิดต่างๆ

แหล่งเงินทุนอุดหนุนภายในสถาบันประจำปี 2554

ได้ดำเนินการวิจัยลุล่วงไปแล้วประมาณ 30 %

ประวัตินักวิจัยและคณะ พร้อมหน่วยงานสังกัด

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Education

1999	D.Sc. (Biotechnology), King Mongkut's University of Technology Thonburi, Thailand
1997-1998	Doctoral research at International Institute of Genetics and Biophysics (IIGB), Naples, Italy
1990	B.Sc. (Nursing and Midwifery, 1st class honors, Mahidol University, Thailand

Professional Experience

May 2010	Visiting Researcher, Department of Biochemical Technology, Institute of Biotechnology and Food Sciences, Faculty of Chemical and Food Technology, Slovak University of Technology
2006-present	Senior Researcher, Biochemical Engineering and Pilot Plant Research and Development Unit (BEC), National Center for Genetic Engineering and Biotechnology, Thailand
2000-2005	Researcher, Biochemical Engineering and Pilot Plant Research and Development Unit (BEC), National Center for Genetic Engineering and Biotechnology, Thailand
2001-Present	Adjunct Lecturer at Biotechnology department, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Thailand (Molecular Biotechnology, Advance Molecular Biology and Molecular Biochemistry courses)
2005-present	Invited reviewer in the international journals (Microbiology, Enzyme and Microbial Technology, Journal Research in Microbiology, Food Technology and Biotechnology, Indian Journal of Microbiology)

Honors and Awards

1997-1999	A fellowship for D.Sc. study from Graduate Research and Education Consortium (GREC) in Biotechnology and Molecular Biology, National Sciences and Technology Development Agency, Thailand
2000	*Taguchi prize 2000 for outstanding doctoral degree thesis, a young scientist in the field of biotechnology by Thai Society for Biotechnology*
2008	L'Oreal Thailand "For Women in Science 2008" fellowship (Life Science) with the support of the Thai National Commission for UNESCO, April 24, 2008
2008	Outstanding alumni 2008, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Thailand, November 29, 2008
2008-present	Member of Thai Academy of Science and Technology Foundation (TAST)
2010	Inclusion in the 27th edition of "Who's Who in the World", Marquis Who's Who, New Providence, U.S.A.
2010	A scholarship for research stay from The National Scholarship Programme of the Slovak Republic (NSP), Minister of Education, Slovak Republic, May 1-31, 2010.

Publications

In peer reviewed international journals

1. **Laoteng, K.**, Anjard, C., Rachadawong, S., Tanticharoen, M., Maresca, B. and Cheevadhanarak, S. 1999. *Mucor rouxii* Δ^9-desaturase gene is transcriptionally regulated during cell growth and by low temperature. Mol. Cell. Biol. Res. Commun. 1(1): 36-43.
2. Passorn, S., **Laoteng, K.**, Rachadawong, S., Tanticharoen, M. and Cheevadhanarak, S. 1999. Heterologous expression of *Mucor rouxii* Δ^{12}-desaturase gene in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 263(1): 47-51.
3. **Laoteng, K.**, Mannontarat, R., Tanticharoen, M. and Cheevadhanarak, S. 2000. Δ^6-desaturase gene of *Mucor rouxii* with high similarity to plant Δ^6-desaturase and its heterologous expression in *Saccharomyces cerevisiae*, Biochem. Biophys. Res. Commun. 279(1):17-22.
4. **Loateng, K.**, Pongchuachidthai, R., Rueksomtawin, K., Dandusitapunth, Y., Tanticharoen, M. and Cheevadhanarak, S. 2003. A *Mucor rouxii* mutant with high accumulation of an unusual *trans*-linoleic acid (9c,12t-C18:2). FEMS Microbiol. Lett. 223(2):159-165.

5. Wongsumpanchai, W., Anamnart, S., **Laoteng, K.** and Petsom, A. 2004. Elongation of C16:0 to C18:0 fatty acids in methylotrophic yeast *Hansenula polymorpha* CBS1976 and fatty acid auxotrophic mutants. FEMS Microbiol. Lett. 237(2): 213-218.
6. **Laoteng, K.**, Ruenwai, R., Tanticharoen, M. and Cheevadhanarak, S. 2005. Genetic modification of essential fatty acids biosythesis in *Hansenula polymorpha*. FEMS Microbiol. Lett. 245(1): 169-178.
7. Na-Ranong, S., **Laoteng, K.**, Kittakoop, P., Tanticharoen, M. and Cheevadhanarak, S. 2005. Substrate specificity and preference of Δ^6-desaturase of *Mucor rouxii*. FEBS Lett. 579(12): 2744-2748.
8. **Laoteng, K.**, Cheevadhanarak, S., Tanticharoen, M. and Maresca, B. 2005. Promoter analysis of *Mucor rouxii* Δ^9-desaturase: Its implication for transcriptional regulation in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 335(2): 400-405.
9. Na-Ranong, S., **Laoteng, K.**, Kittakoop, P., Tanticharoen, M. and Cheevadhanarak, S., 2006. Targeted mutagenesis of a fatty acid delta-6 desaturase from *Mucor rouxii*: Role of amino acid residues adjacent to histidine-rich motif II. Biochem. Biophys. Res. Commun. 339(4): 1029-1034.
10. Jeennor, S., **Laoteng, K.**, Tanticharoen, M. and Cheevadhanarak, S., 2006. Comparative fatty acid profiling of *Mucor rouxii* under different stress conditions. FEMS Microbiol. Lett. 259(1): 60-66.
11. Nookaew, I., Meechai, A., Thammarongtham, C., **Laoteng, K.**, Ruanglek, V., Cheevadhanarak, S., Nielsen, J. and Bhumiratana, S., 2007. Identification of flux regulation coefficients from elementary flux modes: A systems biology tool for analysis of metabolic networks. Biotechnol. Bioeng. 97(6): 1535-1549.
12. **Laoteng, K.**, Jitsue, S., Dandusitapunth, Y. and Cheevadhanarak, S., 2008. Ethanol-induced changes in expression profiles of cell growth, fatty acid and desaturase genes of *Mucor rouxii*. Fungal Genet. Biol. 45(1): 61-67.
13. Jeennor, S., **Laoteng, K.**, Tanticharoen, M. and Cheevadhanarak, S., 2008. Evaluation of inoculum performance for enhancing gamma-linolenic acid production from *Mucor rouxii*. Lett. Appl. Microbiol. 46(4): 421-427.
14. Khoomrung, S., **Laoteng, K.**, Jitsue, S. and Cheevadhanarak, S. 2008. Significance of fatty acid supplementation on profiles of cell growth, fatty acid and gene expression of three desaturases in *Mucor rouxii*. Appl. Microbiol. Biotechnol. 80(3): 499-506.
15. Nookaew, I., Jewett, M.C. Meechai, A., Thammarongtham, C., **Laoteng, K.**, Cheevadhanarak, S., Nielsen, J. and Bhumiratana, S., 2008. The genome-scale metabolic

model iIN800 *of Saccharomyces cerevisiae* and its validation: A scaffold to query lipid metabolism. BMC Syst. Biol. 2(1):71.

16. Ruenwai, R., Cheevadhanarak, S. and **Laoteng, K.** 2009. Overexpression of acetyl-CoA carboxylase gene of *Mucor rouxii* enhanced fatty acid content in *Hansenula polymorpha*. Mol. Biotechnol. 42(3): 327-332.
17. Jangbua, P., **Laoteng, K.**, Kitsubun, P., Nopharatana, M. and Tongta, A., 2009. Gamma-linolenic acid production of *Mucor rouxii* by solid-state fermentation using agricultural by-products. Lett. Appl. Microbiol. 49(1):91-97.
18. Ruenwai, R., Cheevadhanarak, S., Rachdawong, S., Tanticharoen, M. and **Laoteng, K.** 2010. Oxygen-induced expression of Δ^6-, Δ^9- and Δ^{12}-desaturase genes modulates fatty acid composition and lipid content in *Mucor rouxii*. Appl. Microbiol. Biotechnol. 86(1):327-334.
19. Jeamton, W., Dulsawat, S., **Laoteng, K.**, Tanticharoen, M. and Cheevadhanarak, S. 2010. Phycocyanin promoter of Spirulina platensis controlling heterologous expression in cyanobacteria. J. Appl. Phycol. (in press)
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21. **Laoteng, K.**, Certik, M. and Cheevadhanarak, S. 2011. Mechanisms controlling lipid accumulation and polyunsaturated fatty acid synthesis in oleaginous fungi. Chem. Pap. 65(2): 97-103.
22. Khongto, B., **Laoteng, K.** and Tongta, A. 2011. Enhancing the production of gamma-linolenic acid in *Hansenula polymorpha* by fed-batch fermentation using response surface methodology. Chem. Pap. 65(2): 124-131.
23. Cheawchanlertfa, P., Cheevadhanarak, S., Tanticharoen, M., Maresca, B. and **Laoteng, K.** 2011. Up-regulated expression of desaturase genes of *Mucor rouxii* in response to low temperature associates with pre-existing cellular fatty acid constituents. Mol. Biol. Rep. (in press)
24. Ruenwai, R., Neiss, A., **Laoteng, K.**, Vongsangnak, W., Dalfard, A.B., Cheevadhanarak, S., Petranovic, D. and Nielsen, J. 2011. Heterologous production of polyunsaturated fatty acids in *Saccharomyce cerevisiae* causes a global transcriptional response resulting in reduced proteasomal activity and increased oxidative stress. Biotechnol. J. 6: 343-356.

Book Chapter

- **Laoteng, K.** and Certik, M. 2010. Biotechnological production and application of high-value microbial oils. In: Industrial Fermentation: Food Processes, Nutrient Sources and Production Strategies, Krause, J and Fleischer, O (eds.), Nova Science Publishers, Inc., New York, pp. 187-215.

Invited Speaker

1. **Laoteng, K.** and Cheevadhanarak, S. 2006. Regulation of fatty acid and lipid biosynthesis in *Mucor rouxii*: Implication for essential oils production. The 18th Annual Meeting of the Thai Society for Biotechnology (TSB2006): Biotechnology, Benefits and Bioethics, November 2-3, The Montien Riverside Hotel, Bangkok, Thailand.
2. **Laoteng, K.** 2008. Regulation of gamma-linolenic acid synthesis in *Mucor rouxii*. Thai-German Special Joint Seminar on "Molecular Cell Biology", March 18, National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand.
3. **กอบกุล เหล่าเท้ง** 2008. การศึกษาการควบคุมการสังเคราะห์กรดไขมันในรามิวคอร์ รูซิไอ, งานเสวนา คุยกันฉันท์วิทย์ เรื่อง "ผลงานสตรีเด่นด้านวิทยาศาสตร์ ประจำปี 2551", 23 พฤษภาคม 2551, ณ อาคารพระจอมเกล้า กระทรวงวิทยาศาสตร์และเทคโนโลยี, กรุงเทพมหานคร
4. **Laoteng, K.** 2008. Progress towards the essential fatty acid production from microbes: Molecular and physiological mechanisms of gamma-linolenic acid biosynthesis in *Mucor rouxii*, July 15, Faculty of Science, Mahidol University, Bangkok.
5. **Laoteng, K.** 2010. Molecular mechanisms controlling fatty acid synthesis in *Mucor rouxii*. May 5, Institute of Animal Biochemistry and Genetics, Slovak Academy of Science, Ivanka pri Dunaji, Slovak Republic.
6. **Laoteng, K.**, Cheawchanlertfa, P., Cheevadhanarak, S. and Sooksai, S. 2010. Probing fatty acid metabolism in *Hansenula polymorpha* by comparative analysis of defective and overexpressing strains. 38th Annual Conference on Yeasts, May 11-14, Congress Centre SAS, Smolenice castle, Slovak Republic.
7. กอบกุล เหล่าเท้ง น้ำมัน GLA: เทคโนโลยีการผลิตและการประยุกต์ใช้, งานสัมมนา สุขภาพดีด้วย PUFAs, 24 กันยายน 2553 ศูนย์ฝึกอบรมเขตอุตสาหกรรมซอฟต์แวร์ประเทศไทย กรุงเทพมหานคร
8. **Laoteng, K.** 2010. Heterologous expression and process development in *Hansenula polymorpha*. Workshop on Heterologous expression in *Pichia pastoris*, October 4, Mahidol University, Saraya, Thailand.

9. **Laoteng, K.** 2010. Mechanisms controlling fatty acid biosynthesis in oleaginous fungi and biotechnological production of essential fatty acids, October 29, Betagro Science Center LTD., Pathumthani, Thailand.

International Proceeding

: Poster presentation (Full paper)

1. Vorapreeda, T., Thammarongtham, C., **Laoteng, K.**, Meechai, A., Cheevadhanarak, S., Bhumiratana, S. 2002. In silico analysis of *Saccharomyces cerevisiae*: Lipid biosynthesis pathway. The International Conference on Bioinformatics 2002: North-South Networking, 6-8 February 2002, Le Royal Meridien, Bangkok, Thailand.
2. Nookaew, I., Liamprawat, C., Meechai, A., **Laoteng, K.**, Cheervadhanarak, S., Bhumiratana, S. 2002. Elementary flux mode analysis of fatty acid metabolism in *Saccharomyces cerevisiae*. The International Conference on Bioinformatics 2002: North-South Networking, 6-8 February 2002, Le Royal Meridien, Bangkok, Thailand.
3. Meechai, A., Nookaew, I., Thamarongtham, C., **Laoteng, K.**, Ruanglek, V., Cheevadhanarak, S., and Bhumiratana, S. 2003. Pathway analyses of yeast metabolic reaction network for potential improvement of fatty acids production. Asia Pacific Biochemical Engineering Conference 03, 30 November-4 December 2003, Carlton Crest Hotel, Brisbane, Australia
4. Vorapreeda, T., Thamarongtham, C., **Laoteng, K.**, Meechai, A., Cheevadhanarak, S., and Bhumiratana, S. 2003. Insights into the lipid biosynthesis of *Saccharomyces cerevisiae* via pathway reconstruction from genome data. Asia Pacific Biochemical Engineering Conference 03, 30 November-4 December 2003, Carlton Crest Hotel, Brisbane, Australia.

National Proceeding

: Poster presentation (Full paper)

1. Nookaew, I., Liamprawat, C., Meechai, A., **Laoteng, K.**, Cheevadhanarak, S., and Bhumiratana, S. 2002. Topology analysis of fatty acid metabolism in *Saccharomyces cerevisiae*. การประชุมวิชาการวิศวกรรมเคมีและเคมีประยุกต์แห่งประเทศไทย ครั้งที่ 12, 8-9 พฤศจิกายน 2545 โรงแรมโซล ทวิน ทาวเวอร์ กรุงเทพ
2. Na-Ranong, S., Defernez, M., Mellon, F.A., Roberts, I.N., **Laoteng, K.**, Tanticharoen, M., Cheevadhanarak, S., and MacKenzie, D.A. 2006. Discrimination of changes in metabolic profile of transgenic yeasts containing the *Mucor rouxii* fatty acid delta-6

desaturase by metabolic footprinting. The 18th Annual Meeting of the Thai Society for Biotechnology (TSB2006): Biotechnology, Benefits and Bioethics, November 2-3, The Montien Riverside Hotel, Bangkok, Thailand.

3. Ruenwai, R., **Laoteng, K.**, Tanticharoen, M. and Cheevadhanarak, S. 2006. Isolation of an acetyl-CoA carboxylase gene from *Mucor rouxii*: An attractive tool for studying fatty acid metabolism. The 18th Annual Meeting of the Thai Society for Biotechnology (TSB2006): Biotechnology, Benefits and Bioethics, November 2-3, The Montien Riverside Hotel, Bangkok, Thailand.

Published Abstracts

27 presentations at international conferences

49 presentations at national conferences