REFERENCES

Aires-Barros, M., Taipa, M.A., and Cabral, J.M.S. 1994. Isolation and purification of lipases. In P. Wooley and P. B. Steffen (eds.), *Lipases: their structure, biochemistry and application*, 363p. London: Cambridge University Press.

Albergel, C., Fontecilla, J., and Cambillau, C. 1990. Crystallization of gastric lipases. In C. C. Akoh and D. B. Min (eds.), *Food lipids: Chemistry, nutrition and biotechnology*, pp. 670-671.

Alberghiya, L., Gradori, R., Lorghi, S., Lotti, M., Fusetti, F., and Vanoni, M. 1990. Molecular cloning of a lipase and of a lipase related gene from ***candida cylindracea***. In L. Alberghina, R. D. Schimid and R. Verger (eds.), *Lipases: Structure, mechanism and genetic engineering*, pp. 231-235: GBF monographs.

Alford, J. A., and Steinle, E.E. 1967. A double layered plate method for the detection of microbial lipolysis. *J. Appl. Bacteriol.* 30: 488-494.

Ammaranon, P. 1996. *Purification and characterization of thermotolerant lipase from a microorganism isolated from raw milk.* Master' Thesis, Department of Biotechnology, Graduate School, Mahidol University.

Andee, H., Muller, W. R., and Schid, R. D. 1980. Lipases as detergent components. *J. Appl. Biochem.* 2: 218-229.

Antonian, E. 1988. Recent advances in purification, characterization and structure determination of lipases. *Lipids.* 23: 1101.

Aoyama, S., Yoshida, N., and Inouye, S. 1988. Cloning, Sequencing and expression of the lipase gene from ***Pseudomonas fragi*** IFO-12049 in ***E.coli***. *Febs Letters.* 242(1): 36-40.

Bjorkling, F., Godtfredsen, S. E., and Kirk, O. 1991. The future impact of industrial lipases. *TIBTECT.* 9: 360-363.

Bourne, Y., Martinez, C., Kerfelec, B., Lombardo, D., Chapus, C., and Cambillau, C. 1994. Horse pancreatic lipase. *J. Mol. biol.* 238: 707.

Brady, L., and others. 1990. Serine protease triad forms the catalytic center of a triacylglycerol lipase. *Nature.* 343: 767.

Bremner, S. 1998. The molecular evolution of genes and protein: a tale of two serines. Nature. 334: 524-530.

Cambillau, C., and Boyrne, Y. 1990. Crystallographic studies of The pancreatic lipase / colipase system. In L. Alberghina, R. D. Schimid and R. Verger (eds.), *Lipases: Structure, mechanism and genetic engineering*, pp. 31-34: GBF monographs.

Chen, J., Shimura, S., Kirimura, K., and Uramis, S. 1993. Enhancement of lipase production from hydrocarbons by mutation of ***Trichosporon fermentans***. *Appl. Microbiol. Biotechnol.* 38:714-718.

Chung, G. H., Lee, P. Jeohn, G.H., Yoo, O.J., and Rhee, J. S. 1991. Cloning and nucleotide sequence of thermostable lipase gene from ***Pseudomonas Pluores cences*** SIKW1. *Agric. Biol. Chem.* 55:2359-2365.

Claon, P. A., and Ashok, C. C. 1994. Effect of reaction parameters on SP435 lipase-catalysed synthesis of citronnellyl acetate in organic solvent. *Enzyme Microbiol. Technol.* 16: 853-838.

Cleasley, A., Garwran, E., Emond, M. R., and Batenburg, M. 1992. Crystallization and preminary X-ray Study of lipase from ***Pseudomonas glumae***. *J. Mol. Biol.* 224: 281.

Collins, M. A., and Hammer, B. W. 1934. The action of certain bacteria on some simple triglycerides and neutral fats, as show by Nile blue sulphate. *J. Bacteriol.* 27: 473-485.

Conn, E. E., and Stumpf, P. K. 1989. Introduction. In Outlines of biochemistry, 3nd ed., pp. 535. Johm Wieley and sons.

Dartois, V., Baulard, A., Schanch, K., and Calson, C. 1992. Cloning, nucleotide sequence and expression in ***Escherichia coli*** of a lipase gene from ***Bacillus subtilis***. *Biochim. Biophys. Ac.* 1131: 253-260.

Derewenda, Z., and Derewenda, U. 1991. Relationships among serine hydrolases: evidence for a common structural motif in triglyceride lipases and esterases. *Biochem. Cell. Biol.* 69: 842-851.

Derewenda, U., Brzozowski, A. M., Lawson, D. M., and Derewenda Z. S. 1992. Catalysis at the interface: The anatomy of a conformational change in a triacylglyceride lipase. *Biochemistry* 31: 1532.

Falch, E. A. 1991. Industrial enzymes-developments in production and application. *Biotech. Advan.* 9: 643-658.

Gawel, L. J., amd Chen, C. S. 1977. Industrial enzyme from microbial sources. *U. S. patent* 4,019,959.

Gilbert, E. J., Cornish, A., and Jones, C. W. 1991. Purification and properties of extracellular lipase from ***Pseudomonas aeroginisa*** EF2. *J. Gen. Microbiol.* 137: 2223-2229.

Gotz, F., Pop, F., Korn, E., and Shleifer, H. 1985. Complete Nucleotide sequence of the lipase gene from ***Staphylococcus hyicus*** cloned in ***Staphylococcus carnosus***. *Nucleie Acids Res.* 13: 5895.

Grochulski, P., Li, Y., Schrag, J. D., Bouthellier, F., Smith, P., Harrison, D., Rubin, B., and Cygler, M. 1993. Insights into interfacial activation from and open structure of ***Candida rugosa*** lipase. *J. Biol. chem.* 268: 12843.

Gubernator, K., Muller, K., and Winkler, F. K. 1990. The structure of human pancreatic lipase suggests a locally inverted, trysin-like mechanism. In L. Alberghina, R. D. Schimid and R. Verger (eds.), *Lipases: Structure, mechanism and genetic engineering*, pp. 8-16: GBF monographs.

Hanahan, D. J. 1960. Introduction. In *Lipide Chemistry*, pp. 1-9. New York: John wiley&sons..

Hardley, N. F. 1985. Lipids: Classilication, structure and properties. In *the adaptive role of lipids in biological systems*, p. 1-25. New York: John weiley & sons.

Hjorth, A., and others. 1993. A structure domain (the lid) found in pancreatic lipase is absent in the guinea pig (phospholipase) lipase. *Biochemistry* 32: 4702.

Huang, A. H. C., Lin, Y. H., and Wang, S. M. 1988. Characteristics and biosynthesis of seed lipases in maize and other plant species. *J. Am. Oil. Chem. Soc.* 65: 897.

Iisumi, T., Nakamura, K, and Fukase, T. 1990. Purificationand characterization of a thermostable lipase from newly isolated ***Pseudomonas sp.*** KWI-56. *Agric. Biol. Chem.* 54(5): 1253-1258.

Iizumi, T., Nakamura, K., Sugihara, A., Tominaga, y., and Fukase, T. 1991. Cloning, nucleotide sequencing, and expression in

Escherichia coli of lipase and its activated genes from ***Pseudomonas sp. KWI-56***. *Agric. Biol. chem.* 55: 2349.

Iwai, M., Tsujisaka,Y., Okamoto, Y., and Fukumoto, J. 1993. Lipid requirement for the lipase production by ***Geotrichum candidum*** Link. *Agi. Biol. Chem.* 37(4): 929-931.

Jensen, R. G., Marks, T. S., Sampugna, J., Quinn, J. G., and Carpenter, D. L. 1966. Purification of triglycerides with alumina column. *Lipids.* 1(6): 451-452.

Jorgensen, S., Skov, K. W., and Dideriehsen, B. 1991. Cloning, sequence and expression of lipase gene from ***Pseudomonas cepacia***: lipase production in heterogeneous hosts requires two ***Pseudomonas*** genes. *J. Bacteriol.* 173: 559.

Kates, M. 1960. Lipolytic enzymes. In B. Konrad, *Lipid metabolism*, pp. 167-180. London: Weiley and Sons.

Kakinuma, A., and others. 1969. Conformation of the structure of surfactin by mass spectrometry. *Agric. Biol. Chem.* 33: 1669.

Kim, K. K., and others. 1992. Crystallization and premilinary X-ray crystallographie analysis of lipase from ***Pseudomonas cepacia***. *J. Mol. Biol.* 227: 1258.

Kok, R. G., Nudel, B. C., Gonzalez, R. H., Nugteren-Roodzant, I. M., and Hellingwerf, K. J. 1996. Physiological factors affecting production of extracellular lipase (lipA) in ***Acinetobacter calcoaceticus*** BD413: fatty acid repression of lipA expression and degradation of Lip A. *J. Bacteriol.* 178: 6025-6035.

Kok, R. G., Van-Thar, J. J., Nugteren-Roodzant, I. M., Vosman, B., and Hellingwerf, K. J. 1995. Characterization of lipase deficient mutants of ***Acinetobacter calcoaceticus*** BD 413: identification of periplasmic lipase chaperone essential for

production of extracellular lipase. *J. Bacteriol.* 177: 3295-3307.

Kugimiya, W., Otani, Y., Hashimoto, Y., and Takagi,Y. 1986. Molecular cloning and nucleotide sequence of the lipase gene from ***Pseudomonas fragi***. *Biochem. Biophys. Res. Comom.* 141(1): 185-190.

Kokusho, Y., Machida, H., and Iwasaki, S. 1982. Production and Properties of alkaline lipase from Alcaligenes sp. Strain No. 679. *Agric. Biol. Chem.* 46(7): 1743-1750.

Kouker, G., and Jaeker, K. E. 1987. Specificand sensitive plate assay for bacterial lipase. *App. Environ. Microbiol.* 53(1): 211-213.

Kwon, D. Y., and Rhee, J. S. 1986. A simple and rapid Colorimetric Method for determination of free fatty acid for lipase assay. *J. Am. Oil Chem. Chem. Soc.* 63: 89-82.

Larson, S., Day, J., Greenwood, A., Oliver, J., Rubingh, J. and Mcpherson, A. 1991. Premilinary investigation of crystals of neutral lipase from ***Pseudomanas fluorescences***. *J. Mol. Biol.* 222: 21.

Lawrence, R. C., Fryer, T. F., and Reiter, B. 1967. The production and characterization of Lipase from a ***Micrococus*** and a ***Pseudomonad***. *J. Gen. Microbiol.* 48: 401-418.

Lawson, D. M., and others. 1994. Three-dimensional structures of two lipases from filamentous fungi. In P. Woolley and S. B. Petersen, (eds.), *Lipases*, pp. 77-94. London: Cambridge.

Lee, C. Y., and Iandolo, J. J. 1986. Lysogenic conversion of ***Stephylococeal*** lipase is caused by insertion of the bacteriophage L54a genome into the lipase structural gene. *J. Bacteriol.* 166: 385.

Lesuisse, E., Schanck, K., and Colson, C. 1993. Purification and preliminary characterization of the extracellular lipase of ***Bacillus subtilis*** 168, an extremely basic pH-tolerant enzyme. *Eur. J. Biochem.* 216: 155-160.

Lin, S. F., Chiou, C. M., Yeh, C. M., and Tsai, Y. C. 1996. Purification and partial Characterization of an alkaline lipase from ***Pseudomonas pseudoalcalignes*** F-111. *Appl. Environ. Microbiol.* 62(3): 1093-1095.

Lortrakul, P., and Dharmsthiti, S. 1997. Lipase production by ***Aeromonas sobia*** LP004 in a medium containing whey and soybean meal. *J. Microbiol. Biotechnol.* 13: 163-166.

Malcata, F. X., Reyes, H. R., Garcia, H. S., Hill, C. G., and Amundson, C. H. 1990. Immobilizedlipase reactors for modificationof fats and oils. *J. Am. Oil Chem. Soc.* 67: 890-910.

Macrae, A. R. 1983 a. Extracellular microbial lipases. In W. M. Forgarty (ed.), *Microbial enzymes and biotechnology*, pp. 266-250.

Macrae, A. R. 1983 b. Lipase catalyzed interesferification of oils and fats. *JAOCS.* 60(2): 291-294.

Macrae, A. R., and Hammond, R. C. 1985. Present and future application of lipases. *Biotech. Engin.* Rev. 3: 193-217.

Mahler, G. F., Kok, R. G., Cordenous, A., Hellingwert, K. J., and Nudel, B. C. 2000. Effects of carbon sources on extracellular lipase production and *lip*A transcription in ***Acinetobacter calcoaceticus***. *J. Indust. Miorobiol. Biotechnol.* 24: 25-30.

Makherjee, K. D., and Hills, M. J. 1994. Lipases from plants. In P. Wooly and S. B. Petersen, *Lipases: their structure, biochemistry and application*, 363p. London: Cambrigde.

Mourey, A., and Kilburtus, G. 1976. Simple media containing stabilized tributyrinfor demonstrating lipolytic bacteria in foods and soils. *J. Appl. Bact.* 40: 47-51.

Nishio, T., Chikano, T., and Kamimvra, M. 1987. Produced by Pseudomonas fragi 22.39 B. *Agric. Biol. Chem.* 51(1): 181-186.

Omar, I. C., Nishio, N., and Nagai, S. 1987. Production of a thermostable lipases by ***Humicola lamuginosa*** grown on sorbital-corn steep liguor medium. *Agric. Biol. chem.* 51: 2145-2151.

Pencreac, G., Leullier, M., and Baratti, J. C. 1997. Properties of free and immobilized lipase from ***Pseudomonas cepacia***. *Biotechnol. Bioeng.* 56(2): 181-189.

Schrag, J. D., and Cygler, M. 1993. A refined structure of the lipase from ***Geotrichum candidum***. *J. Mol. Biol.* 230: 575.

Schrag, J. D., Li, Y., Wes, S., and Cygler, M. 1991. Ses-His-Gly triad forms the catalytic site of the lipase from ***Geotrichum candidum***. *Nature.* 351: 761.

Shabtai, Y, and Wang, D. I. C. 1990. Production of emulsion in a fermentation process using soybean oil (SBO) in a carbon-nitrogen coordinated feed. *Biotechnol. Bioeng.* 53: 753.

Shimada, Y., Sugihara, A., and Tominaga, Y. 1994. Microbial lipase: structure and production. In Y. Nuooka and I. Inanaka (eds.), *Recombinant microbes for industrial and agricultural applications*, pp. 359-371.

Shimada, Y., Sugihara, A., Iizumi, T., and Tominaga,.Y. 1990. cDNA cloning and characterization of ***Geotrichum candidum*** lipase II. *J. Biochem.* 107: 703.

Shimada, Y., Sugihara, A., Iizumi, T., and Tsunasawa, S. 1989. cDNA molecular cloning of ***Geotrichum candidum*** lipase. *J. Biochem.* 106: 383.

Sierra, G. 1957. A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact betweencells and fatty substances. *J. Microbiol. Serol.* 23: 15-22.

Staueffer, C. E. 1989. Ester hydrolases. In *enzyme assays for food Scientists*, pp. 187-195.

Sugihara, A., Tani, T., and Tominaga, Y. 1991. Purification and characterization of a novel thermostable lipase from ***Bacillus sp.*** *J. Biochem.* 109: 211-216.

Suzuki, T., Mushiga, Y., Yamane, T., and Shimiza, S. 1988. Mass production of lipase by fed-batch culture ***of Pseudomanas fluorescens.*** *Appl. Micnbiol.* 27: 417-422.

Wang, Y., Srivastava, K. C., Shen, G. J., and Wang, H. Y. 1995. Thermostable alkaline lipase from a newly isolated thermophilic ***Bacillus,*** Strain A 30-1 (ATCC53841). *J. Ferment. Bioeng.* 79(5): 433-438.

Weete, J. D. 1998. Microbial lipases. In C. C. Aksh and D. B. Min eds.), *Food lipids: chemistry, nutrition and biotechnology*, pp. 641-664.

Willis, W. M., and Mrangori, A. G. 1998. Enzymitic interesterified in In C. C. Aksh and D. B. Min (eds.), *Food lipids: chemistry, nutrition and biotechnology*, pp. 670-768.

Winkler, U. K., and Stuckmann, M. 1979. Glycogen, hyaluronate and some other polysaccharides greatly enhance the formation of exolipase by ***Serratia***. *J. Bacteriol.* 138(3): 663-670.

Wong, D. N. S. 1995. Lipolytic enzymes. In *food enzymes : structure and mechanism*, p.171-189.

Yamagushi, T., Muroya, N., Isobe, M., and Zugiura, M. 1973. Production and properties of lipase from a newly isolated Chromobacterium. *Agic. Biol. Chem.* 37: 999-1005.

Yamane, T. 1987. Enzyme technology for the lipids industry: An engineering overview. *JAOCS.* 64(12): 1657-1661.

Yang, D., and Rhee, J. S. 1992. Continuous hydrolysis of olive oil by immobilized lipase in organic solvent. *Biotechnol. Bioeng.* 40: 748-752.

Zaks, A., and Klibamov, A. M. 1988. Enzymatic catalysis in nonaqueous solvents. *J. Biol. Chem.* 263: 3914-3021.

APPENDICES

APPENDIX A

BACTERIAL SOURCES

In this present study , 45 samples were selected as bacterial sources. They were sequentially collected during April 1998 to October 1999, and were divided into 2 groups as follows:

Types of samples	Sampling sites
Soil	S1
	S2
	S3
	S4
	S5
	S6
	S7
	S8
	S9
	S10
	S11
	S12
	S13
	S14
	S15
	S16
	S17
	S18
	S19
	S20

Type of samples	Sampling sites
Soil	S21
	S22
	S23
	S24
	S25
	S26
	S27
	S28
	S29
	S30
	S31
	S32
	S33
	S34
	S35
	S36
	S37
	S38
	S39
	S40
Wastewater	WW1
	WW2
	WW3
	WW4
	WW5

APPENDIX B

CULTURE MEDIA

1. Lipid-rhodamine B Agar (LRA)

(slightly modified from Wang and et al.,1995)

Formula in milliliter and gram per 1 liter

-Corn oil	10
-$MgSO_4.7H_2O$	0.5
-NH_4Cl	1.0
-$CaCl_2.2H_2O$	0.05
-NaCl	1.0
-Trace mineral solution	10
-Vitamin solution	10
-Rhodamine B (0.1% solution)	10
-Agar	25
-phosphate buffer(KH_2PO_4+Na_2HPO_4, 1M, pH9.0)	10
-Glycine-NaOH solution (1M, pH 9.0)	10

Trace mineral solution composed of (mg/l):

-H_3BO_3	0.5
-$CaCl_2.2H_2O$	20
-$CoCl_2.6H_2O$	200
-$CuSO_4.5H_2O$	0.4
-$FeSO_4.7H_2O$	130
-KI	0.1
-$MnCl_2.4H_2O$	100
-Na_2MoO_4	10
-Na_2SeO_4	20
-Na_2WO_4	20

-$NiSO_4.6H_2O$	30
-$ZnCl_2.2H_2O$	100

The vitamin solution contained (mg/l):

-Biotin	2
-Crystalline cyanocobalamin (B_{12})	0.1
-Folic acid	2
-Nicotinic acid (niacin)	5
-Pantothenic acid	5
-p-aminobenzoic acid (PABA)	5
-pyridine-HCl (B_6)	10
-Riboflavin (B_2)	5
-Thiamine-HCl (B_1)	5

Lipid rhodamine B agar (LRA) was prepared bas follow. A stock solution of 0.1% (wt/vol) rhodamine B was prepared in deionized water and then sterlized by filtration. The stock solution was stored in refrigerator. The medium (LRA) was prepared by suspending all mineral salts including agar according to formula and adjusted to pH 9.3 followed by heating to dissolve completely. After the mixture was autoclaved at 121 C for 15 min and then cooled to 60 C, all of the remaining ingredients were aseptically added. All media were dispended in plates and before used plate was incubated overnight.

2. Lipid-Oil Broth (LOB)

(slightly modified from Wang and et al.,1995)

Formula in milliliter and gram per 1 liter

-Corn oil	10
-$MgSO_4.7H_2O$	0.5
-NH_4Cl	1.0
-$CaCl_2.2H_2O$	0.05
-NaCl	1.0
-Trace mineral solution	10
-Vitamin solution	10
-phosphate buffer(KH_2PO_4+Na_2HPO_4, 1M, pH9.0)	10
-Glycine-NaOH solution (1M, pH 9.0)	10
-Yeast Extract	0.1

Final pH 9.0

The LOB medium was prepared by suspending all mineral salts described above including yeast extract. After the medium was autoclaved at 121 C for 15 min, and then cooled down to 60 C, 10 ml of corn oil including vitamin solution as well as all buffer solutions were aseptically added. All media were dispended into each test tube.

3. Lipase Production Medium (LPM)

(Schmidt-Dannert and et al.,1994)

Formula im gram per 1 liter

-Nutrient Broth	3.25
-$CaCl_2$	1.0
-Gum arabic	10
-Olive oil	25

The LPM was prepared by suspending all ingredients in ditilled water and then warm slightly to dissolve completely. Later, the medium was autoclaved at 121 C for 15 min.

4. Measuring Growth Medium (MGM)

(Wang and et al.,1995)

Formula in milliliter and gram per 1 liter

-Olive oil	10
-Tween 80	1
-Yeast extract	1

The MGM was prepared by suspending all ingredients in distilled water and then adjusted to desired pH. After that, the mixture was autoclaved at 121 C for 15 min.

5. Nutrient Broth (Difco)

Formula gram per 1 liter

Bacto Beef Extract	3
Bacto Peptone	5

Final pH 6.8±0.2 at 25 C

6. Nutrient Agar

Formular in gram per 1 liter

Bacto Beef Extract	3
Bacto Peptone	5
Agar	15

Final pH 6.8±0.2 at 25 ^{0}C

The NB was prepared by dissolving 8 grams of NB in 1 liter distilled water or deionized water and added 15 grams of agar when prepare NA medium and warm slightly to dissolve completely. After the medium was autoclaved at 121^{0}C for 15 minutes, they were dispended in plates. Before used, plates were incubated overnight.

APPENDIX C

MEDIA FOR BIOCHEMICAL TESTS

1. Pseudomonas Selective Isolation Agar(PSIA)

(adapted from Krulger and Sheikh, 1986)

Formula milliliter andgram per 1 liter

-Nitrofurantoin (5% solution)	7
-Crystal violet (0.1% solution)	2
-Trypic Soy Broth	30
-Agar	15
-Distilled water	990

Pseudomonas selection isolation agar (PSIA) was prepared as follow. A stock solution of 5% (wt/vol) nitrofurantoin (Sigma, Steinheim, Germany), was prepared in N,N-dimethylformamide (Merk, Darmsatadt, Germany). A stock solution of 0.1% (wt/vol) crystal violet (Merk) was prepared in distilled water. The stock solution were stored at room temperature, and nitrofurantoin solution was protected from expose to light. The medium (PSIA) was prepared by suspending 30 gram of TSB and 15 gram of the agar in 990 ml distilled water and added 2 ml of crystal violet stock solution. After the mixture was autoclaved at 121 C for 15 min and then cooled to 50 C, 7 ml of nitrofurantoin stock solution was added. All media were dispended in plates and before used plate was incubated overnight.

2. Shigella and Salmonella Agar (SSA, Difco)

Formula in gram per 1 liter

-Bacto Beef Extract	5
-Bacto Proteose Peptone	5
-Bacto Lactose	10
-Bacto Bile Salt No.3	8.5
-Sodium Citrate	8.5
-Ferric Citrate	1
-Bacto Agar	13.5
-Brilliant Green	0.33 mg
-Neutral Red	0.025

Final pH 7.0±0.2 at 25 C

Suspend 60 gram in 1 liter distilled water or deionized water and boil carefully for no more than 2-3 minutes to dissolve completely. Avoid overheating. Do not autoclaved.

3. Simmons Citrate Agar

Formula in gram per liter

Magnesium Sulfate	0.2
Ammonium Dihydrogen Phosphate	1
Dipotassium Phosphate	1
Sodium Citrate	2
Sodium Chloride	5
Bacto Agar	15
Bacto Brom Thymol Blue	0.08

Final pH 6.8 at 25 ° C

To rehydrate the medium, suspend 24.2 grams in 1L, cold freshly distilled water and heat to boiling to dissolve the medium completely. Sterilize in the autoclave for 15 minutes at 15 pounds pressure (121° C).

4. Triple Sugar Iron Agar (TSI)

Formula in gram per liter

Bacto Beef Extract	3	
Bacto Yeast Extract	3	
Bacto Peptone	15	
Proteose Peptone	5	
Bacto Dextrose	1	
Bacto Lactose	10	
Saccharose	10	
Ferrous Sulfate	0.2	
Sodium Sulfate	5	
Sodium Thiosulfate	0.3	
Bacto Agar	12	
Bacto Phenol Red	24	mg

Final pH 7.4 at 25 ° C

To rehydrate the medium, suspend 65 grams in 1000 ml, cold freshly distilled water and heat to boiling to dissolve the medium

completely. Sterilize in the autoclave for 15 minutes at 15 pounds pressure. Allow the tubes to solidify in a slanting position in a manner which will give a generous butt.

5. MacConkey agar

For isolating and differentiating lactose-fermenting from lactose-non fermenting gram negative enteric bacilli.

Formula in gram per liter

Bacto Peptone	17	
Bacto Proteose Peptone	3	
Bacto Lactose	10	
Bacto Bile Salt No.3	1.5	
Sodium Chloride	5	
Bacto Agar	13.5	
Neutral Red	30	mg
Bacto Crystal Violet	1	mg

Final pH 7.1± 0.2 at 25 °C

Direction : suspend 50 grams in 1 liter, distilled or deionize water and boil to dissolve completely. Sterilize in the autoclave for 15 minutes at 15 pounds pressure. Avoid overheating.

6. Motility test medium

Formula in gram per 1 liter

Beef extract	3
Peptone	10
NaCl	5
Agar	4

Final pH 7.3

7. MR/VP broth

Formula in gram per 1 liter

Polypeptone	7
Glucose	5
Dipotassium phosphate	5

Final pH 6.9 ± 0.2

APPENDIX D

p-NITROPHENOL METHOD

(Winkler and Stuckmann, 1979)

Reagent :

1) 50 mM Tris-HCl

2) 0.1 % (w/v) p-nitrophenol

Procedure :

1. To prepare the stock standard solution , dissolved 100 mg p-nitrophenol in 100 ml distilled water (conc. 1 mg/ml). Diluted 1:100 in 50 mM Tris-HCl just before use to give a solution containing 100 μg p-nitrophenol per ml. Prepared standard (1-6 μg/ml).
2. Read the absorbance of each tube at 410 nm against the blank without p-nitrophenol using the spectrophotometer.
3. Determinated the concentration of p-nitrophenol in the samples from a standard curve prepared by plotting the absorbances of the standards versus the concentration of p-nitrophenol.

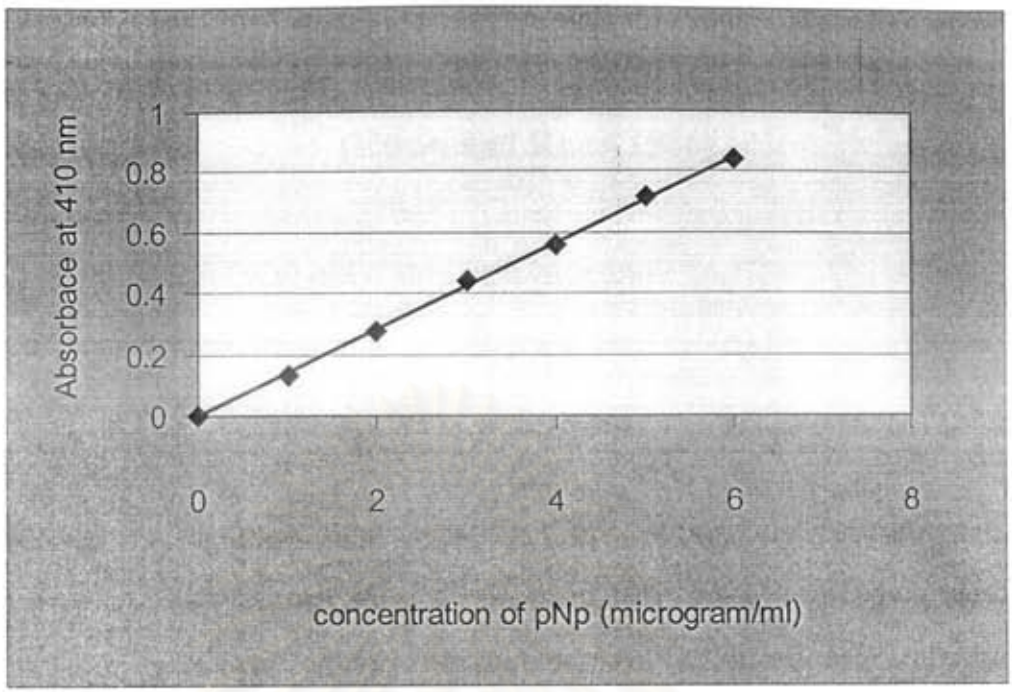

Figure D-1 A linear standard curve of p-nitrophenol detected by this method.

Calculation Method

$$\text{p-nitrophenol (nmol)} = \frac{\text{Absorbance read x 1000}}{\text{Slope constant x 139.11}}$$

Where slope constant equals to 0.1416

$$\text{Unit} = \frac{\text{p-nitrophenol (nmol)}}{\text{ml of enzyme used x incubation time}}$$

APPENDIX E

CUPRIC ACETATE-PYRIDINE METHOD

(Kwon and Rhee, 1986)

Reagent :

1) 2% (v/v) oleic acid in n-propanol
2) isooctane
3) 5% cupric acetate

Procedure :

1. To prepare the standard, place a portion of the sample containing 2.0-10.0 μmol of oleic acid in a screw cap culture tube and remove any solvent present at 50 °C using oven.
2. To prepare 5% cupric acetate reagent, dissolve 5gram of cupric acetate in 100 ml distilled water, and then filter as well as adjust to pH 6.0 with pyridine.
3. Acculately added 5.0 ml of isooctane and swirl to dissolve the sample.
4. added 1 ml of cupric acetate-pyridine reagent, vortexed for 2 min and then centrifuge for 5 min.
5. Read the absorbance of upper layer in each tube at 715 nm against the blank without oleic acid using the spectrophotometer.
6. Determine the concentration of free fatty acid in the samples from standard curve prepared by plotting absorbabce of the standards versus concentration of oleic acid.

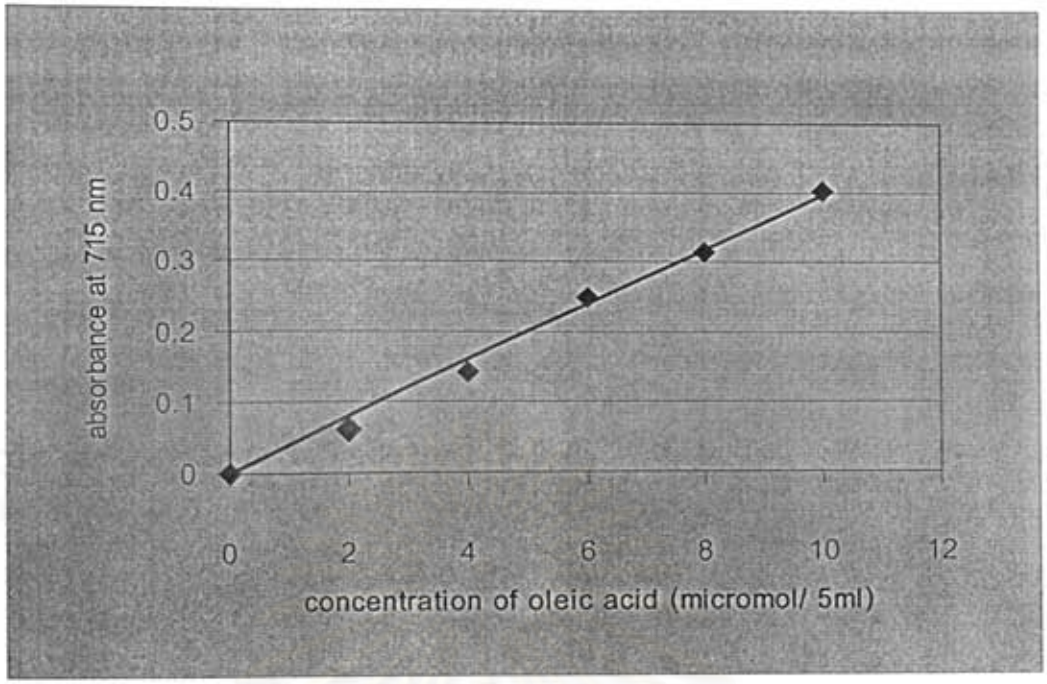

Figure E-1 A linear standard curve of oleic acid detected by this method.

Calculation Method

$$\text{oleic acid (nmol)} = \frac{\text{Absorbance read x 1000}}{\text{Slope constant}}$$

Where slope constant equals to 0.0397

$$\text{Unit} = \frac{\text{oleic acid (nmol)}}{\text{ml of enzyme used x incubation time}}$$

BIOGRAPHY

Mr.Pises Liawsakul was born in Surathani province. He received a Bachelor degree in General Science, Faculty of Science, Chulalongkorn University in 1994. After working as a production chemist at Hoechst Chemical Industries for 2 years, he entered the Graduate School of Chulalongkorn University in 1996. He earns a master degree in Biotechnology, Faculty of Science in 2000.