Journal of Biochemistry and Molecular Biology, Vol. 33, No. 4, July 2000, pp. 332-336

JOURNAL OF
Biochemistry
and
Molecular Biology

© BSRK & Springer-Verlag 2000

Phenazine 1-carboxylic acid resistance in phenazine 1-carboxylic acid producing *Bacillus* sp. B-6

Kyoung-Ja Kim
Department of Life Science, Soonchunhyang University, Asan 336-745, Korea

Received 26 May 2000, Accepted June 28 2000

Phenazine 1-carboxylic acid (PCA) is an antifungal antibiotic isolated from a culture filtrate of *Bacillus* sp. B-6 producing an acyl CoA synthetase inhibitor. This antibiotic is reported as an inhibitor of an acyl CoA synthetase from *Pseudomonas* sp.. *Bacillus* sp. B-6 was resistant to PCA up to 350 μg/ml. We investigated the mechanism of the resistance of *Bacillus* sp. B-6 to PCA. The rate of growth in a medium containing up to 100 μg/ml was as rapid as the PCA-free medium. At a PCA concentration of 300 μg/ml, the growth rate was more than half that of the control. In this work, we purified acyl CoA synthetase from *Bacillus* sp. B-6 and found that this acyl CoA synthetase was much less sensitive to PCA than the acyl CoA synthetase from other source. These findings suggested that the insensitivity of *Bacillus* sp. B-6 acyl CoA synthetase plays an important role in the PCA resistance of this bacterium.

Keywords: *Bacillus* sp. acyl CoA synthetase, acyl CoA synthetase inhibitor, antibiotic resistance, phenazine 1-carboxylic acid

Long chain fatty acyl-CoA, an activated form of fatty acids is essential for most organisms (Bar-Tana *et al.*, 1971; Hosaka *et al.*, 1979; Kamiryo *et al.*, 1976). It plays an important role as metabolic intermediates in lipid biosynthesis and β-oxidation of fatty acids (Paul *et al.*, 1992; Watkins *et al.*, 1998). Recently, it was demonstrated that fatty acyl-CoA serves as a regulator, not only in the above processes, but also in other metabolisms (Konrad and Ronald, 1994; Kameda and Nunn, 1981). The conversion of free long-chain fatty acids to acyl CoAs is catalyzed by acyl CoA synthetase (EC 6.2.1.3, Fontes and Silero, 1998; Hiroshi *et al.*, 1991). In previous papers (Kim and Kim, 1996; Kim and Kim, 1998), we reported phenazine 1-carboxylic acid (PCA), a new acyl CoA synthetase (from *Pseudomonas* sp.) inhibitor (Aatoshi *et al.*, 1986; Hiroshi *et al.*, 1987; Kathleen and Michael, 1992; Masahiko *et al.*, 1992; Sachiko *et al.*, 1992; Salaha *et al.*, 1992) that was isolated from the culture filtrate of *Bacillus* sp. B-6. PCA showed antifungal activity against *C. albicans* (Kim and Kim, 1998). *Bacillus* sp. B-6 was resistant to PCA (Messenger and Turner, 1983; Thomshow and Weller, 1988) up to 350 μg/ml. We investigated the mechanism of the resistance (Park, *et al.*, 1998) of *Bacillus* sp. B-6 to PCA. In this work, we purified acyl CoA synthetase from *Bacillus* sp. B-6 and found that this acyl CoA synthetase was much less sensitive to PCA than acyl CoA synthetase from other source. This insensitivity of the enzyme to PCA may be responsible for the protection of *Bacillus* sp. B-6 from inhibitory effects of this antibiotic (McKay *et al.*, 1994; Russell and Day, 1996).

*To whom correspondence should be addressed.
Tel: 82-41-530-1352; Fax: 82-41-530-1350
E-mail: kyoungjakim@hotmail.com

Materials and Methods

Reagents Acyl CoA synthetase of *Pseudomonas* sp., enzyme assay reagents and enzyme purification reagents were obtained from Sigma Chemical Co. (St. Louis, USA).

Microorganisms and Growth Conditions The bacterial strain used in this study was *Bacillus* sp. B-6, which is reported as a PCA producer (Kim and Kim, 1998). The growth medium was a chemically defined one with the following composition (in grams per liter): KH_2PO_4 (13.6), $(NH_4)_2SO_4$ (2.0), $MgSO_4 \cdot 7H_2O$ (0.25), $FeSO_4 \cdot 7H_2O$ (0.0005), biotin (0.001), and octanoic acid (2.8). The pH of this medium was adjusted to 7.0 with 50% KOH solution. Incubations were carried out at 250 rpm and 30°C.

Spectrophotometric assay of PCA Chloroform extracts of acidified bacterial cultures were clarified by centrifuging at 1000 g for 5 min, appropriately diluted with chloroform, and spectra were obtained by using Jasco scanning spectrophotometer at 252 nm. The concentration of PCA were assayed by using extinction coefficient 6,000 at 252 nm (Messenger and Turner, 1983; Thomshow and Weller, 1988).

Enzyme purification Cells of *Bacillus* sp. B-6 were harvested

in the late log phase. All subsequent operations were carried out at 0-5°C. The cells were washed with 0.01 M K_3PO_4, 10^{-3} M mercaptoethanol, the pH 8.0 was suspended in 1.4 volumes of the same buffer, and disrupted by 0.1 mm glass beads. The supernatant obtained after centrifugation of the resulting suspension at 15,000 × g for 15 minutes was centrifuged at 78,000 × g for 60 minutes. The resulting supernatant (crude extract) was adjusted to pH 6.3 with cold 1 M acetic acid. A solution of protamine sulfate (10 mg/ml, pH 5.0) was added so that the bacterial protein-protamine ratio was 20 : 1. The resulting solution was stirred for 10 minutes and the insoluble material was removed by centrifugation at 15,000 × g for 10 minutes. To the supernatant, 0.1 volume of 1 M K_3PO_4, pH 8.0 was added. The extract was then treated with $(NH_4)_2SO_4$ in order to obtain the protein fraction precipitating between 40 and 65% $(NH_4)_2SO_4$ saturation. The precipitated protein was dissolved in 0.05 M K_3PO_4, pH 7.5, and then dialyzed for 14-20 hours against 300-500 volumes of 0.01 M K_3PO_4, 0.01 M mercaptoethanol, 10^{-4} M EDTA, pH 7.0. After dialysis, an insoluble materials was removed by centrifugation at 15,000 × g for 15 minutes. Then the supernatant was mixed with alumina Cγ gel (gel to protein ratio, 1.0 : 0.62). The pellet obtained upon centrifugation of this mixture was washed with 0.01 M K_3PO_4, pH 7.0, and extracted several times with 0.1 M K_3PO_4, pH 7.0. The combined extracts were dialyzed against 0.01 M K_3PO_4, 0.01 M mercaptoethanol, 10^{-4} M EDTA, pH 7.0 for 11 hours. The dialyzed solution was chromatographed on a DEAE-cellulose column using a linear gradient apparatus containing 0.01 M K_3PO_4, 0.001 M mercaptoethanol, pH 7.5 and 0.8 M K_3PO_4, 0.001 M mercaptoethanol, pH 7.5. The fractions containing enzyme activity were combined and concentrated by using an Amicon pressure filtration stirred-cell with a PM10 ultrafiltration membrane.

Acyl CoA synthetase activity Acyl CoA synthetase activity (Bar-Tana *et al.*, 1971) was assayed at 37°C in a mixture containing 150 mM Tris-HCl buffer, pH 7.4, 0.25 mg of Triton X-100, 2 mM EDTA, 50 mM $MgCl_2$, 20 mM ATP, 200 M linoleic acid, 300 M CoASH and 5-15 g of enzyme protein in a total volume 0.25 ml. Incubations were carried out for 10 min and terminated by the addition of 0.75 ml of 0.5 mM 5,5'-dithiobis-(2-nitrobenzoic acid) in 0.1 M potassium phosphate buffer, pH 8.0. Addition of enzyme at the termination of the reaction served as a blank. The decrease in E413 was measured in a spectrophotometer. The molar extinction coefficient given by CoASH under these conditions was assumed to be $1.36 \times 10^4 cm^{-1}$. Enzyme inhibitiory activity was assayed as described previously (Kim and Kim, 1998).

SDS-polyacrylamide gel electrophoresis and molecular weight determination A SDS-slab gel electrophoresis in a 10% gel was carried out according to Laemmli (1970). Protein samples in 10% (v/v) glycerol, 1% (w/v) SDS, 2% (v/v) β-mercaptoethanol and 0.0025% (w/v) bromophenol blue were heated in a boiling water bath for 5 min and then subjected to electrophoresis. Electrophoresis was carried out at 4°C and the voltage applied was 70 volts at stacking gel, 100 volts at running gel. A β-galactosidase, *E. coli* (116,000), phosphorylase b (97,400), bovine serum albumin (66,000), ovalbumin (45,000), carbonic anhydrase (29,000) were used as protein standards. Proteins were stained with Coomassie brilliant blue R-250 and destained with 5% methanol containing 7.5% acetic acid. For determination of the native enzyme, gel filtration by Sepharose 6B column was carried out. Reference proteins were bovine thyroglobulin (669,000), horse spleen apoferritin (443,000), sweet potato β-amylase (200,000), yeast alcohol dehydrogenase (150,000), bovine serum albumin (66,000), and carbonic anhydrase (29,000).

Protein assay The concentrations of protein were measured by the method of Bradford (1976) and also estimated using the average protein absorptibity of $A_{280\,(0.1\%)} = 0.15$.

Results and Discussion

Effect of PCA on the growth of *Bacillus* sp. B-6. The growth of *Bacillus* sp. B-6 reached the stationary phase after about 4 days of cultivation. The phenazine 1-carboxylic acid (PCA) that was detected in the culture filtrate after 5 days of cultivation, and the amount of PCA, reached a maximum (about 400 μg/ml) at 8 days of cultivation (Fig. 1). To reveal the PCA resistance of *Bacillus* sp. B-6, growth of this bacterium in a medium supplemented with PCA was carried out and the results are shown in Fig. 2. Early (Fig. 2A) and late (Fig. 2B) log phase cells were transferred to the medium supplemented with PCA and the growth was followed by measuring the absorbance at 600 nm. Both the length of the lag phase and the rate of the growth were slightly affected by the age of the inoculum, but the growth pattern were essentially the same. The growth rate in the medium containing up to 100 μg/ml PCA was as rapid as that in PCA-free medium and even with 300 μg/ml PCA the growth rate was more than half the rate in the controls. Since the growth of PCA sensitive organisms was stopped completely at the PCA concentration of 100 μg/ml, the above results suggested that *Bacillus* sp. B-6 exhibited a higher resistance to PCA.

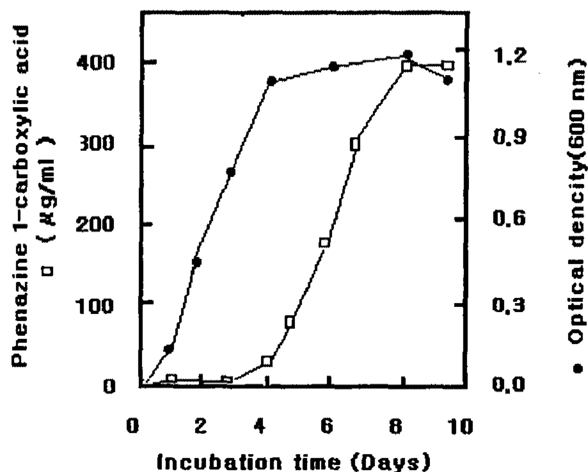

Fig. 1. Relationship of growth and production of PCA of *Bacillus* sp. B-6.

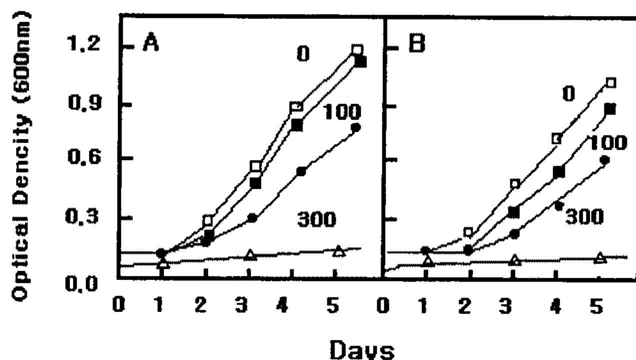

Fig. 2. PCA effect on the growth of *Bacillus* sp. B-6. Cells grown for 12 h (A) and 60 h (B) were inoculated into the 50 ml medium supplemented with various concentration of phenazine-1-carboxylic acid (numbers indicate μg/ml). The growth of *E. coli* cells which were inoculated into the same medium supplemented with 100 μg/ml of PCA was indicated as tringle (△).

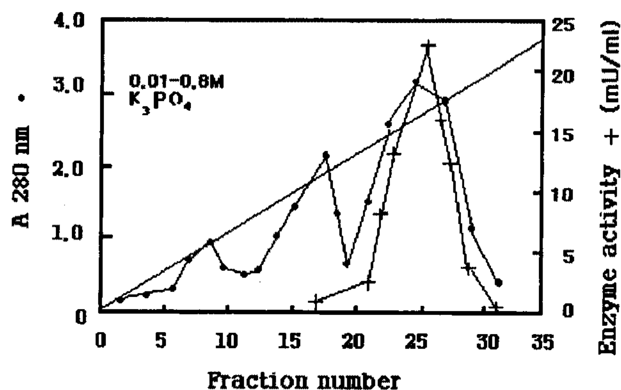

Fig. 3. Chromatogram of the acyl CoA synthetase on DEAE cellulose. The column was eluted with 0.01 M K_3PO_4, 0.001 M mercaptoethanol, pH 7.5 and 0.8 M K_3PO_4, 0.001 M mercaptoethanol, pH 7.5 at a flow rate 25 ml/h; Fractions of 6.0 ml were collected.

Malik and Vinig (1971) studied the chloramphenicol resistance of chloramphenicol producing *Streptomyces* and observed that late log phase cells showed a higher resistance to chloramphenicol than early log phase cells. To explain these observations, they postulated that membrane permeability for the antibiotic (Kim, *et al.*, 1998) changed after the exposure to chloramphenicol. In the case of *Bacillus* sp. B-6, it is hard to consider such changes of membrane permeability after exposure to PCA, because the growth patterns were not dependent on the age of the inoculum.

Purification and properties of acyl CoA synthetase Antibiotic-producing organisms should have appropriate resistance to their own products (Francois *et al.*, 1977; Katsukiyo *et al.*, 1994). There have been several studies on the mechanisms of resistance that producing organisms display toward their own toxic metabolites and three different mechanisms were reported (Konstatin and Shahriar, 1995; McKay *et al.*, 1994; Ying *et al.*, 1996). Permeability barriers prevent access of antibiotics to their target sites. The second mechanism, involving the resistance of the producers to their own antibiotics depends on inactivating enzymes of the antibiotics, such as streptomycin kinase, neomycin kinase, kanamycin acetyltransferase and chloramphenicol hydrolase. We could not obtain data on the presence of PCA-inactivating enzymes in *Bacillus* sp. B-6. The third mechanism is the

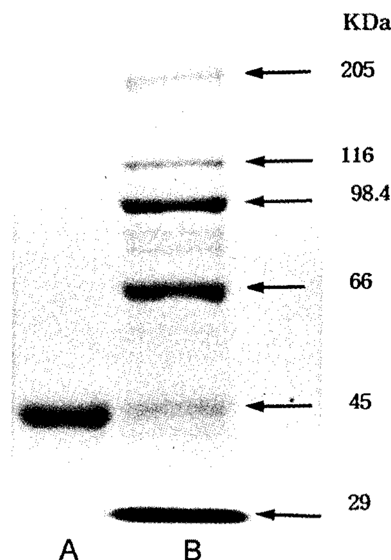

Fig. 4. SDS-polyacrylamide gel electrophoresis of of the acyl CoA synthetase from *Bacillus* sp. B-6. A: Purified acyl CoA synthetase, B: Molecular weight marker; myosin from rabbit muscle (205 kDa), β-galactosidase from *E. coli* (116 kDa) phosphorylase b (98 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa).

insensitivity of the target enzyme, or a lack of the target site for the antibiotics. To reveal the PCA resistance of *Bacillus* sp. B-6, the target enzyme, acyl CoA synthetase was purified.

Table 1. Purification steps of acyl CoA synthetase from *Bacillus* sp. B-6

Step	Total protein (mg)	Total activity (mU)	Specific activity (mU/mg)	Yield (%)	Purification fold
Crude extract	3,800	5,700	1.5	100	1
$(NH_4)_2SO_4$	950	4,275	4.5	75	3
Alumina Cγ	70	3,969	56.7	69	37.8
DEAE-cellulose	10	1,060	106	18	70.6

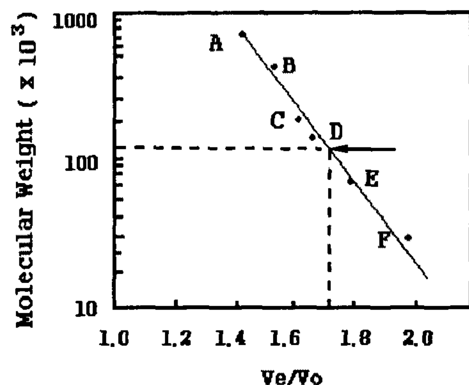

Fig. 5. Native molecular weight determination of acyl CoA synthetase from *Bacillus* sp. B-6 by Sepharose 6-B gel filteration. Molecular standards were A: bovine thyroglobulin (669 kDa), B: horse spleen apoferritin (443 kDa), C: sweet potato β-amylase (200 kDa), D: yeast alcohol dehydrogenase (150 kDa), E: bovine serum albumin (66 kDa), F: carbonic anhydrase (29 kDa). The arrow shows molecular weight of acyl CoA synthetase purified from *Bacillus* sp. B-6.

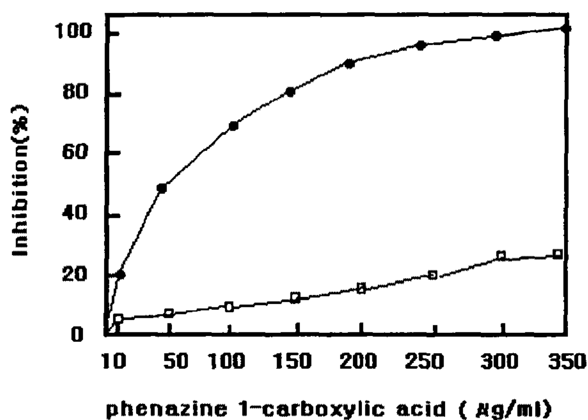

Fig. 6. Inhibition percentage of acyl CoA synthetases by PCA. The symbols in the graph are: ❑, *Bacillus* sp. B-6 acyl CoA synthetase, ●, *Pseudomonas* sp. acyl CoA synthetase. Assay of acyl CoA synthetase was as described under Materials and Methods.

Table 1. summarizes the results of the purification procedures of acyl CoA synthetase from *Bacillus* sp. B-6. By the use of 40-65% saturated ammonium sulfate precipitation, Alumina Cγ, and DEAE-cellulose column chromatography (Fig. 3), the acyl CoA synthetase was purified. The overall purification was 70.6 fold, with a specific activity of 106 mU/mg protein.

Molecular weight determination Purified acyl CoA synthetase runs in 10% SDS-PAGE as a single band corresponding to a mass of 45 kDa (Fig. 4). This molecular mass was confirmed by gel filtration with Sepharose 6-B column calibrated with several proteins with known molecular masses (Fig. 5). By this procedure, we estimated a mass of 132 kDa for acyl CoA synthetase. These results suggest that in its native form, the enzyme is a trimer. A similar molecular mass has been reported for the subunits of the acyl CoA synthetase of *Escherichia coli* (Kameda and Nunn, 1981), and acetyl CoA synthetases isolated from *Methanothrix soehngenii* (Jetten *et al.*, 1989), baker's yeast (Frenkel and Kichens, 1977) and *P. chrysogenum* (Martinez-Blank *et al.*, 1992), as well as for the acetyl-CoA ligase of *Neurospora crassa* and *Aspergillus nidulans* (Connerton *et al.*, 1990). The molecular weight of acyl CoA synthetase I from *Candida lypolytica* was reported as 84 kDa (Hosaka *et al.*, 1979).

Inhibition of acyl CoA synthetase by PCA Fig. 6. shows the effects of PCA on acyl CoA synthetase purified from *Bacillus* sp. B-6 and *Pseudomonas* sp. The enzyme from *Pseudomonas* sp. was 50% inhibited by PCA at concentration higher than 42 µg/ml. The activity of acyl CoA synthetase from *Bacillus* sp. B-6 was not affected up to 15 µg/ml PCA and inhibition was not complete even at a concentration of 350 µg/ml. The purified acyl CoA synthetase from *Bacillus* sp. B-6 was much less sensitive to PCA than acyl CoA synthetase from *Pseudomonas* sp. This insensitivity of the enzyme to PCA may be responsible for the protection of *Bacillus* sp. B-6 from inhibitory effects of this antibiotic.

References

Aatoshi, O., Hiroshi, T., Qin, M. X., Yoko, T. and Yuzuru, I. (1986) Triacins, new inhibitors of Acyl-CoA Synthetase produced by *Streptomyces* sp. *J. Antibiotics.* **39**, 1211-1218.

Bar-Tana, J., Rose, G. and Shapiro, B. (1971) The Purification and Properties of Microsomal Palmitoyl-Coenzyme A Synthetase. *Biochem. J.* **12**, 353-362.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the priciple of protein-dye binding. *Anal. Biochem.* **72**, 248-254.

Choi, Y. -H., Yang, C. -H., Kim, H. -W. and Jung, S. H. (2000) Molecular dynamic stimulations of the fatty acid bilayer contaning very long chain transmembrane dicarboxylic acids. *J. Biochem. Mol. Biol.* **33**(1), 54-58.

Connerton, I. F., Fincham, J. R. S., Sandeman, R. A. and Hynes, M. J. (1990) Comparison and cross-species expression of the acetyl-CoA synthetase genes of the ascomycete fungi *Aspergillus nidulans* and *Neurospora crassa. Mol. Microbio.* **4**, 453-460.

Fontes, R., Silero, M. A. and Silero, A. (1998) Acyl coenzyme A synthetase from *Pseudomonas fragi* catalyzes the synthesis of adenosine 5'-polyphosphates and dinucleoside polyphosphates. *J. Bacteriol.* **180**, 3152-3158.

Fontes, R., Silero, M. A. and Silero, A. (1999) Acyl coenzyme A synthetase catalyzes the synthesis of diadenosine hexaphosphate (Ap6A). *Biochimie.* **81**, 229-33.

Francois, L. G., Marie, L. C., Didier, B., Claude, C., Claude, S., Alain, D. and Duval, J. (1977) Plasmid-mediated pristinamycin resistance. PAC IIA: a new enzyme which modifies pristinamycin IIA. *J. Antibiotics.* **30**, 665-669.

Frenkel, E. P. and Kitchens, R. L. (1977) Purification and properties of acetyl coenzyme A synthetase from Baker's

yeast. *J. Biol. Chem.* **252**, 504-507.

Gargiulo, C. E, Stuhlsatz, K. S. M. and Sahaffer, J. E. (1999) Localization of adipocyte long-chain fatty acyl-CoA synthetase at the plasma membrane. *J. Lipid. Res.* **40**, 881-92.

Hiroshi, T., Kazuaki, I. and Satoshi, O. (1987) Inhibition of acyl-CoA synthetase by triacsins. *Biochim Biophys Acta.* **921**, 595-598.

Hiroshi, T., Kazuaki I, Cyong, J. C. and Satoshi O. (1991) Evidence for an Essential Role of Long Chain Acyl-CoA Synthetase in Animal Cell Proliferation. *J. Biol Chem.* **266**, 4214-4219.

Hosaka, K., Mishina, M., Tanaka, M., Kamiryo, T. and Numa, S. (1979) Acyl-coenzyme A synthetase I from *Candida lipolytica. Eur. J. Biochem.* **93**, 197-203.

Jetten, M. S. M., Stams, A. J. M. and Zehnder, A. J. B. (1989) Isolation and characterization of acetyl-coenzyme A synthetase from *Methanothrix soehngenii. J. Bacteriol.* **171**, 5430-5435.

Kameda, K. and Nunn, W. D. (1981) Purification and charaterization of acyl coenzyme A synthetase from *Escherichia coli. J. Biol. Chem.* **256**, 5702-5707.

Kamiryo, T., Parthasarothy, S. and Numa, S. (1976) Evidence that acyl coenzyme A synthetase activity is required for repression of yeast acetyl coenzyme A carboxylase by exogeneous fatty acids. *Proc. Natl. Acad. Sci. U.S.A.* **73**, 386-390.

Kasuya, F., Yamaoka, Y. Igarshi, K. and Fukui, M. (1998) Molecular specificity of a medium chain acyl-CoA synthetase for substrates and inhibitors; conformational analysis. *Biochem. Pharmacol.* **55**, 1769-75.

Kathleen, M. K. and Michael, E. J. (1992) Inhibition kinetics of hepatic microsomal long chain fatty acid-CoA ligase by 2-Arylpropionic acid non-steroidal anti-inflammatory drugs. *Biochem. Pharmacol.* **43**, 1465-1471.

Katsukiyo, Y., Yuzuru, M., Akio, M., Naoko, M. and Shigeo, I. (1994) Phosphorylative inactivation of rifampicin by Nocardia otitidiscaviarum. *J. Antimicrob. Chemother.* **33**, 1127-1135.

Kim, K.-J. and Kim, T.-S. (1996) Isolation and characterization of soil microorganism producing acyl CoA synthetase inhibitor. *Yakhak Hoeji.* **40**, 713-719.

Kim, K.-J. and Kim, T.-K. (1998) Isolation and structure elucidation of acyl CoA synthetase inhibitor from *Bacillus* sp. B-6. *Yakhak Hoeji.* **42**, 552-557.

Kim, K. T., Hong, S. W., Lee, J. H., Park, K. B. and Cho, K. S. (1998) Mechanism of antibiotic action and biosynthesis of centipedin purified from scalopendra subspinipes koch (centipede). *J. Biochem. Mol. Biol.* **31**(4), 328-332.

Konrad, E. T. and Ronald, L. M. (1994) In vitro evidence for involvement of CoA thioesters in peroxisome proliferation and hypolipidaemia. *Biochim Biophys Acta.* **1120**, 118-124.

Konstantin, M. and Shahriar, M. (1995) Purification, Characterization and Investigation of the Mechanism of Aminoglycoside 3-Phosphotransferase Type Ia. *Biochemistry.* **34**, 12681-12688.

Laemmli, U. K. (1970) Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature.* **227**, 680-685.

Malik, V. S. and Vinig, L. C. (1971) *Can. J. Microbiol.* **17**, 1287-1290.

Martinez-Blanco, H., Regiero, A., Fernandez-Valverde, M., Ferrero, M. A., Moreno, M. A., Penalva, M. A. and Luengo, J. M. (1992) Isolation and characterization of the acetyl-CoA synthetase from *Penicillium chrysogenum*. Involvement of this enzyme in the biosynthesis of penicillins. *J. Biol. Chem.* **267**, 5474-5481.

Masahiko, H., Yohsuke, I., Hiroaki, N., Hiroshi, T., Satoshi, O. and Sachiko, O. I. (1992) Enhanced production of platelet-activating factor in stimulated rat leukocytes pretreated with Triacsin C, a novel Acyl-CoA Synthetase Inhibitor. *Biochem. Biophys. Res. Commun.* **188**, 1280-1285.

McKay., G. A., Tompson, P. R. and Wright, G. D. (1994) Broad Spectrum Aminoglycoside Phosphotransferase Type III from *Enterococcus*: Overexpression, Purification, and Substrate Specificity. *Biochemistry.* **33**, 6936-6944.

Messemger, A. J. M. and Turner, J. M. (1983) Effect of growth conditions on phenazine production by *Pseudomonas phenazinium. J. Gen. Microbiol.* **129**, 1013-1018.

Park, N. S., Lee, N. J., Choi, Y. J., Kim, T. H. and Kim, K. W. (1998) Effect of α-glycosidase inhibitor in multidrug resistant cell lines. *J. Biochem. Mol. Biol.* **31**(3), 269-273.

Paul A. G., Susan, M. K., Richard, R. W. and Nada, A. A. (1992) Induction of aP2 gene expression by nonmetabolized long-chain fatty acids. *Proc. Natl. Acad. Sci. USA.* **89**, 10930-10934.

Russell, A. D. and Day, M. J. (1996) Antibiotic and biocide resistance in bacteria. *Microbios.* **85**, 45-65.

Sachiko, O.-I., Kohji, Y., Mitsuhiro, A., Hiroshi, T. and Satoshi, O. (1992) The Acyl-CoA Synthetase Inhibitor Triacsin C Enhanced Eicosanoid Release in Leukocytes. *Japan. J. Pharacol.* **59**, 417-418.

Salah, A., Jihan, Y., Mostafa, B., Paul, M. and Crist, F. (1992) The Inhibition of Long-Chain Fatty Acyl-CoA Synthetase by Enoximone in Rat Heart Mitochondria. *J. Cardiovasc. Pharmacol.* **19**, 899-904.

Steinberg, S. J., wang, S. J., Kim, D. G., Mihalik, S. J. and watkins, P. A. (1999) Human very-long-chain acyl-CoA synthetse: cloning, topography, and relevance to branched-chain fatty acid metabolism. *Biochem. Biophys. Res. Commun.* **257**, 615-21.

Tomashow, L. S. and Weller, D. M. (1988) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis var. tritici. J. Bacteriol.* **70**, 3499-3508.

Watkins, P. A., Lu, J. F., Steinberg, S. J., Gould, S. J., Smith, K. D. and Braiterman, L. T. (1998) Disruption of the Saccharomyces cerevisiae FAT1 gene decreases very long-chain fatty acyl-CoA synthetase activity and elevates intracellular very long chain fatty acid concentration. *J. Biol. Chem.* **273**, 18210-18219.

Ying, C. Y., Benjamin K, Victor L. Y, and Ding J. J. (1996) A mechanism of rifampicin and resistance in *Pseudomonas aeruginosa. J. Antimicrob Chemother.* **38**, 133-137.