

# Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids

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**Abstract** Long-chain unsaturated fatty acids, such as linoleic acid, show antibacterial activity and are the key ingredients of antimicrobial food additives and some antibacterial herbs. However, the precise mechanism for this antimicrobial activity remains unclear. We found that linoleic acid inhibited bacterial enoyl-acyl carrier protein reductase (FabI), an essential component of bacterial fatty acid synthesis, which has served as a promising target for antibacterial drugs. Additional unsaturated fatty acids including palmitoleic acid, oleic acid, linolenic acid, and arachidonic acid also exhibited the inhibition of FabI. However, neither the saturated form (stearic acid) nor the methyl ester of linoleic acid inhibited FabI. These FabI-inhibitory activities of various fatty acids and their derivatives very well correlated with the inhibition of fatty acid biosynthesis using [<sup>14</sup>C] acetate incorporation assay, and importantly, also correlated with antibacterial activity. Furthermore, the supplementation with exogenous fatty acids reversed the antibacterial effect of linoleic acid, which showing that it target fatty acid synthesis. Our data demonstrate for the first time that the antibacterial action of unsaturated fatty acids is mediated by the inhibition of fatty acid synthesis.

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## 1. Introduction

The antibacterial activity of long-chain unsaturated fatty acids have been well known for many years. Fatty acids function as the key ingredients of antimicrobial food additives which inhibit the growth of unwanted microorganisms [1]. Linoleic and oleic acids are antibacterial components in the herbs (*Helichrysum pedunculatum* and *Schotia brachypetala*) used for dressing wounds during male circumcision rituals in South Africa [2,3]. Besides normal fatty acids, fatty acid derivatives showing potent antimicrobial activities exist in nature. These are mainly found in microorganisms, algae, or plants, which may mediate chemical defense against microorganisms [4–6].

Additionally, long-chain unsaturated fatty acids are bactericidal to important pathogenic microorganisms, including Methicillin-resistant *Staphylococcus aureus* [7–9], *Helicobacter pylori* [10,11], and *Mycobacteria* [12]. These antibacterial actions of fatty acids are usually attributed to long-chain unsaturated fatty acids including oleic acid, linoleic acid, and linolenic acid, while long-chain saturated fatty acids, including palmitic acid and stearic acid, are less active [7,9,10,12]. However, their primary molecular target still remains unknown.

Fatty acid synthesis (FAS) in bacteria is essential to the production of a number of lipid-containing components, including the cell membranes [13]. Bacterial fatty acid synthesis is carried out by a set of individual enzymes which are collectively known as type II, while mammalian fatty acid is mediated by a single multifunctional enzyme-acyl carrier protein (ACP) complex referred to as type I. FabI is an enoyl-ACP reductase which catalyzes the final and rate-limiting step of the chain elongation process of the type II FAS. Since there is a lack of an overall sequence homolog with the corresponding one of humans, FabI has been identified as a target for antibacterial drug development [14]. Indeed, FabI has been revealed as a target of the broad spectrum biocide, triclosan, which is used as an antibacterial additive in a wide range of consumer goods and was widely thought to be a non-specific biocide which attacks bacterial membranes [15,16]. The site of action of isoniazid, used in the treatment of tuberculosis for 50 years, was also determined to be the mycobacterial FabI homolog (termed InhA) [17].

During the course of our screening for FabI inhibitors from natural resources for new antibacterial-drug development, we frequently experienced the isolation of unsaturated fatty acids as potent inhibitors of FabI. This led us to examine whether unsaturated fatty acids are selective inhibitors of FabI and this inhibition is related to the inhibition of fatty acid synthesis and their antibacterial activity.

## 2. Materials and methods

### 2.1. Materials

Oleic acid, linoleic acid, linolenic acid, stearic acid, palmitic acid, palmitoleic acid, arachidonic acid, oleic acid methyl ester, linoleic acid methyl ester, and arachidonic acid methyl ester were purchased from Sigma. The unsaturated fatty acids are purchased all as the *cis* form. [<sup>1-14</sup>C] acetate (57 μCi/μmol) and L-[U-<sup>14</sup>C] leucine (306 μCi/μmol) were purchased from Amersham.

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## 2.2. Synthesis of *trans*-2-octenoyl *N*-acetylcysteamine thioester (*t*-o-NAC-thioester)

To a solution of 2-octenoic acid (1138 mg, 8 mmol), *N*-acetylcysteamine (954 mg, 8 mmol), and 4-dimethylaminopyridine (2064 mg, 18.4 mmol) in dichloromethane at room temperature was added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (3527 mg, 18.4 mmol). After overnight the reaction solution was concentrated under vacuum, and usual aqueous workup. The residue was purified by flash chromatography on silica gel (Hexane/EA = 3:1) to give the title compound (1363 g, 70%) as a solid.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.90 (t, *J* = 6.5 Hz, 3H), 1.26–1.33 (m, 4H), 1.45–1.50 (m, 2H), 1.97 (s, 3H), 2.20 (q, *J* = 7.2 Hz, 2H), 3.09 (t, *J* = 6.2 Hz, 2H), 3.46 (q, *J* = 6.2 Hz, 2H), 6.03 (br s, 1H), 6.07–6.15 (m, 1H), 6.89–6.99 (m, 1H).

## 2.3. Cloning of the *fabI* gene

The full length the *fabI* gene was amplified from genomic DNA obtained from *Escherichia coli* or *S. aureus* (ATCC 26695). After confirming the DNA sequence, the gene was cloned into a modified pET21b vector (Novagen, USA) which has 6 His-tag coding regions at the N-terminus of the insert. After transforming the ligated mixture into BL21(DE3) with ampicillin resistance, the resultant colonies were screened for their ability to overexpress proteins of the correct size after induction by IPTG. To obtain FabI, the bacteria were cultured in Luria–Broth media and induced by 0.5 mM IPTG at 18 °C for 18 h. The cells were collected by centrifugation, resuspended in a buffer A (50 mM Tris(pH 8.0) + 300 mM NaCl + 5 mM imidazole) and disrupted by microfluidizer (Model M-110L, Microfluidics, USA). Soluble fraction was applied onto a Ni-NTA (Hi-trap, 5 ml, Amersham) and washed by buffer A, followed by elution in buffer A containing 500 mM imidazole. The fusion protein was further purified by Superdex 200 (Amersham) in a buffer B (50 mM Tris (pH 8.5) + 200 mM NaCl + 2 mM DTT), and the purified protein was stored at –20 °C until use.

## 2.4. FabI assay

Assays were carried out in half-area, 96-well microtitre plates. Compounds were evaluated in 100 μl assay mixtures containing components specific for each enzyme (see below). Reduction of the *t*-o-NAC substrate analog was measured spectrophotometrically by following the utilization of NADH or NADPH at 340 nm at 30 °C for the linear period of the assay. *S. aureus* FabI assays contained 50 mM sodium acetate, pH 6.5, 400 μM *t*-o-NAC, 200 μM NADPH, and 150 nM *S. aureus* FabI. The rate of decrease in the amount of NADPH in each reaction well was measured by a microtiter ELISA reader using SOFTmax PRO software (Molecular Devices, California, USA). The inhibitory activity was calculated by the following formula: % of inhibition = 100 × [1 – (rate in the presence of compound/rate in the untreated control)]. IC<sub>50</sub> values were calculated by fitting the data to a sigmoid equation. An equal volume of dimethyl sulfoxide solvent was used for the untreated control. *E. coli* FabI assays contained 50 mM sodium phosphate, pH 7.5, 200 μM *t*-o-NAC, 200 μM NADH, and 150 nM *E. coli* FabI. The inhibitory activity was determined in the same methods as for *S. aureus* FabI, as described above.

## 2.5. Determination of MIC

The antibacterial activities of the test compounds were evaluated using clinically isolated bacterial strains (Hoechst, Germany). The strains were inoculated into 3 ml of Fleisch extract broth (Beef extract 1%, peptone 1%, NaCl 0.3%, Na<sub>2</sub>HPO<sub>4</sub> · 12 H<sub>2</sub>O 0.2%, pH 7.4–7.5. For *Streptococcus pyogenes*, 10% horse serum was supplemented.) and cultured on a shaking incubator at 37 °C for 18 h. Test compounds were serially diluted in 2-fold dilutions from 2 mM to 15 μM. The 1.5 ml volume of each diluted solution was mixed with 13.5 ml of Muller Hinton agar (Difco, USA) and plated. The overnight-cultured strains were 100-fold diluted with broth on a 96-well plate. The diluted bacterial culture media were then inoculated (10<sup>4</sup> CFU/spot) on the prepared agar plates by an automatic inoculator (Dynatech, USA). The plates were incubated at 37 °C for 18 h. The lowest concentration that prevented the growth of each bacterium was determined to be MIC.

## 2.6. [<sup>14</sup>C]acetate incorporation

*S. aureus* was grown to mid-log phase in LB medium. Each 1 ml culture was treated with drugs for 10 min. An equal volume of DMSO solvent was added to the untreated control. 2 μCi of [<sup>14</sup>C] acetate was then added to the cultures and incubated at 37 °C for 1 h in a shaker. After being harvested by centrifugation, the cell pellets were washed twice with PBS buffer. The total cellular lipids were then extracted with chloroform–methanol–water. The incorporated radioactivity in the chloroform phase was measured by scintillation counting. Data were expressed as percentage inhibition of incorporation compared with the untreated control.

## 2.7. L-[<sup>14</sup>C]leucine incorporation

*S. aureus* were prepared in the same manner as [<sup>14</sup>C]acetate incorporation. Each 1 ml culture was treated with drugs and an equal volume of DMSO solvent as the untreated control for 10 min. 0.6 μCi of L-[<sup>14</sup>C] leucine was then added to the cultures and incubated at 37 °C for 1 h in a shaker. The incorporation was terminated by the addition of 10% (wt/vol) trichloroacetic acid (TCA) and cooling on ice for 20 min. The precipitated material was collected on Whatman GF/C glass microfiber filters, washed with TCA and ethanol, dried, and counted in a scintillation counter.

## 2.8. Supplementation of exogenous fatty acids

*S. aureus* was grown to mid-log phase in LB medium and diluted 1000-fold in the same medium. An 100 μl of the diluted cell suspension (2 × 10<sup>5</sup> cells) were used to inoculate to each well of a 96-microtiter plate containing 95 μl of LB media with inhibitors at the concentration of MIC. 5 μl of the serially diluted fatty acid solution was supplemented, and the cell suspension was incubated at 37 °C for 18 h. The bacterial growth was determined by measuring at 650 nm using a microtiter ELISA reader. Since fatty acids are easily transported into cells from the medium as an ethanolic suspension [18], the fatty acids were added as ethanolic solutions. An equal volume of ethanol solvent was added for the untreated control.

## 3. Results

### 3.1. Inhibition of FabI by linoleic acid

The ability of linoleic acid to specifically inhibit FabI was investigated in an in vitro spectrophotometric assay using *S. aureus* FabI and the enoyl-ACP substrate analog, *t*-o-NAC-thioester. As shown in Fig. 1, the addition of increasing concentrations of linoleic acid to the reaction potentially inhibited the reduction of *t*-o-NAC-thioester by NADPH with an IC<sub>50</sub> of 39 μM. We expanded our analysis to include 4 additional unsaturated fatty acids, palmitoleic acid, oleic acid, linolenic acid, and arachidonic acid, to investigate whether the inhibition of FabI was a characteristic of this class of antibacterial compound. All of the tested unsaturated fatty acids exhibited inhibition of *S. aureus* FabI with IC<sub>50</sub>s between 25 and

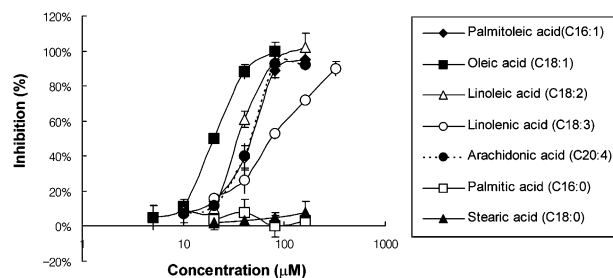


Fig. 1. Inhibitory effects of long-chain fatty acids on *S. aureus* FabI. The values were represented as the means ± S.D. in triplicates obtained from two independent experiments.

Table 1

Comparison of effects of long-chain fatty acids and their derivatives on enoyl reductase, bacterial viability, and [ $^{14}\text{C}$ ] acetate incorporation

Compounds	IC <sub>50</sub> (mM)	MIC (mM)		[ $^{14}\text{C}$ ]acetate incorporation inhibition in <i>S. aureus</i> (IC <sub>50</sub> (mM))
	<i>S. aureus</i> FabI	<i>S. aureus</i>	<i>S. pyogenes</i>	
Palmitic acid (C16:0)	>2	>2	>2	>2
Stearic acid (C18:0)	>2	>2	>2	>2
Palmitoleic acid (C16:1)	0.041	0.4	0.1	0.028
Oleic acid (C18:1)	0.020	0.4	0.1	0.027
Linoleic acid (C18:2)	0.035	0.2	0.05	0.011
Linolenic acid (C18:3)	0.080	0.4	0.1	0.029
Arachidonic acid (C20:4)	0.041	0.2	0.1	0.030
Oleic acid, Me ester	>2	>2	>2	>2
Linoleic acid, Me ester	>2	>2	>2	>2
Arachidonic acid, Me ester	>2	NT	NT	>2

73  $\mu\text{M}$ . To see the structure–activity relationship, the tested saturated fatty acid forms and methyl ester forms of the unsaturated fatty acids were assayed. Interestingly, even at 2 mM, saturated fatty acids, stearic acid and palmitic acid, did not inhibit FabI activity. Methyl ester forms of oleic acid, linoleic acid, and arachidonic acid were also not active (Table 1). Thus, these data clearly show that unsaturated fatty acids were solely active and, importantly, both the double bond and carboxylic acid moiety of the unsaturated fatty acids are critical for their activity. For a double check of the fatty acid inhibition of FabI, assays were carried out using *E. coli* FabI and resulted in a very similar phenomena as in the case of *S. aureus* FabI (data not shown).

### 3.2. Mode of inhibition of FabI by linoleic acid

The kinetic mechanism for inhibition of FabI was investigated using linoleic acid as a model compound. The FabI reaction mechanism is obligatory ordered sequential with the nucleotide cofactors, NADH or NADPH, as the first substrates. In order to determine whether linoleic acid binds to the free enzyme, the enzyme–substrate complex, or both, the inhibition pattern with respect to the substrate and the cofactor was examined with a Lineweaver–Burk plot. The inhibition of *S. aureus* FabI by linoleic acid was mixed with respect to *t*-o-NAC with a  $K_i$  value of 14.6  $\mu\text{M}$  (Fig. 2A and C). Additionally, linoleic acid exhibited mixed inhibition respective to NADPH (Fig. 2B). Thus, linoleic acid binds to the free enzyme to prevent the binding of the nucleotide cofactor, and also binds to the FabI–NADPH complex to prevent the binding of the substrate.

### 3.3. Antibacterial activities of fatty acids

Fatty acids tested in enzyme assays were tested for their antibacterial activity against the Gram positive pathogens, *S. aureus* and *S. pyogenes*, and against Gram negative bacteria, including *E. coli* and *Pseudomonas aeruginosa* (Table 2). The antibacterial activity of fatty acids tested in enzyme assays was monitored by the ability (MIC) of the fatty acids to inhibit cell growth. Unsaturated fatty acids tested exhibited the antibacterial activity with MIC values of 0.05–0.4 mM against Gram positive bacteria of *S. aureus* and *S. pyogenes*, while they did not show antibacterial activity on Gram negative bacteria of *E. coli* and *P. aeruginosa*. The MIC values are similar to the reported values (0.05–0.5 mM) [9]. The saturated fatty acids tested were not active on either Gram positive or Gram negative bacteria, even at 2 mM. This differential antibacterial

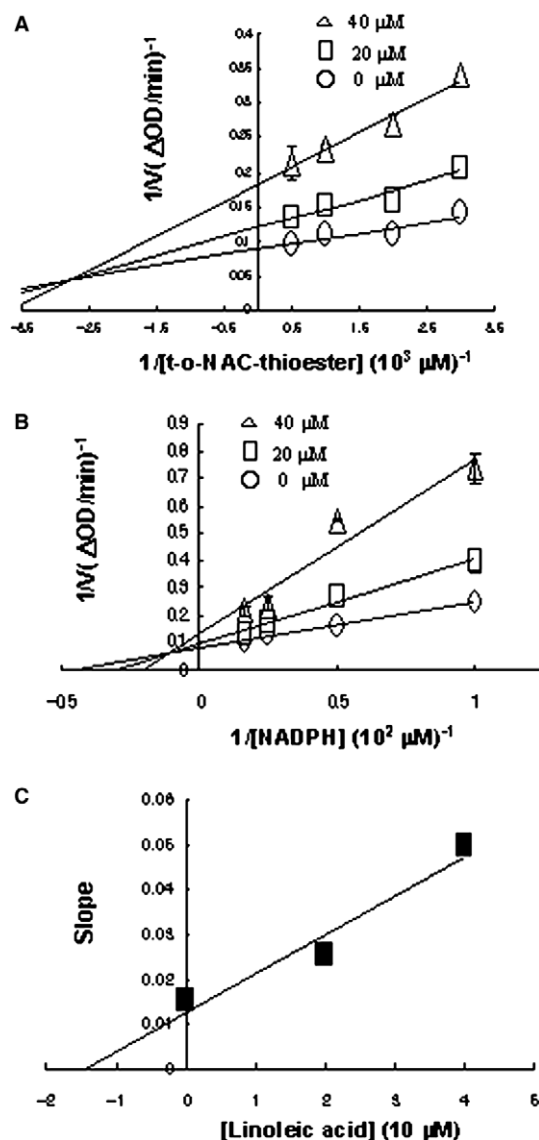


Fig. 2. The mechanism of inhibition of *S. aureus* FabI by linoleic acid respective to *t*-o-NAC thioester (A) and NADPH (B), and  $K_i$  determination of linoleic acid (C). (A, B) The reciprocals of the initial reaction and substrate (A) and cofactor (B) concentrations are plotted. (C) The slope values of the lines from graph A are plotted versus the inhibitor concentrations affording a line obtained by linear regression. The intercept point of this line with the x-axis gives an approximate  $K_i$  value of 14.6  $\mu\text{M}$  for linoleic acid. The values were represented as the means  $\pm$  S.D. in triplicates.

Table 2  
Antibacterial effects of long-chain fatty acids and their derivatives<sup>a</sup>

	1	2	3	4	5	6	7	8	9
<i>S. pyogenes</i> 308A	>1	>1	0.1	0.1	0.05	0.1	0.1	>1	>1
<i>S. pyogenes</i> 77A	>1	>1	0.1	0.2	0.1	0.2	0.1	>1	>1
<i>S. aureus</i> SG5 11	>1	>1	0.4	0.4	0.4	0.4	0.2	>1	>1
<i>S. aureus</i> 285	>1	>1	0.4	0.4	0.2	0.4	0.2	>1	>1
<i>S. aureus</i> 503	>1	>1	0.4	0.4	0.2	0.4	0.2	>1	>1
<i>E. coli</i> 078	>1	>1	>1	>1	>1	>1	>1	>1	>1
<i>E. coli</i> DCO	>1	>1	>1	>1	>1	>1	>1	>1	>1
<i>P. aeruginosa</i> 9027	>1	>1	>1	>1	>1	>1	>1	>1	>1
<i>P. aeruginosa</i> 1592E	>1	>1	>1	>1	>1	>1	>1	>1	>1

1, Palmitic acid; 2, stearic acid; 3, palmitoleic acid; 4, oleic acid; 5, linoleic acid; 6, linolenic acid; 7, arachidonic acid; 8, oleic acid methyl ester; 9, linoleic acid methyl ester.

<sup>a</sup>Results are given in mM.

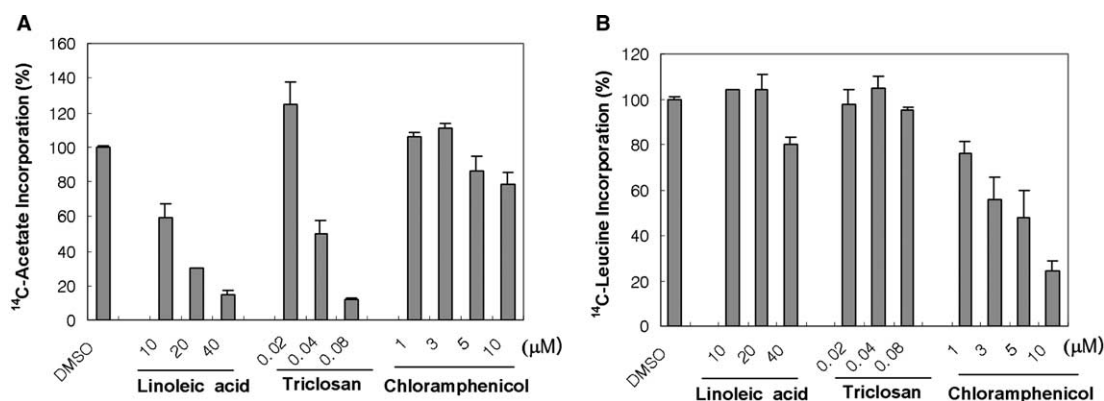


Fig. 3. Effects of linoleic acid on fatty acid biosynthesis (A) and protein biosynthesis (B) of *S. aureus*. Triclosan (a known FabI inhibitor) and chloramphenicol (a known protein synthesis inhibitor) were used as positive and negative controls, respectively. The values were represented as the means  $\pm$  S.D. in duplicates obtained from two independent experiments.

activity between unsaturated fatty acids and saturated fatty acids on Gram positive bacteria of *S. aureus* and *S. pyogenes* was well correlated with the inhibition of *S. aureus* FabI in vitro (Table 1).

### 3.4. Inhibition of cellular fatty acid synthesis by linoleic acid

To evaluate whether unsaturated fatty acids inhibit cellular fatty acid synthesis, we determined whether the compounds inhibited the incorporation of acetate into membrane fatty acids in vivo. We measured the effects of fatty acids on the incorporation of [1-<sup>14</sup>C] acetate into the membrane fatty acids in *S. aureus*. In agreement with their antibacterial activity, unsaturated fatty acids indeed blocked fatty acid synthesis in vivo compared to the untreated cells, while saturated fatty acid and esters of fatty acid did not affect fatty acid synthesis (Table 1). As shown in Fig. 3A, with an IC<sub>50</sub> value of 10.9 μM, linoleic acid selectively inhibited incorporation of radioactively labeled acetate into chloroform/methanol-extractable phospholipids in a dose-dependent fashion, as the known FabI inhibitor, triclosan, inhibited acetate incorporation. The potency of unsaturated acid tested in the inhibition of [1-<sup>14</sup>C] acetate incorporation was similar to that against FabI. In contrast, the incorporation of leucine into proteins was not inhibited by linoleic acid at the concentrations showing the inhibition of fatty acid synthesis, whereas the protein synthesis inhibitor, chloramphenicol, inhibited incorporation (Fig. 3B).

### 3.5. Reversion of the antibacterial effect of linoleic acid by the supplementation with exogenous fatty acids

To see whether the antibacterial effect of linoleic acid is due to the inhibition of fatty acid synthesis, we examined whether *S. aureus* in linoleic acid medium could grow by the supplementation with exogenous fatty acids. *S. aureus* in medium containing linoleic acid at the MIC value of 200 μM did not grow at all compared to the untreated control cells. However, when either saturated fatty acids (stearic acid and palmitic acid) or unsaturated fatty acid (oleic acid) at sub-antibacterial concentration were supplemented to the final concentration of 50, 100, and 200 μM, the *S. aureus* cells in linoleic acid medium grew well in a dose-dependent manner (Fig. 4A). Similarly, *S. aureus* cells were rescued from the growth-inhibitory effect of triclosan, a FabI inhibitor, by addition of the exogenous fatty acids (Fig. 4B). As a negative control, *S. aureus* in medium containing chloramphenicol, a protein synthesis inhibitor, did not grow in supplementation of the same fatty acids (Fig. 4C). This result indicated that unsaturated fatty acids target fatty acid synthesis.

## 4. Discussion

The antibacterial activity of long-chain unsaturated fatty acids (C16–C20) against *Staphylococci*, *Streptococci*, *Mycobacteria*, *Helicobacter*, and *Bacilli* are well documented as the



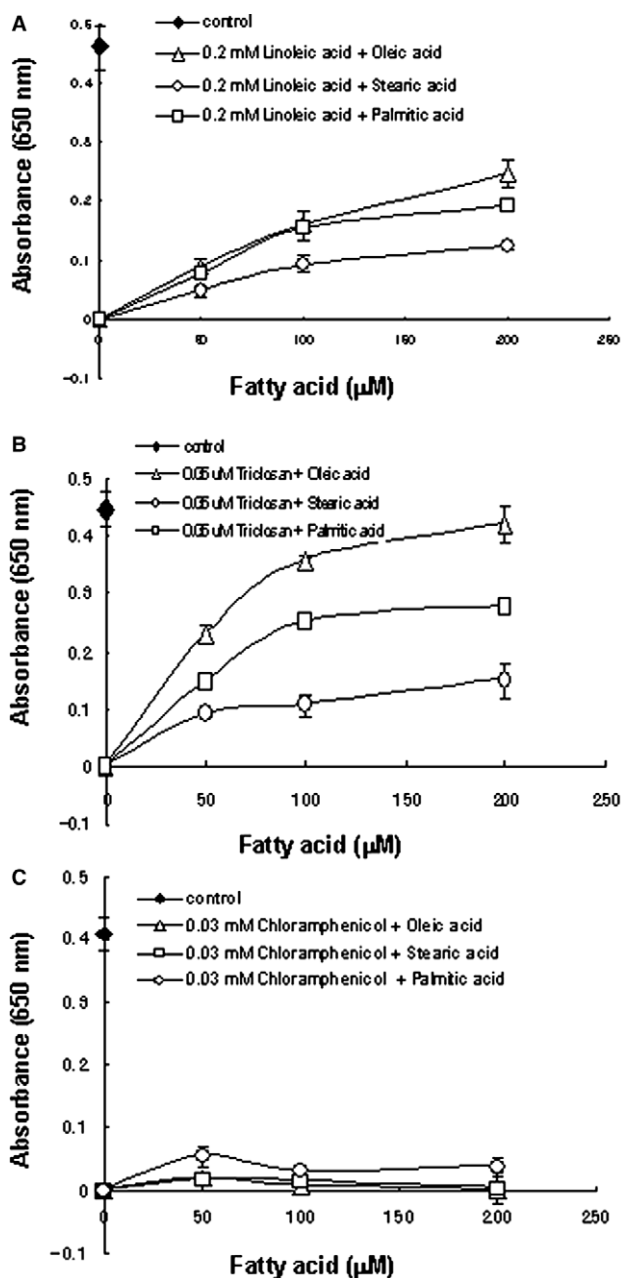


Fig. 4. Growth of *S. aureus* in linoleic acid medium by supplementations with exogenous fatty acids (A). Triclosan (B) and chloramphenicol (C) were used as positive and negative controls, respectively. The values were represented as the means  $\pm$  S.D. in triplicates obtained from two independent experiments.

ingredients of most antimicrobial food additives, key ingredients of traditional antibacterial and antituberculosis herbs, antimicrobial components of milk fat effective for stomach ulcers, and antibacterial compounds in plants or microorganisms [1–3,7–12]. Even though there have been several reports regarding the mode of action of long-chain unsaturated fatty acids, the precise mechanism for this antimicrobial activity remains unclear. Fatty acid synthesis in bacteria is essential to the production of a number of lipid-containing components, including the cell membranes. The bacterial fatty acid synthesis is carried out by a set of individual enzymes in conjunction with acyl carrier protein (ACP)-associated substrates. FabI

catalyzes the final, rate-limiting step of the chain elongation process in bacterial fatty acid synthesis; it has been validated as an excellent target for antibacterial drug development [13,14].

In this study, we found that linoleic acid is a model compound of unsaturated fatty acids which selectively inhibits FabI of *S. aureus* and *E. coli*. Other long-chain unsaturated fatty acids also inhibit FabI, whereas long-chain saturated fatty acids were not active. Methyl esterification of long-chain unsaturated fatty acids results in the loss of FabI-inhibitory activity. In the antibacterial assay using whole cells, unsaturated fatty acids showed greater inhibition than saturated fatty acids, which is consistent with the results seen by several other investigators [1,19]. Methyl ester derivatives were less active than the corresponding acids. This is consistent with the observations of Kabara et al. [9], who showed that the free carbonyl group is necessary for activity. It is important to note that the differential action of these fatty acids and their derivatives in the FabI enzyme assay is highly significantly correlated with that in their antibacterial activity against the Gram positive bacteria, strongly suggesting that the FabI step in bacterial type II fatty acid synthesis could be a molecular target for long-chain unsaturated fatty acids. This hypothesis was supported by the incorporation assay of [ $^{14}$ C] acetate into membrane fatty acids in bacterial cells, showing that long-chain unsaturated fatty acids inhibit fatty acid synthesis, while their methyl esters and saturated fatty acids do not. Importantly, antibacterial effect of linoleic acid was reversed by the supplementation with either saturated fatty acids (stearic acid and palmitic acid) without antibacterial activity or unsaturated fatty acid (oleic acid) at sub-antibacterial concentration. This result indicated that unsaturated fatty acids exerted their antibacterial effect by inhibiting fatty acid synthesis.

Greenway and Dyke [19] had suggested that linoleic acid probably inhibited growth by increasing the permeability of the bacterial membrane as a result of its surfactant action. This hypothesis had not explained the reason why saturated fatty acids did not inhibit bacterial growth. However, this question could be answered by our findings that there were the differences between unsaturated fatty acids and saturated fatty acids in terms of the inhibition of fatty acid synthesis and FabI.

Unsaturated fatty acids also inhibit *E. coli* FabI at a similar dosage as with *S. aureus*. However, this is not correlated with a lack of, or very weak, antibacterial activity of unsaturated fatty acids against *E. coli*. Long-chain unsaturated fatty acids, including linoleic acid, are well known to not inhibit Gram negative bacteria such as *E. coli* [1,2,9,10]. This large difference in the fatty acid sensitivities between Gram positive and Gram negative bacteria may result from the impermeability of the outer membrane of Gram negative bacteria since the outer membrane of Gram negative bacteria is an effective barrier against hydrophobic substances [20–22].

Unsaturated fatty acids have been known to reduce tumor growth, but their molecular mechanism has remained elusive [23–25]. Type I fatty acid synthase (FAS I), by which the de novo synthesis of fatty acids in mammalian cells is accomplished, is overexpressed in some breast cancer cells; inhibition of FAS I has been shown to induce apoptosis in breast cancer cells, and, consequently, is a potential chemotherapeutic target [26,27]. Triclosan, a well-known inhibitor of FabI, has been shown to be toxic to breast cancer cells [28]. Since fatty acids selectively inhibit fatty acid synthesis, the anticancer activity

of fatty acids can be speculated to arise from the inhibition of fatty acid synthesis.

In summary, long-chain unsaturated fatty acids are selective inhibitors of FabI and antibacterial effect of long-chain unsaturated fatty acids is due to their inhibition of fatty acid biosynthesis. Also, the differential activity was found between unsaturated fatty acids and saturated fatty acids in the inhibition of fatty acid synthesis and FabI, which could explain the unsolved question why saturated fatty acids did not have antibacterial activity.

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