J. Microbiol. Biotechnol. (2016), 26(9), 1579–1585
http://dx.doi.org/10.4014/jmb.1601.01045

jmb

Antibacterial Effect of Fructose Laurate Synthesized by *Candida antarctica* B Lipase-Mediated Transesterification[S]

Ki Ppeum Lee and Hyung Kwoun Kim*

Department of Biotechnology, The Catholic University of Korea, Bucheon 14662, Republic of Korea

Received: January 19, 2016
Revised: May 25, 2016
Accepted: June 1, 2016

First published online
June 10, 2016

*Corresponding author
Phone: +82-2-2164-4890;
Fax: +82-2-2164-4865;
E-mail: hkkim@catholic.ac.kr

Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

Copyright© 2016 by
The Korean Society for Microbiology and Biotechnology

Sugar esters are valuable compounds composed of various sugars and fatty acids that can be used as antibacterial agents and emulsifiers in toothpaste and canned foods. For example, fructose fatty acid esters suppress growth of *Streptococcus mutans*, a typical pathogenic bacterium causing dental caries. In this study, fructose laurate ester was chosen as a target material and was synthesized by a transesterification reaction using *Candida antarctica* lipase B. We performed a solvent screening experiment and found that a *t*-butanol/dimethyl sulfoxide mixture was the best solvent to dissolve fructose and methyl laurate. Fructose laurate was synthesized by transesterification of fructose (100 mM) with methyl laurate (30 mM) in *t*-butanol containing 20% dimethyl sulfoxide. The conversion yield was about 90%, which was calculated based on the quantity of methyl laurate using high-performance liquid chromatography. Fructose monolaurate (M_r 361) was detected in the reaction mixture by high-resolution mass spectrometry. The inhibitory effect of fructose laurate on growth of oral or food spoilage microorganisms, including *S. mutans*, *Bacillus coagulans*, and *Geobacillus stearothermophilus*, was evaluated.

Keywords: Lipase, fructose laurate, antimicrobial activity, transesterification, sugar ester

Introduction

Sugar esters are diverse compounds composed of a hydrophilic carbohydrate moiety and one or more fatty acids as lipophilic moieties [15, 18]. These molecules are usually tasteless and odorless nonionic surfactants. They are not only biodegradable, non-toxic, and non-irritating but they have emulsifying, stabilizing, and detergent effects as well. Some sugar esters also have antibacterial activities [3, 6, 17, 22]. Some studies have reported that gram-positive bacteria are more susceptible to inhibition by sugar esters than gram-negative bacteria [10]. For example, fructose and galactose esters strongly inhibit growth of *Streptococcus mutans*. The hydroxyl group in the sugar moiety of ester compounds is very important for their antimicrobial properties [23]. In addition, sucrose palmitate ester has bacteriostatic activities against *Bacillus coagulans* and *Geobacillus stearothermophilus* [10]. Thus, sugar esters have received attention in the food, cosmetics, pharmaceutical, and dental care industries [8, 10, 11].

S. mutans is a well-known opportunistic pathogenic bacterium that causes dental caries [10]. It grows in the presence of sucrose and produces a glucosyltransferase enzyme that synthesizes a water-insoluble glucan to form the carious tooth. Some fructose fatty acid esters inhibit growth of *S. mutans* [17, 22].

B. coagulans and *G. stearothermophilus* are representative putrefying bacteria in canned foods. *B. coagulans* is a non-pathogenic, facultative anaerobic, thermotolerant, and acidophilic bacterium [3], whereas *G. stearothermophilus* is a thermophilic spore-forming bacterium that frequently causes spoilage of canned vegetables. Its spores can survive thermal processing of commercial products. Some sugar esters are possible candidate materials to prevent growth of these bacteria [12].

Some sugar esters are currently being manufactured chemically. However, chemical methods generally produce several isomers with different degrees of acylation and different acylated positions. Therefore, enzymatic synthesis is expected to be a promising method [5], as bioconversion

reactions can be performed at relatively low temperatures, so energy is saved and side reactions are prevented. Enzymes have substrate specificity and regio-selectivity, so target compounds can be synthesized with a high degree of purity [21].

Lipases not only perform hydrolysis reactions in aqueous systems but also esterification and transesterification reactions in non-aqueous systems. Thus, lipases can be used to produce sugar esters based on their regioselectivity and stereoselectivity [4, 7, 9]. Among many microbial lipases, *Candida antarctica* lipase and its immobilized form, Novozym 435, are the most frequently used enzymes in various bioconversion reactions [2, 20].

In this study, we performed a *C. antarctica* B lipase (CalB)-mediated transesterification reaction to produce fructose laurate, which we evaluated for its antibacterial activity against *S. mutans*, *B. coagulans*, and *G. stearothermophilus*.

Materials and Methods

Reagents

Fructose, lauric acid, methyl laurate, nutrient broth, brain heart infusion broth, lipase acrylic resin from *Candida antarctica*, 2-methyl-2-butanol, *n*-heptane, and acetone were purchased from Sigma (USA). *t*-Butanol, dimethyl sulfoxide (DMSO), and *n*-hexane were purchased from Junsei Chemical (Japan). Water and acetonitrile were supplied by J.T. Baker (USA). Glass beads were purchased from Glastechnik (Germany).

Bacterial Strains

Freeze-dried type cultures of *B. coagulans* (KCTC 3625), *G. stearothermophilus* (KCTC 2107), and *S. mutans* (KCTC 3065) were purchased from the Korean Collection for Type Cultures (South Korea).

Lipase Assay

To measure transesterification activity, enzyme samples (5 mg of immobilized CalB) were added to 0.5 ml of 10 mM *p*-nitrophenyl palmitate (*p*NPP) in hexane and 30 µl ethanol [19]. The reaction was performed at 30°C for 10 min with shaking (230 rpm). After settling, 25 µl of the supernatant was taken and mixed immediately with 1 ml of 0.1 M NaOH. The liberated *p*-nitrophenol (*p*NP) was extracted using the aqueous alkaline phase, and the amount of *p*NP was determined by absorbance at 410 nm. One unit of lipase activity (U) was defined as the lipase amount needed to liberate 1 µmol *p*NP per minute.

Hydrolytic activity was measured using the pH stat method [4]. An olive oil emulsion was prepared by emulsifying a mixture containing 1% olive oil, 1% arabic gum, 20 mM NaCl, and 5 mM $CaCl_2$ for 2 min at maximum speed in a Waring blender. The pH of the substrate emulsion (20 ml) was adjusted to 8.0 with 10 mM NaOH solution, and 5 mg of lipase was added. The fatty acid release rate was measured using a pH titrator (718 Stat Titrino; Metrohm, Switzerland) for 3 min at 30°C. One unit of lipase activity (U) was defined as the amount of enzyme required to liberate 1 µmol of fatty acid per minute.

Enzymatic Synthesis of Fructose Laurate Ester

Fructose laurate was synthesized by two different enzymatic reactions. The first reaction was transesterification of fructose (100 mM) with methyl laurate (30 mM) in *t*-butanol (3 ml) including 20% DMSO. The second reaction was esterification of fructose (100 mM) with lauric acid (30 mM) in the same organic solvent system. CalB lipase (100 mg) was used as the enzyme catalyst and 10 glass beads were added for mixing. Reactions were performed at 40°C for 48 h with shaking (150 rpm).

The conversion yields of those reactions were calculated by measuring the decrease in the quantities of methyl laurate and lauric acid. After the reaction finished, 5 µl of the reaction mixture was injected into a high-performance liquid chromatography (HPLC) system. The Agilent HP 1100 series HPLC system (Agilent Technologies, USA) and 4.6 mm × 250 mm Cogent C_{18} HPLC column were used for the analysis of methyl laurate and lauric acid. Acetonitrile and water (90:10 (v/v)) were used as the mobile phase (flow rate, 1.0 ml/min) and a refractive index detector was used. Then, the molecular weights of the reaction products were measured by high-resolution mass spectrometry (Thermo Scientific, LTQ Orbitrap XL; USA). The reaction mixture (2 µl) was injected into an Accelar UHPLC system. An ACQUITY UPLC@ BEH C 18 column (1.7 µm, 2.1 mm × 100 mm) was used to analyze fructose laurate. Acetonitrile (0.1% formic acid) and water (0.1% formic acid) was used as the mobile phase (90:10 (v/v), flow rate 0.6 ml/min).

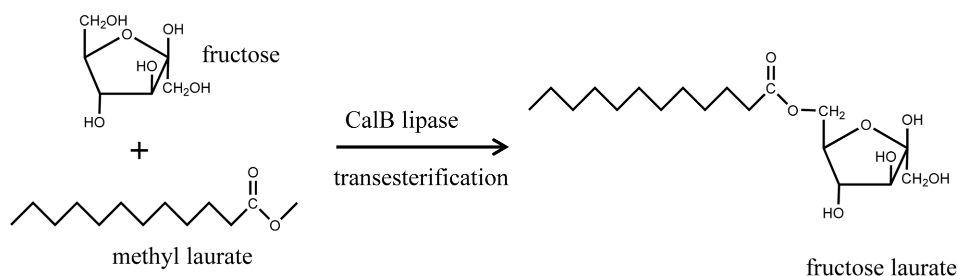
The organic solvent in the reaction mixture was evaporated completely in a speed vacuum concentrator, and the dried reaction product was stored at 4°C.

Optimizing the Fructose Laurate Synthesis

Various organic solvents (*t*-butanol, 2-methyl-2-butanol, acetone, *n*-heptane, and *n*-hexane) were screened for the target transesterification reaction. CalB lipase (100 mg) and methyl laurate (final concentration, 30 mM) were dissolved in 2.4 ml of each of the organic solvents. Then, 0.6 ml of fructose (stock concentration, 500 mM in DMSO) and 10 glass beads were added. The reaction was performed at 40°C for 48 h with shaking (150 rpm), and the reaction products were analyzed by HPLC.

Then, the same transesterification reactions were performed in the absence of DMSO to check the effect of DMSO as a co-solvent. Bioconversion yields were calculated and compared with those obtained in the presence of DMSO.

To determine the optimum quantity of CalB lipase, different quantities of CalB lipase (5, 10, 20, 40, 60, 80, and 100 mg) were added to the transesterification reaction. In this experiment, methyl laurate (30 mM) and fructose (100 mM) were dissolved in 3 ml of *t*-butanol (or 2-methyl-2-butanol) including 20% DMSO,



Scheme 1. Schematic presentation of *Candida antarctica* B lipase (CalB)-mediated synthesis of fructose laurate.

and 10 glass beads were added. The reaction was performed at 40°C for 48 h, and the reaction products were analyzed by HPLC.

The time course of bioconversion yield was measured for the transesterification reactions in the *t*-butanol system using 100 mg of CalB. At predetermined times (0.5, 1, 2, 3, 12, 24, and 48 h), reaction samples were taken and analyzed by HPLC.

Analysis of Antimicrobial Properties Using Fructose Laurate Ester

After these enzymatic transesterification reactions, various concentrations (96.3, 193, 386, and 770 μM) of fructose laurate ester were prepared in 1 ml of DMSO, and their antimicrobial properties were tested against oral or food spoilage microorganisms. *S. mutans* was pre-cultured in brain heart infusion broth for 16 h at 37°C. Then, *S. mutans* (1% (v/v)) was inoculated in 100 ml of brain heart infusion broth. The fructose laurate preparation was added, and the cells were cultured for 24 h at 37°C. *S. mutans* was also cultured with DMSO (1 ml) or without the fructose laurate preparation as negative controls. The substrate (0.9 mM methyl laurate) was also tested for *S. mutans* growth. Growth curves were drawn by measuring the cell mass of the organism as absorbance at 600 nm with a spectrophotometer.

B. coagulans and *G. stearothermophilus* were cultured in nutrient broth at 37°C and 55°C, respectively. Cell growth in the presence of the fructose laurate preparation was tested as described for *S. mutans*.

Results and Discussion

Use of Lipase in the Synthesis Reaction

Lipases are widely used in esterification and transesterification to produce various ester compounds and hydrolyze fats and oils [11, 18]. Lipase-catalyzed synthesis of sugar esters by transesterification has received attention in many industries [5]. In this study, CalB was used to synthesize the fructose laurate ester (Scheme 1). CalB lipase was an immobilized form and it had hydrolytic activity of 15.63 U/g toward olive oil, measured using the pH stat method. It had transesterification activity of 13.33 U/g toward *p*NPP, measured using a spectrophotometer.

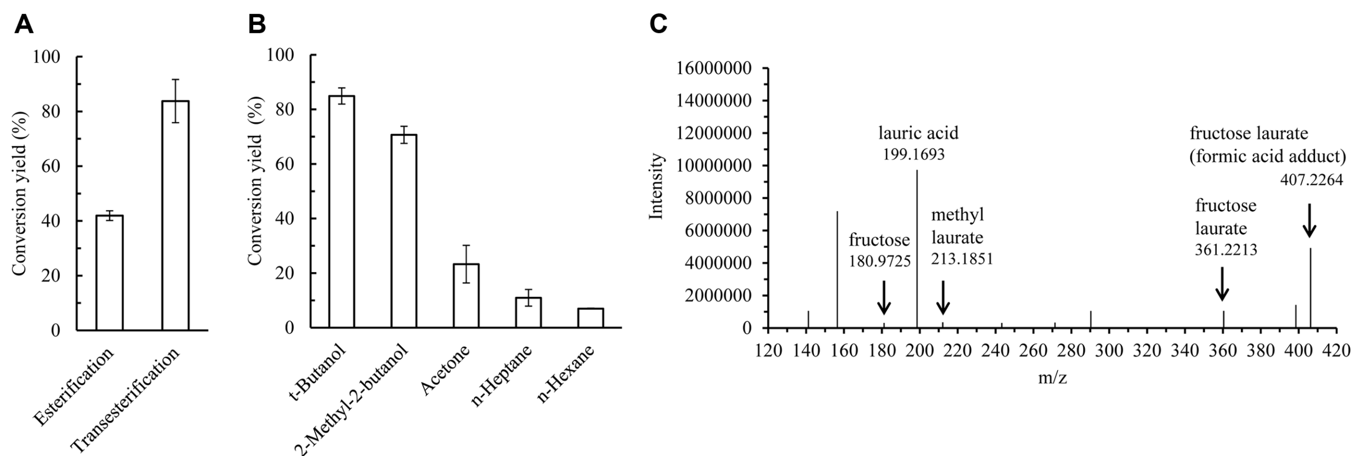


Fig. 1. Synthesis of fructose laurate ester.

(**A**) *Candida antarctica* B lipase (CalB)-mediated esterification and transesterification were performed in *t*-butanol/DMSO using fructose and lauric acid (or methyl laurate) at 40°C for 24 h. (**B**) CalB-mediated transesterification was performed in various organic solvents using fructose and methyl laurate at 40°C for 24 h. (**C**) Analysis of reaction products by high-resolution mass spectrometry.

Synthesis of the Fructose Laurate Ester

The fructose laurate ester was synthesized by esterification and transesterification. In these two reactions, fructose and laurate (or methyl laurate) were dissolved in *t*-butanol including 20% DMSO, and CalB lipase-mediated bioconversion reactions were performed at 40°C. After the 48 h reaction, the quantities of the remaining laurate (or methyl laurate) were analyzed by HPLC, and conversion yields were calculated. As shown in Fig. 1A, a higher conversion yield (83.79%) was obtained by the transesterification reaction, whereas a lower conversion yield (41.93%) was obtained by the esterification reaction. Water molecules were produced together with fructose laurate during the esterification reaction. These water molecules seemed to induce a reverse (hydrolysis) reaction. Therefore, we chose the transesterification reaction to optimize the enzymatic reaction process.

Before optimization, the reaction products were confirmed using high-resolution mass spectrometry. As shown in Fig. 1C, peaks corresponding to fructose laurate (*m/z*, 361.2213) and its formic adduct (*m/z*, 407.2264) were detected. In addition, methyl laurate (*m/z*, 213.1851) and lauric acid (*m/z*, 1,999.1693) were also detected. Lauric acid seemed to be produced from methyl laurate during the HPLC analysis.

Influence of the Solvent System on the Transesterification Reaction

A suitable non-aqueous solvent is very important for efficient synthesis of sugar esters by the transesterification reaction. Sugars usually dissolve well in water or a polar solvent, whereas medium/long-chain fatty acids dissolve

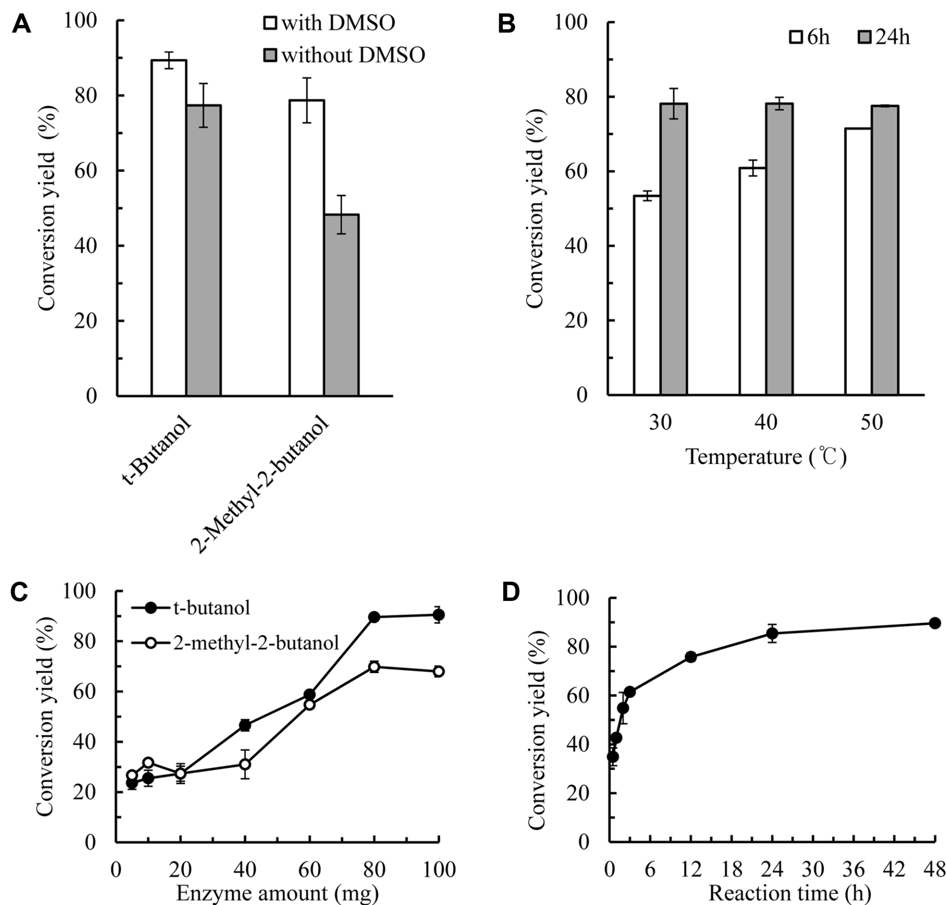


Fig. 2. Optimization of lipase-mediated transesterification.

(**A**) *Candida antarctica* B lipase (CalB)-mediated transesterification was performed in *t*-butanol (or 2-methy-2-butanol) with or without DMSO. (**B**) CalB-mediated transesterification was performed in *t*-butanol/DMSO at different temperatures (30–50°C) using 100 mg of CalB lipase. (**C**) Effect of enzyme quantity on the transesterification reaction. CalB-mediated transesterification was performed in *t*-butanol/DMSO (or 2-methy-2-butanol/DMSO) using different quantities (5–100 mg) of CalB lipase. (**D**) Time course of CalB-mediated transesterification. CalB-mediated transesterification was performed in *t*-butanol/DMSO at 40°C using 100 mg of CalB lipase.

in nonpolar solvents. Thus, a suitable solvent system should be used to dissolve both sugars and fatty acids [18]. The effects of various organic solvents on synthesis of fructose laurate ester were tested. As shown in Fig. 1B, among *t*-butanol, 2-methyl-2-butanol, acetone, *n*-heptane, and *n*-hexane, including 20% DMSO, the highest conversion yield (86.99%) was achieved in the *t*-butanol/DMSO system. The transesterification yield in the 2-methyl-2-butanol/DMSO system was the second highest (68.44%). On the other hand, conversion yields in the acetone, *n*-heptane, and *n*-hexane systems were relatively lower. LogP values of *t*-butanol and 2-methy-2-butanol were 0.584 and 1.095, respectively, which appeared to be appropriate to dissolve both substrates. Acetone (logP, −0.042) was too polar, whereas hexane (logP, 3.764) and heptane (logP, 4.274) were too nonpolar.

Co-solvents are frequently used to synthesize fructose esters to improve product yield [13]. In the above enzyme reactions, 20% DMSO was added to each organic solvent as a co-solvent to increase fructose solubility. To confirm the effect of DMSO on transesterification, the enzyme reaction was performed in the absence of DMSO. Fig. 2A indicates that conversion yields decreased considerably compared with those in the presence of DMSO.

A similar result was reported previously [1]. DMSO was used as a co-solvent to synthesize sucrose esters by *Thermomyces lanuginosus* lipase and CalB lipase. In the 2-methyl-2-butanol/DMSO (4:1 (v/v)) system, *T. lanuginosus* lipase synthesized a monoester, 6-*O*-acylsucrose, whereas CalB lipase was employed successfully for selective synthesis of the 6,6′-diester. This solvent/co-solvent system was chosen as a compromise between enzyme activity and sugar solubility.

Effect of Reaction Temperature on the Synthesis Reaction

It is also important to check the influence of temperature on enzyme reactions. The reaction temperature can affect the solubility of substrates and products as well as enzyme activity. Immobilized CalB lipase is a thermostable enzyme. One study [16] reported that CalB lipase can be used at up to 80°C without loss of enzyme activity.

In this study, the CalB lipase-mediated reaction was performed at various temperatures. As shown in Fig. 2B, conversion yields were 53%, 61%, and 71% at 30°C, 40°C, and 50°C, respectively after a 6 h reaction. When the reaction continued for 24 h, all conversion yields reached 77%, regardless of temperature, suggesting that the initial conversion rate at 50°C was the highest and reached a plateau within 24 h.

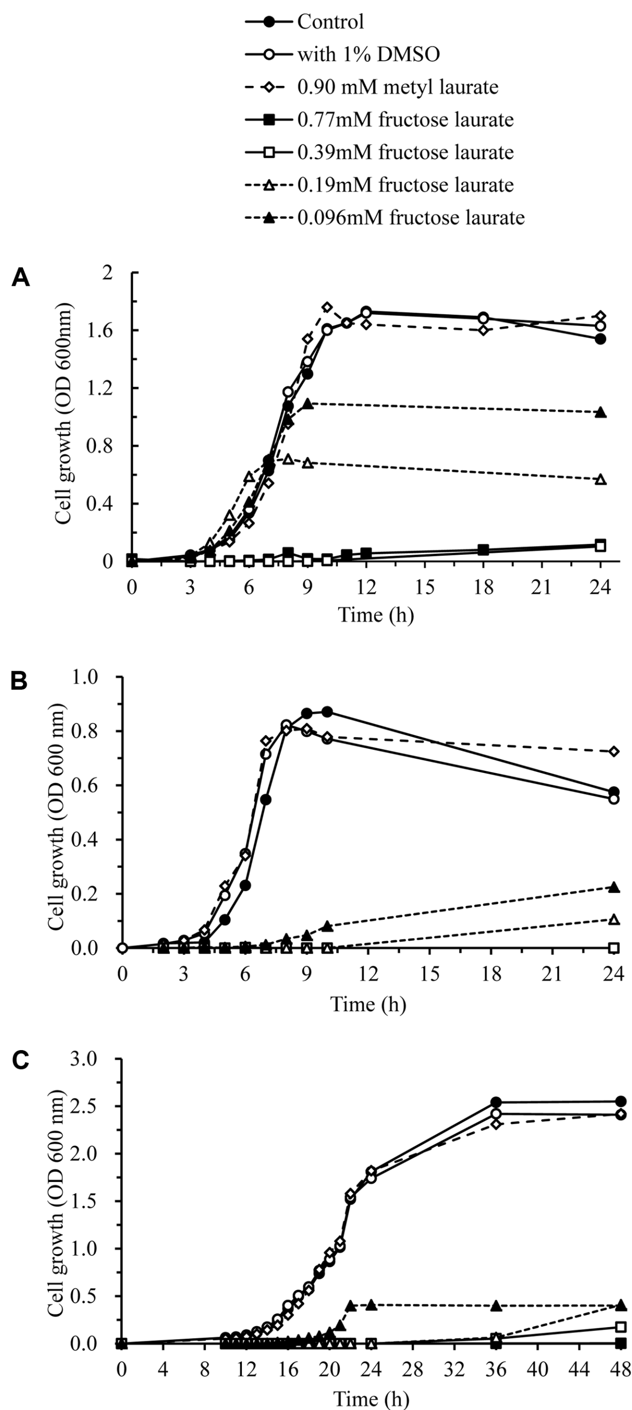


Fig. 3. Antimicrobial properties of fructose laurate ester.
Growth of *Streptococcus mutans* (**A**), *Bacillus coagulans* (**B**), and *Geobacillus stearothermophilus* (**C**) was measured in various culture conditions.

Effect of Enzyme Quantity on the Synthesis Reaction

Enzyme quantity is another important parameter in the lipase-mediated synthesis of fructose laurate [21]. Fig. 2C

shows that the conversion yield increased continuously when the quantity of lipase was increased from 20 to 80 mg in both the *t*-butanol/DMSO and 2-methyl-2-butnaol/DMSO systems. The conversion yield reached a plateau when more than 80 mg of enzyme was used. The conversion yields were 90.52% and 67.96% in the *t*-butanol and 2-methyl-2-butnaol systems, respectively, when 100 mg of enzyme was used.

The conversion yield at 40°C was measured over time (Fig. 2D). At the initial stage (up to 3 h) of the reaction, the conversion yield increased rapidly with a conversion rate of 10.5 nmol/min/mg CalB. After 3 h, the conversion rate decreased gradually, and the conversion yield reached to 90% at 48 h.

Antimicrobial Properties of Fructose Laurate

In this study, we performed a cell growth inhibition assay with three bacterial strains using the fructose laurate preparation. First, *S. mutans* was cultured with different concentrations of fructose laurate. As shown in Fig. 3A, 0.39 mM fructose laurate inhibited cell growth completely and inhibited growth moderately at 0.096 mM. The concentration that prevented 50% cell growth (IC_{50}) was approximately 0.15 mM (Fig. S1). However, a rather higher concentration (0.9 mM) of methyl laurate (substrate) showed no inhibitory effect, suggesting that combining a lauric group with fructose increased the solubility in the culture medium and enhanced its antibacterial activity. Fructose laurate has previously shown antibacterial activity against *S. mutans* [22]. The growth inhibitory effect of fructose laurate was determined using absorbance at 620 nm. The inhibitory concentration was 0.55 mM, which was higher than our present experimental result (Table 1).

B. coagulans and *G. stearothermophilus* were cultured in the presence of different concentrations of fructose laurate. As shown in Fig. 3B and 3C, fructose laurate inhibited cell growth of the two strains at 0.39 mM and the IC_{50} values were lower than 0.096 mM (Fig. S1). However, a 10-fold higher concentration (0.9 mM) of methyl laurate did not have any inhibitory effects.

A previous study reported that 200 μg/ml (corresponding to 0.34 mM) of a sucrose palmitate ester had bacteriostatic activities against *B. coagulans* and *G. stearothermophilus* [10]. Another study investigated the minimum inhibitory concentration of monoacyl erythrols and xylitols with different acyl chains toward the two bacterial strains [14]. They confirmed that the monomyristoyl ester had the best antimicrobial activity, and the minimal inhibitory concentration was 12.5 μg/ml (0.035–0.038 mM). Additionally, another group investigated inhibition against *G. stearothermophilus* using various sugar esters; 2 mg/ml 6′-*O*-laurylmaltose (3.8 mM) and 6′-*O*-palmitoylmaltose (3.4 mM) inhibited growth by 44% and 31%, respectively [1]. As summarized in Table 1, sugar esters show quite different inhibitory effects on bacterial growth, depending on the fatty acid and carbohydrate moieties. In general, fatty acid sugar esters show higher antibacterial activity than do fatty acid or fatty acid methyl esters. In addition, medium-chain (C_{12} and C_{14}) sugar esters seem to have higher activity than long-chain sugar esters. Although the detailed mechanism of antibacterial activity has not been elucidated, one study suggested that sucrose monocaprate affected the permeability of the bacterial cell membrane, leading to leakage of proteins, reducing sugars, and 260-nm-absorbing materials

Table 1. Antibacterial activities of sugar esters towards three gram-positive bacterial strains

Bacterial strain	Sugar ester	Inhibitory Conc.	Reference
S. mutans	Fructose laurate	<0.39 mM	This study
	Fructose laurate	0.55 mM	[22]
B. coagulans	Fructose laurate	<0.39 mM	This study
	Sucrose palmitate	0.34 mM	[10]
	Erythrol myristate	0.035–0.038 mM	[14]
	Xylitol myristate	0.035–0.038 mM	[14]
G. stearothermophilus	Fructose laurate	<0.39 mM	This study
	Sucrose palmitate	0.34 mM	[10]
	Erythrol myristate	0.035–0.038 mM	[14]
	Xylitol myristate	0.035–0.038 mM	[14]
	Maltose laurate	3.8 mM (44%)	[1]
	Maltose palmitate	3.4 mM (31%)	[1]

[23]. Further study is required to elucidate the detailed mechanism of fructose laurate on growth inhibition.

In this study, fructose laurate was produced efficiently by the CalB-mediated transesterification reaction in nonpolar solvent systems and inhibited the growth of three gram-positive bacteria. These results suggest that this fructose laurate preparation may be useful as a component in toothpaste or a preservative in canned foods.

Acknowledgments

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea Government (MSIP) (2014R1A2A2A01006978).

References

1. Ferrer M, Soliveri J, Plou FJ, López-Cortés N, Reyes-Duarte D, Christensen M, *et al*. 2005. Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their antimicrobial properties. *Enzyme Microb. Technol*. **36:** 391-398.
2. Gumel AM, Annuar MSM, Heidelberg T, Chisti Y. 2011. Lipase mediated synthesis of sugar fatty acid esters. *Process Biochem*. **46:** 2079-2090.
3. Haberbeck LU, da Silva Riehl CA, Salomão BDCM, de Aragao GMF. 2012. *Bacillus coagulans* spore inactivation through the application of oregano essential oil and heat. *LWT Food Sci. Technol.* **46:** 267-273.
4. Jo JC, Kim S, Kim HK. 2014. Transesterification of plant oils using *Staphylococcus haemolyticus* L62 lipase displayed on *Escherichia coli* cell surface using the OmpA signal peptide and EstA-8 anchoring motif. *Enzyme Microb. Technol*. **67:** 32-39.
5. Kobayashi T. 2011. Lipase-catalyzed syntheses of sugar esters in non-aqueous media. *Biotechnol. Lett.* **33:** 1911-1919.
6. Koga T, Hamada S, Murakawa S, Endo A. 1982. Effect of a glucosyltransferase inhibitor on glucan synthesis and cellular adherence of *Streptococcus mutans*. *Infect. Immun.* **383:** 882-886.
7. Lee KP, Kim HK. 2015. Transesterification reaction using *Staphylococcus haemolyticus* L62 lipase crosslinked on magnetic microparticles. *J. Mol. Catal. B Enzym*. **115:** 76-82.
8. Lee SM, Sandhu G, Walsha MK. 2015. Growth inhibitory properties of lactose fatty acid esters. *Saudi J. Biol. Sci.* doi:10.1016/j.sjbs.2015.10.013.
9. Li L, Ji F, Wang J, Li Y, Bao Y. 2015. Esterification degree of fructose laurate exerted by *Candida antarctica* lipase B in organic solvents. *Enzyme Microb. Technol*. **69:** 46-53.
10. Lou X. 2014. Antimicrobial structure-efficacy relationship of sugar fatty acid esters. *J. Chem. Pharm. Res.* **6:** 944-946.
11. Neta NS, Teixeira JA, Rodrigues LR. 2015. Sugar ester surfactants: enzymatic synthesis and applications in food industry. *Crit. Rev. Food Sci. Nutr.* **55:** 595-610.
12. Ng TM, Schaffner DW. 1997. Mathematical models for the effects of pH, temperature, and sodium chloride on the growth of *Bacillus stearothermophilus* in salty carrots. *Appl. Environ. Microbiol.* **63:** 1237-1243.
13. Nott K, Brognaux A, Richard G, Laurent P, Favrelle A, Jérôme C, *et al*. 2012. (Trans) Esterification of mannose catalyzed by lipase B from *Candida antarctica* in an improved reaction medium using co-solvents and molecular sieve. *Prep. Biochem. Biotechnol.* **42:** 348-363.
14. Piao J, Kawahara-Aoyama Y, Inoue T, Adachi S. 2006. Bacteriostatic activities of monoacyl sugar alcohols against thermophilic sporeformers. *Biosci. Biotechnol. Biochem.* **70:** 263-265.
15. Pöhnlein M, Slomka C, Kukharenko O, Gärtner T, Wiemann LO, Sieber V, *et al*. 2014. Enzymatic synthesis of amino sugar fatty acid esters. *Eur. J. Lipid Sci. Technol.* **116:** 423-428.
16. Poojari Y, Clarson SJ. 2013. Thermal stability of *Candida antarctica* lipase B immobilized on macroporous acrylic resin particles in organic media. *Biocatal. Agric. Biotechnol.* **2:** 7-11.
17. Šabeder S, Habulin M, Knez Ž. 2006. Lipase-catalyzed synthesis of fatty acid fructose esters. *J. Food Eng*. **77:** 880-886.
18. Szüts A, Pallagi E, Regdon G, Aigner Z, Szabó-Révész P. 2007. Study of thermal behaviour of sugar esters. *Int. J. Pharm.* **336:** 199-207.
19. Teng Y, Xu Y. 2007. A modified para-nitrophenyl palmitate assay for lipase synthetic activity determination in organic solvent. *Anal. Biochem*. **363:** 297-299.
20. Torres MG, Foresti ML, Ferreira ML. 2014. CLEAs of *Candida antarctica* lipase B (CALB) with a bovine serum albumin (BSA) cofeeder core: study of their catalytic activity. *Biochem. Eng. J.* **90:** 36-43.
21. Vitisant T, Chulalaksananukul W, Piumthongkum R, Sinbuathong N, Mekthong P, Chulalaksananukul S. 2012. Synthesis of sugar ester by local yeast lipase in solvent free system. *Int. J. Sci. Technol.* **2:** 773-777.
22. Watanabe T, Katayama S, Matsubara M, Honda Y, Kuwahara M. 2000. Antibacterial carbohydrate monoesters suppressing cell growth of *Streptococcus mutans* in the presence of sucrose. *Curr. Microbiol*. **413:** 210-213.
23. Zhao L, Zhang H, Hao T, Li S. 2015. In vitro antibacterial activities and mechanism of sugar fatty acid esters against five food-related bacteria. *Food Chem*. **187:** 370-377.