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Article

Antibacterial Activity of Bioconverted Linoleic Acid Produced by *Pseudomonas aeruginosa* PR3

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Crude extract of bioconverted linoleic acid using *Pseudomonas aeruginosa* PR3 was evaluated for its antibacterial activity against food-borne pathogenic bacteria. Crude extract showed antibacterial activity against four Gram-positive bacteria, *Staphylococcus aureus* (ATCC 6538), *S. aureus* (KCTC 1916), *Listeria monocytogenes* (ATCC 19166), and *Bacillus subtilis* (ATCC 6633), and one Gram-negative bacterium, *Pseudomonas aeruginosa* (KCTC 2004), with minimum inhibitory concentrations ranging from 750 to 1,500 μg · m*l*$^{-1}$. *S. aureus* and *B. subtilis* were selected for growth inhibition assays with bioconverted linoleic acid. Major antibacterial effects occurred at lag phase.

Key words: *antibacterial activity, linoleic acid, disc diffusion, MIC, Pseudomonas aeruginosa* PR3

Hydroxy fatty acids are produced mainly in plants, and their hydroxyl groups are well known to give the fatty acids special properties, such as higher viscosity and reactivity, compared with normal fatty acids such that they are used in a wide range of industrial products including resins, waxes, nylons, plastics, lubricants, cosmetics, and additives in coatings and paintings.[1,2] Microbial conversion of unsaturated fatty acids has been widely exploited to produce new, value-added hydroxy products. Microbial oxidation of unsaturated fatty acids was recently reviewed.[3] Among those unsaturated fatty acids used for microbial production of hydroxy fatty acids oleic acid, linoleic acid and linolenic acid were well studied as substrates to produce mono-, di-, and trihydroxy fatty acids. Strain PR3, isolated from a wastewater stream on a pig farm in Morton, Illinois, was found to convert oleic acid to a novel compound, 7,10-dihydroxy-8(*E*)-octadecenoic acid[4], and to convert ricinoleic acid to a novel compound, 7,10,12-trihydroxy-8(*E*)-octadecenoic acid.[5] Several reports have been published on the production and anti-fungal activity of hydroxy linoleic acids by microbial bioconversion.[4-12]

However, antibacterial activity of linoleic acid hydroxylated by microbial conversion has not yet been investigated. In this paper we report the neutraceutical and industrial potentials of the bioconverted linoleic acid(s) by determining their antibacterial activity against a range of food-borne pathogenic bacteria.

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Materials and Methods

Microorganisms. *Pseudomonas aeruginosa* PR3, kindly provided by Dr. Hou of USDA/ARS/NCAUR, was grown at 28°C aerobically at 200 rpm on a standard medium containing per liter 4 g dextrose, 2 g K_2HPO_4, 2 g $(NH_4)_2HPO_4$, 1 g NH_4NO_3, 0.5 g yeast extract, 0.014 g $ZnSO_4$, 0.01 g $FeSO_4 \cdot 7H_2O$, and 0.01 g $MnSO_4 \cdot 7H_2O$.

Ten strains of food-spoiling bacteria tested, *Bacillus subtilis* (ATCC 6633), *Enterobacter aerogenes* (KCTC 2190), *Escherichia coli* (ATCC 8739), *E. coli* O157:H7 (ATCC 43888), *Listeria monocytogenes* (ATCC 19166), *Pseudomonas aeruginosa* (KCTC 2004), *Salmonella enteritidis* (KCCM 12021), *S. typhimurium* (KCTC 2515), *Staphylococcus aureus* (ATCC 6538), and *S. aureus* (KCTC 1916), were obtained from the Korea Food & Drug Administration, Daegu, Korea. The stock cultures were maintained on Luria broth (LB) agar medium at 4°C.

Bioconversion of linoleic acid. Bioconversion was carried out in the standard medium as mentioned above. Linoleic acid (0.5 g · 50 m*l*$^{-1}$) as substrate was added to 24-h-old cultures, followed by continued incubation for an additional 72 h. The culture broth was acidified to pH 2.0 with 6N HCl, followed by immediate extraction twice with an equal volume of ethyl acetate and diethyl ether (1 : 1, v/v). The solvent was evaporated from the combined extract with a rotary evaporator, and the crude lipid extracts (0.37 g) were obtained.

Antibacterial assay. Antibacterial tests were carried out by a disc diffusion method[13] using 10 m*l* of suspension containing

10^5 CFU · ml^{-1} bacteria, which was poured onto LB agar. The discs (6 mm in diameter) were impregnated with 1.5 μ*l* (1,400 μg crude extract) bioconverted linoleic acid and placed on the inoculated agar. The inoculated plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone against the test microorganisms. Each assay was performed in triplicate.

Minimum inhibitory concentration (MIC). MIC, minimum inhibitory concentration at which no visible growth was observed in the tube, as expressed in μg · ml^{-1}, of bioconverted linoleic acid was tested by the two-fold dilution method.[13] A loopful of bacterial culture from a slant was inoculated into LB and incubated at 37°C for 24 h, and a two-fold serial dilution method was performed as follows. The crude extract was first dissolved in 5% dimethyl sulfoxide (DMSO), further diluted with 5% DMSO, and added to LB to final concentrations of 0, 500, 1000, and 2000 μg · ml^{-1}. The bacterial suspension was inoculated into 25 m*l* LB medium and incubated for 24 h at 37°C. A set of tubes containing only seeded liquid medium and 5% DMSO was kept as control. All tests for MIC determinations were performed in triplicates.

Bacterial growth kinetics. The effect of bioconverted crude oil on the growth kinetics of bacteria tested in broth culture was determined. Cultivation conditions for this study were same as those mentioned above for MIC determination. For each bacterial strain, the experimental tubes and a control set with no antimicrobial supplements were monitored spectrophotometrically at 600 nm for 24 h. All experiments were performed in triplicates, and the mean values were plotted as growth curves.

Results and Discussion

The *in vitro* antibacterial activity of bioconverted linoleic acid against the food-borne pathogenic bacteria was qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values. The crude extract of bioconverted linoleic acid showed antibacterial activity against four Gram-positive bacteria, *S. aureus* (ATCC 6538), *S. aureus* (KCTC 1916), *L. monocytogenes*, and *B. subtilis*, and one Gram-negative bacterium, *P. aeruginosa* (KCTC 2004) (Table 1), whereas non-bioconverted linoleic acid as negative control had no antibacterial effect (data not shown). The maximal inhibition zones and MIC values for bacterial strains were in the ranges of 10-13 mm and 750-1,500 μg · ml^{-1}, respectively (Table 1). The blind control with 5% DMSO did not inhibit any of the bacteria tested. Gram-positive bacteria such as *B. subtilis* and *S. aureus* were more susceptible than Gram-negative bacteria such as *S. typhimurium*, *S. enteritidis*, and *E. coli* except for *P. aeruginosa*. These results are similar to the results of other studies previously reported.[14,15]

Table 1. Antibacterial activity of bioconverted linoleic acid

Bacteria tested	Zone of inhibition (mm)	MIC (μg · ml^{-1})
Bacillus subtilis (ATCC 6633)	11	750
Listeria monocytogenes (ATCC 19166)	11	1,500
Pseudomonas aeruginosa (KCTC 2004)	11	750
Staphylococcus aureus (ATCC 6538)	13	1,500
Staphylococcus aureus (KCTC 1916)	10	1,500
Escherichia coli (ATCC 8739)	ND[a]	ND[b]
Escherichia coli O157:H7 (ATCC 43888)	ND[a]	ND[b]
Enterobacter aerogenes (KCTC 2190)	ND[a]	ND[b]
Salmonella enteritidis (KCCM 12021)	ND[a]	ND[b]
Salmonella typhimurium (KCTC 2515)	ND[a]	ND[b]

[a]ND = no detected antibacterial activity at 2,800 μg · ml^{-1}.
[b]ND = no detected antibacterial activity at 5,000 μg · ml^{-1}.

B. subtilis and *S. aureus*, which displayed different initial sensitivities to the crude extract, were selected for further studies. The effects of crude extract when added at the lag phase on the growth kinetics of these strains in LB are presented in Figs. 1 and 2. *S. aureus* and *B. subtilis* were inhibited up to 100% at 2,000 and 1,000 μg · ml^{-1} of crude extract, respectively, after 24 h incubation. After 20 h incubation *S. aureus* showed growth inhibition up to 50% at 1,000 μg · ml^{-1} crude extract, while *B. subtilis* up to 90% at 500 μg · ml^{-1}. These results showed different initial sensitivities of two bacterial strains tested at different concentrations of the crude extract of bioconverted linoleic acid along with their antibacterial activities.

In conclusion, we report the *in vitro* antibacterial properties of bioconverted crude extract produced from linoleic acid by *P. aeruginosa* PR3. These results suggested the possible use of bioconverted crude oil of natural vegetable fatty acid for trials in controlling food safety. To produce the bioconverted oil from linoleic acid in large quantities and to render the bioprocess feasible and practical, we are performing experiments to isolate single active novel components from the crude

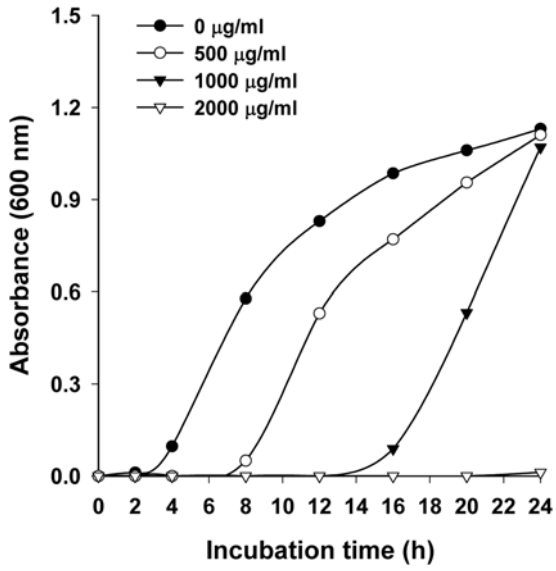

Fig. 1. Growth curve of *Staphylococcus aureus* (ATCC 6538) as affected by the addition of bioconverted linoleic acid.

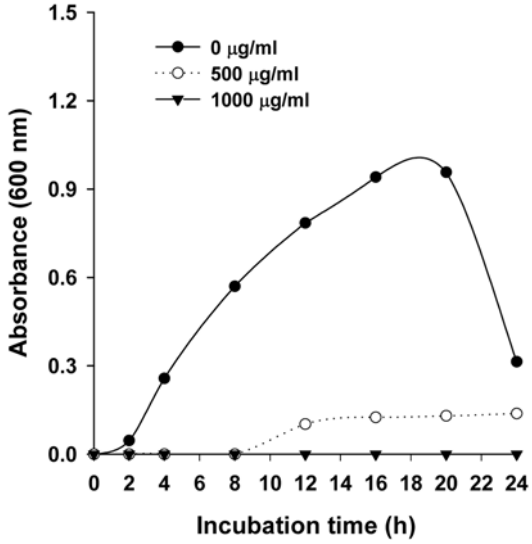

Fig. 2. Growth curve of *Bacillus subtilis* (ATCC 6633) as affected by the addition of bioconverted linoleic acid.

extract and verify their chemical structures. The bioconversion process is quite competitive for commercial point of view as it can provide higher productivity and percentage of yields at low cost processing. During the microbial bioconversion of unsaturated fatty acids 50% production yield for 7,10,12-TOD was obtained from ricinoleic acid, 75% for 7,10-DOD from oleic acid, and 45% for 9,10,13- and 9,12,13-THOD from linoleic acid.[16,17] Further study is needed to improve the production yield and the process cost. Studies on the production of the bioconverted oil of linoleic acid in a bioreactor are in progress as well.

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