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Food and Chemical Toxicology 44 (2006) 1184–1190
www.elsevier.com/locate/foodchemtox

Effect of bacteriocin produced by *Lactococcus* sp. HY 449 on skin-inflammatory bacteria

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Received 31 January 2005; accepted 12 August 2005

Abstract

This study was carried out to evaluate the effect of bacteriocin produced by *Lactococcus* sp. HY 449 against skin-inflammatory bacteria such as *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 65389, *Streptococcus pyogenes* ATCC 21059, and *Propionibacterium acnes* ATCC 6919. The spot-on-the-lawn method was used to determine the antimicrobial activity of bacteriocin against indicator strains on the human skin. The bacteriocin produced by *Lactococcus* sp. HY 449 inhibited the growth of *S. epidermidis* ATCC 12228, *S. aureus* ATCC 65389, *Strep. pyogenes* ATCC 21059, and *P. acnes* ATCC 6919. The treatment of crude bacteriocin caused a rapid inactivation of *P. acnes* ATCC 6919. The LC_{50} of bacteriocin on human fibroblast was ca. 50 mg/ml at which the inhibition of cell proliferation was not observed. Neither any irritations nor allergic reactions by the bacteriocin were evident in a human patch test. The bacteriocin produced by *Lactococcus* sp. HY 449 may be a useful antimicrobial substance to control the growth of *P. acnes* and to prevent skin inflammation and acne.

Keywords: Bacteriocin; Cosmetics; Inflammation; *Lactococcus*; Skin

1. Introduction

Acne vulgaris is one of the most common skin diseases which can result in comedos or severe inflammatory lesions in the face, back, and chest with a large number of sebaceous follicles, and the conditions of the disease is associated with the elevated rate of sebum excretion (Leyden and Kligman, 1976). Sebum, which is accumulated in the pilosebaceous channel, facilitates the proliferation of skin bacteria (Armold et al., 1990). *Staphylococcus epidermidis* and *Propionibacterium acnes* have been recognized as major skin bacteria that cause the formation of acne comedos (Leyden and Kligman, 1976). In addition, these bacteria have the ability to synthesize lipases that degrade sebum triglycerides into free fatty acids which trigger inflammatory responses (Armold et al., 1990; Leyden and Kligman, 1976). For these reasons, various antimicrobial substances including antibiotics, chemicals, and herb extracts have been studied for pharmaceutical and cosmetic purposes.

Lactic acid bacteria (LAB) produce a variety of antimicrobial substances including bacteriocin, bacteriocin-like substances, organic acids, and hydrogen peroxide. Among them, bacteriocins are peptides or proteins, which are

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0278-6915/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.fct.2005.08.008

inhibitory against sensitive bacterial strains (Jack et al., 1995; Montville and Kaiser, 1993). A number of bacteriocin produced by various LAB species including *Lactobacillus*, *Lactococcus, Pediococcus*, *Propionibacterium*, *Leuconostoc*, and *Carnobacterium*, has been reported (Klaenhammer, 1993).

Bacteriocins have been grouped into three main classes based on their chemical and genetic properties (Klaenhammer, 1993). The first class comprises small peptides with dehydrated or modified residues such as dehydroalanine and lanthionine. The second class includes small heat stable bacteriocins such as pediocin A and lactacin F. The third group comprises large heat labile bacteriocins such as helveticin J (Montville and Kaiser, 1993).

We previously reported that the bacteriocin produced by *Lactococcus* sp. HY 449 showed stability against heat, high pH, and surfactant treatment (Oh et al., 2001). This bacteriocin had the ability to inhibit the growth of wide spectrum of pathogenic bacteria such as staphylococci and *Listeria* species. The study presented herein was undertaken to determine the efficacy of the bacteriocin from *Lactococcus* sp. HY 449 in controlling skin inflammation and acnes by clinical skin irritation test.

2. Materials and methods

2.1. Bacteria and growth media

S. aureus ATCC 65389, *S. epidermidis* ATCC 12228, *Strep. pyogenes* ATCC 21059, and *P. acnes* ATCC 6919 were obtained from American Type Culture Collection (ATCC, MD, USA). *S. aureus* ATCC 65389, *S. epidermidis* ATCC 12228, and *Strep. pyogenes* ATCC 21059 were grown at 37 °C for 18 h in Brain heart infusion (BHI) broth (Difco, Detroit, MI, USA), and *P. acnes* ATCC 6919 was anaerobically incubated in GAM broth (Gifu Anaerobic Medium; Nissui, Tokyo, Japan) at 37 °C for 18 h. Lactic acid bacteria were grown for 18 h at 37 °C in MRS broth (Difco) and the pathogens were incubated in Trypticase Soy broth (Difco) at either 30 or 37 °C for 18–24 h. All bacteria were propagated three times before use, and stock cultures were stored at −80 °C in 10% (v/v) skim milk containing cryoprotectant.

2.2. Activity test of bacteriocin

A bacteriocin-producing microorganism, *Lactococcus* sp. HY 449, was incubated in MRS (Difco, Detroit, MI, USA) broth at 32 °C for 48 h. The bacterial cells were centrifuged at 6000*g* for 20 min at 4 °C to obtain cell-free supernatant. The pH of the supernatant was adjusted to 6.5 with 10 N NaOH and filtered through a 0.45 mm syringe filter to remove cellular debris. The bacteriocin activity was tested by the spot-on-the-lawn method (Mäyr-Harting et al., 1972). Bacteriocin activity was quantified by spotting 10 μl aliquots of twofold diluted culture supernatant onto the surface of MRS agar plates. The plates were then overlaid with 0.8% MRS agar inoculated with 1% indicator strains and incubated at 37 °C for 24 h. The bacteriocin activity expressed as Activity Units (AU) was determined with the highest twofold dilution showing a clear inhibitory zone on the MRS agar plate.

2.3. Preparation of crude bacteriocin

The crude bacteriocin was prepared as previously described (Oh et al., 2000). The *Lactococcus* sp. HY 449 culture grown in MRS broth for 48 h was centrifuged (6000*g*, 15 min, 4 °C) to obtain cell-free supernatant which was then treated with ammonium sulfate (Sigma, St. Louis, MO, USA) to a final concentration of 30% (w/v) and stirred overnight at 4 °C. The pellet was collected by centrifugation (8000*g*, 30 min, 4 °C) of the supernatant and was subsequently resuspended in 50 ml of 2-(4-morpholino)-ethane sulfonic acid (MES; FisherBiotech, Fair Lawn, USA) buffer (50mM, pH 6.5). The resuspension of crude bacteriocin was subjected to hydrophobic interaction chromatography (HIC) using Octyl-sepharose 4 fast flow (Sigma). The column was equilibrated with 1.7M ammonium sulfate and then eluted with a gradient using H_2O and ethanol. Active fractions from the column chromatography were pooled and concentrated by vacuum evaporator (Eyela UT-1000, Tokyo, Japan). This preparation, designated as Active Crude Bacteriocin (ACB), was stored at −20 °C until used. Bacteroicin-containing cosmetic emulsion for irritation test was prepared as described in Table 1.

2.4. SDS-PAGE and N-terminal amino acid sequences

The active fraction obtained from the column chromatography was electrophoresed using tricine SDS-PAGE for 60 min (125V constant). After electrophoresis, one part of the gel was stained with 0.1% coomassie brilliant blue G250 (Bio-Rad, CA, USA) and destained using a methanol:acetic acid:water (3:1:6) solution. The molecular weight of the bacteriocin was estimated in comparison with molecular mass markers (Invitrogen Corp., CA, USA). The other part of the gel was washed three times in sterile water for 30 min and overlaid on the GAM soft agar (0.8%) inoculated with fresh culture of *P. acnes* ATCC 6919. The band corresponding to inhibitory activity was excised from the gel and used for N-terminal sequencing by Edman degradation.

2.5. Growth inhibition

Overnight cultures of *S. aureus* ATCC 65389, *S. epidermidis* ATCC 12228, *Strep. pyogenes* ATCC 21059, and*P. acnes* ATCC 6919 were centrifuged (3000*g* for 20 min, 4 °C), and the pellets were resuspended in 0.01% fresh broth to ca. 1×10^7 cells/ml. ACB was added at concentrations of 10, 50 and 100 unit/ml. The samples were taken at different time intervals for the measurement of absorbances at 660 nm for the biomass.

2.6. Mode of action

P. acnes ATCC 6919 was used for the study of mode of the bacteriocin action. *P. acnes* ATCC 6919 was anaerobically incubated in GAM broth (Gifu) at 37 °C for 18 h. Five hundreds AU/ml of ACB was added at various growth stage of indicator cell and incubated at 37 °C. The samples were taken at different time intervals to measure absorbances at 660 nm for the biomass and 260/280 nm for the mode of action.

2.7. Chorion allantoic membrane (CAM) assay

CAM-based assay was assessed as an alternative to the Draize skin irritation test (Reinhart et al., 1985). Three hundred microliters of diluted

Table 1
Composition of the ingredients in cosmetic preparation

Ingredients	Composition (g/100 g)
Polysorbate-60	1.5
Sorbitan stearate	0.5
Stearic acid	1.0
Squalane	5.0
Serostearyl alcohol	2.0
Seryl octanoate	5.0
Carbomer	0.15
Triethanolamine	0.2
Glycerine	5.0
Bacteriocin	100 AU
Distilled water	76.5

bacteriocin was applied to the CAM of fertilized hen's eggs. The blood vessels and the remaining parts of the membrane were examined and scored for irritant effects (Hemorrhage, Lysis or Coagulation) at 0.5, 2, and 5 min of treatment. Irritation was determined by the Quotient (Q) value which was expressed as follows (Lüpke, 1986): irritation onset times after test substance treatment/onset times after reference substance treatment.

2.8. Human primary irritation test

Human patch test was performed with 30 healthy female volunteers aged 20–28 (CTFA technical guidelines, 1991). The test was carried out using Finn Chamber secured to the back site with scanpore tape. Test sites were cleaned with 75% ethanol prior to the application. Forty microliters of filter-sterilized bacteriocin (12,800 AU/ml; 0.2 μm, Millipore Corp., Bedford, USA) was dropped into each Finn Chamber and applied to the human skin. The patches were removed after 24 h, and ICDRG (International contact dermatitis research group) standard 5-point scale was used for evaluating each patch site at 0.5, 24, 48, and 72 h after the removal of the patch (Fisher, 1990).

2.9. Effects of he bacteriocin on cytotoxicity and proliferation

Cytotoxicity of the bacteriocin was evaluated by mitochondrial metabolic activity with some modification (Mosmann, 1983). Briefly, human fibroblast (HNF; ATCC CCL-28) was cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Invitrogen Corp., CA, USA) supplemented with 10% fetal bovine serum (Gibco) under the controlled atmosphere (5% CO_2, 100% humidity) at 37 °C. The cells (ca. 10^6 cfu/ml) were inoculated onto a 96-well micro-plate and incubated at 37 °C for 24 h. Filter-sterilized bacteriocin was applied at various concentrations to determine LD_{50} and incubated at 37 °C for 24 h. MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma) was added to each well and incubated at 37 °C for 4 h. The absorbance was measured at 560 nm by using 96-well plate reader (Quant, Bio-Tek instrument Inc., USA). The cytotoxicity was determined compared with a control containing saline solution instead of bacteriocin, and the LC_{50} value was used as the criterion of the evaluation. All the experiments on the bacteriocin activity were performed at three replicates and average values were shown without error bars.

3. Results

3.1. Antimicrobial spectrum

The bacteriocin produced by *Lactococcus* sp. HY449 showed inhibitory activity against a wide spectrum of pathogenic bacteria including skin-inflammatory bacteria (Table 2). The bacteriocin was active against *E. coli* A2, *Pseudomonas aeruginosa* ATCC 15442, *Listeria monocytogenes*, *P. acnes*, *S. aureus*, *S. epidermidis* and *Strep. pyogenes*. Growth inhibition was also observed for lactic acid bacteria species including *Lactobacillus fermentum*, *L. helveticus*, *L. plantarum*, and *Leuconostoc* species. However, the bacteriocin was not effective against *L. casei* and *Salmonella typhimurium*.

Table 2
Antimicrobial spectrum of crude bacteriocin from *Lactococcus* sp. HY 449

Bacteria	Media	°C	Inhibition
Bacillus subtilis ATCC 6633	TSB	37	−
Enterococcus faecalis	M17	37	+
Escherichia coli HB 101	TSB	37	+
Escherichia coli HB A2	TSB	37	+
Klebsiella aerogenes	TSB	37	−
Lactobacillus brevis IFO 3029	MRS	37	+
Lactobacillus casei YIT 9018	MRS	37	−
Lactobacillus delbruekii subsp. *bulgaricus* CH	MRS	37	+
Lactobacillus delbruekii subsp. *lactis* ATCC 4797	MRS	37	+
Lactobacillus fermentum IFO 3023	MRS	37	+
Lactobacillus helveticus 1213	MRS	37	+
Lactobacillus plantarum	MRS	37	+
Lactobacillus sake	MRS	37	+
Leuconostoc mesenteroides KFCC 11324	M17	30	−
Leuconostoc sp. K2	MRS	30	+
Listeria monocytogenes ATCC 9509	TSB	37	+
Propionibacterium acnes ATCC 6919	GAM	37	+
Pseudomonas aeruginosa ATCC 15442	TSB	37	+
Pseudomonas fluorescens	TSB	37	+
Salmonella typhymurium ATCC 14028	TSB	37	−
Staphylococcus aureus ATCC 65389	TSB	37	+
Staphylococcus epidermidis ATCC 12228	TSB	37	+
Streptococcus pyogenes ATCC 21059	TSB	37	+
Streptococcus. thermophilus M1	M17	43	+
Streptococcus. thermophilus Y1	M17	43	+

+, Inhibited by crude bacteriocin; −, not inhibited.

3.2. Purification of bacteriocin

The crude bacteriocin obtained from the cell-free supernatent was concentrated by ammonium sulfate precipitation followed by separation with HIC. The activity and the recovery of bacteriocin at each step are shown in Table 3. The final specific activity of the partially purified fraction was ca. 318.2-fold greater than that of the culture supernatant with 5% of total final recovery. The elution profile of bacteriocin on the Octyl-sepharose column is shown in Fig. 1. From the column chromatography, the bacteriocin was eluted in 6 fractions (fraction no. 50–55) on a linear ethanol gradient. Tricine-SDS PAGE analysis was carried out for the sample obtained from ammonium sulfate

Table 3
Purification of the bacteriocin produced by *Lactococcus* sp. HY 449

Purification step	Total volume (ml)	Total protein (mg)	Total units ($\times 10^3$ AU)	Specific activity ($\times 10^3$ AU/mg protein)	Recovery	Purification fold
Culture	900	3150	1440	0.46	100	1.0
30% Ammonium sulfate	20	105.4	640	6.07	44.4	13.3
HIC[a]	45	0.495	72	145.46	5.0	318.2

[a] Hydrophobic interaction chromatography using Octyl-sepharose 4 fast flow column.

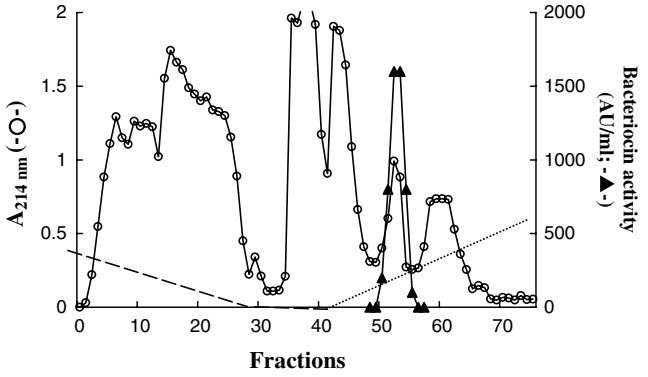

Fig. 1. Seperation of the bacteriocin by Octyl-sepharose 4 fast flow column chromatography; (-□-) absorbance at 214 nm; (-●-) bacteriocin activity (AU/ml) control; (- - -) linear gradient of 22–0% $(NH_4)_2SO_4$; (···) linear gradient of 0–75% ethanol.

precipitation and HIC. The sample from 30% ammonium sulfate precipitation contained a large amount of impurities (data not shown), but the sample from HIC-active fraction showed a single band with the size of 3.4 kDa (Fig. 2A). When the soft agar containing *P. acnes* ATCC 6919 was overlaid on the gel, a clear inhibitory zone at approximately 3.4 kDa was detected (Fig. 2B). Edman degradation method was used to elucidate 10 residues and identified as follows: NH_2-Ile-Leu-Pro-Gln-Try-Tyr-Gly-Asn-Gly-Val.

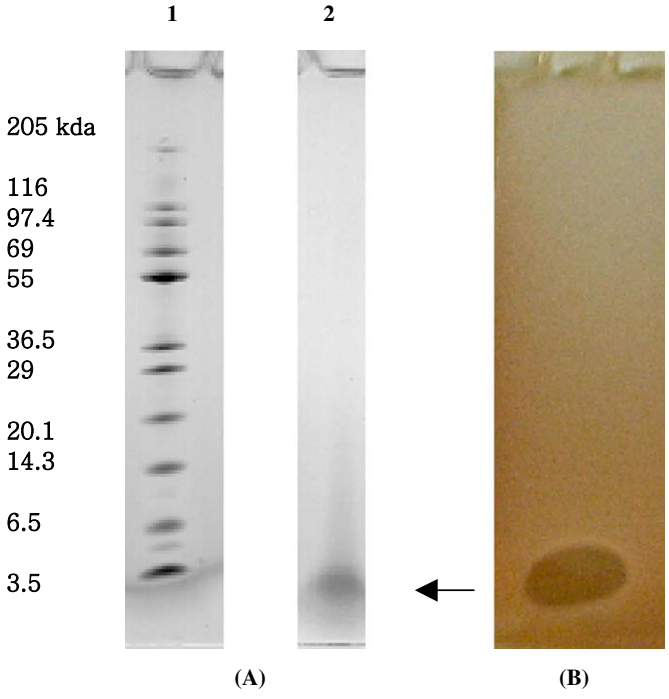

Fig. 2. SDS-PAGE and detection of antimicrobial activity of the purified bacteriocin. (A) Gel stained with CBB; lane 1, molecular weight standatds; lane 2, partially purified bacteriocin by hydrophobic interaction chromatography. (B) Gel overlaid with cells of *Propionibacterium acnes* ATCC 6919.

3.3. Bacteriocin activity

Table 4 shows the antimicrobial activity of bacteriocin against *S. aureus* ATCC 65389, *S. epidermidis* ATCC 12228, *Strep. pyrogenes* ATCC 21059, and *P. acnes* ATCC 6919. Minimal inhibitory activity for indicators ranged between 0.625 and 1.25 μg of bacteriocin, and *S. aureus* ATCC 65389 was shown to be twice as much sensitive as other tested strains to the bacteriocin produced by *Lactococcus* sp. HY 449.

3.4. Mode of bacteriocin actions

To investigate the mode of action of bacteriocin against *S. aureus* ATCC 65389, *S. epidermidis* ATCC 12228, *Strep. pyrogenes* ATCC 21059, and*P. acnes* ATCC 6919, crude bacteriocin was added to the culture of tested strains during logarithmic growth. As shown in Fig. 3, the cell growth was observed after 12 h of incubation without bacteriocin treatment, whereas the cell growth was not detected with bacteriocin at higher than 10 AU/mL.

In order to investigate the mode of bacteriocin action, ACB was added to the liquid culture of *P. acnes* ATCC 6919. The addition of bacteriocin (500 AU/ml) to the logarithmic phase of *P. acnes* ATCC 6919 cells decreased the optical density at 660 nm at each time interval (Fig. 4).

The absorbances at 260 and 280 nm of the cell supernatant after treatment with bacteriocin are shown in Fig. 5. The absorbance at 260 nm was 2–4 times greater for the bacteriocin-treated cells than the control cells (Fig. 5A), and similar patterns were observed at 280 nm (Fig. 5B). These results indicated that the cellular components of *P. acnes* ATCC 6919 may be released when cells were treated with bacteriocin. Therefore, it could be concluded that the bacteriocin may have bacteriolytic action on the cell wall and cell membrane of the *P. acnes* ATCC 6919.

3.5. Change of bacteriocin activity in cosmetic emulsion

The change of the bacteriocin activity during storage in cosmetics was studied with the *P. acnes* ATCC 6919 at 10, 20, and 30 °C. As shown in Table 5, bacteriocin activity on the *P. acnes* ATCC 6919 decreased during storage, however, 3200–6400 AU/ml of bacteriocin activities remained

Table 4
Susceptibility of skin microflora strains to bacteriocin produced by *Lactococcus* sp. HY 449[a]

Strains	Bacteriocin activity (AU/ml)
Streptococcus pyogenes ATCC 21059	3200
Staphylococcus aureus ATCC 65389	6400
Staphylococcus epidermidis ATCC 12228	3200
Propionibacterium acnes ATCC 6919	3200

[a] The minimal inhibition concentration was determined by the highest twofold dilution showing a clear inhibitory zone using 12,800 AU/ml of crude bacteriocin.

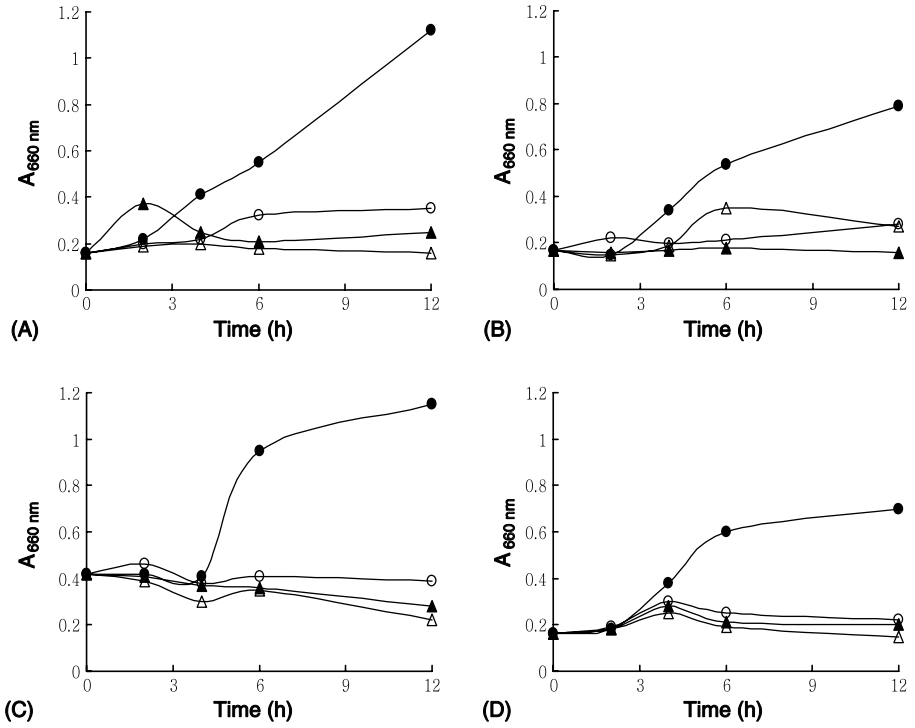

Fig. 3. Inhibitory effect of bacteriocin produced by *Lc. lactis* HY449 on inflammatory bacteria. (-●-) control, (-○-) 10 AU/ml, (-▼-) 50 AU/ml, (-▽-) 100 AU/ml. (A) *Streptococcus pyogenes* ATCC 21059; (B) *Staphylococcus aureus* ATCC 65389; (C) *Staphylococcus epidermidis* ATCC 12228; (D) *Propionibacterium acne* ATCC 6919. *Staphylococcus aureus* ATCC 65389, *Staphylococcus epidermidis* ATCC 12228, and *Strep. pyogenes* ATCC 21059 were grown at 37 °C for 18 h in BHI broth (Difco) and and *Propionibacterium acnes* ATCC 6919 was anaerobically incubated in GAM (Gifu) broth.

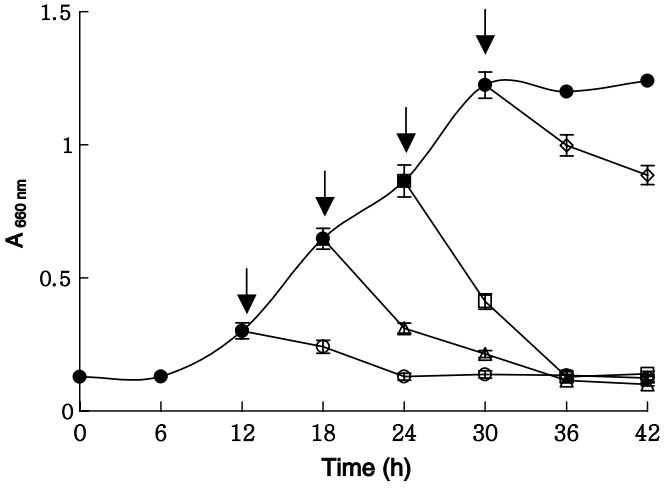

Fig. 4. Effect of the bacteriocin of *Lactococcus* sp. HY449 on the growth of *Propionibacterium acnes* ATCC 6919 at 37 °C in GAM broth. Arrows indicate addition of the bacteriocin (500 AU/ml).

during the storage period at all tested temperature conditions.

3.6. Skin irritation and toxicity

The skin toxicity of the bacteriocin derived from *Lactococcus* sp. HY449 was evaluated by human patch test for the primary skin irritation. As shown in Table 6, no detectable irritation of human skin was observed for the bacteriocin, whereas tea tree oil showed slight irritation at 10% and Triclosan showed moderate irritation at higher than 5%. Any noticeable primary irritation did not appear with bacteriocin in CAM-based assay and human patch test, but minimal erythema and allergic reactions were detected among 2 out of 30 volunteers at 24 h observation and comparative groups (tea tree oil and Triclosan) showed some irritancy in a short term test period (data not shown).

3.7. Cytotoxicity

The cytotoxicity of the bacteriocin produced by *Lactococcus* sp. HY 449 was evaluated with human fibroblasts. Fig. 6 shows the decreased cell proliferation in a dose dependent manner. The presence of 10 and 50 AU/ml of bacteriocin increased cell proliferation after 24 h. However, at the concentrations of 50, 100, and 500 AU/ml of bacteriocin, the proliferation of human fibroblast cells were not affected. In a cytotoxicity test of human fibroblasts, LC_{50} of bacteriocin was about 50 mg/ml after 24 h of treatment.

4. Discussion

P. acnes is a Gram-positive, non-sporeforming, pleomorphic, and anaerobic bacterium that is ubiquitous in nature and commonly found as a normal flora of human gut and this strain has been recognized as a bacterium causing acne and skin inflammation (Meisler and Mandelbaum, 1989). Other gram-positive cocci such as *S. aureus*,

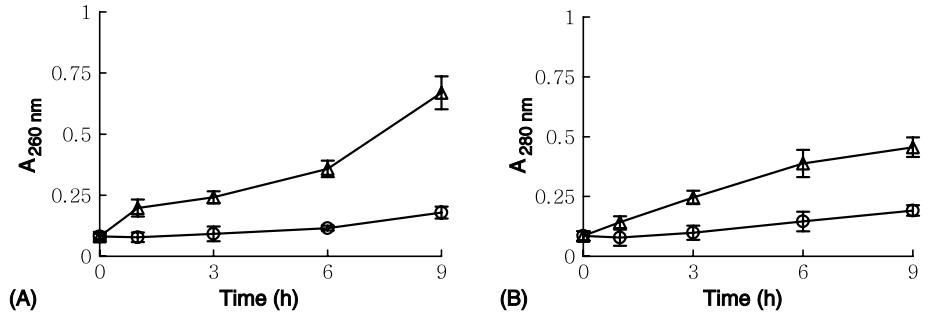

Fig. 5. Changes of UV absorbances of *Propionibacterium acnes* ATCC 6919 after treatment with bacteriocin (100 AU/ml). (A) Measured at 260 nm; (B) measured at 280 nm; (-○-) control; (-△-) 100 AU/ml bacteriocin added.

Table 5
Changes of bacteriocin activity in cosmetic emulsion during storage at different temperatures

Storage temp. (°C)	Storage time (Day)	Bacteriocin activity (AU/ml)
10	0	25,600
	7	12,800
	14	6400
	21	6400
	28	6400
20	0	25,600
	7	12,800
	14	6400
	21	3200
	28	3200
30	0	25,600
	7	6400
	14	3200
	21	6400
	28	3200

Table 6
Irritation tests for the bacteriocin produced by *Lactococcus* sp. HY 449

Samples		Quotient value[a]	Human patch test[c]		
			0.5 h	48 h	72 h
Bacteriocin base	2.5% (6400 AU/ml)	1.0[b]	−	−	−
	5.0% (12,800 AU/ml)	1.0	−	−	−
	10% (25,600 AU/ml)	1.0	−	−	−
Tea tree oil	2.5%	1.0	−	−	−
	5.0%	2.85	−	−	−
	10.0%	4.50	+	?	−
Triclosan	2.5%	6.20	−	−	−
	5.0%	8.00	+	−	−
	10.0%	12.39	++	+	−
Control		1.0	−	−	−

[a] Quotient (Q) values in CAM-based assay; Q values = $\text{score}_{\text{text}}/\text{score}_{\text{reference}}$.

[b] 1: Non irritant; 1–6: slight irritant; 6–12: moderate irritant; 12–16: irritant; 16: severely irritant.

[c] −: negative; +: erythema, infiltration, possibly papules; ++: erythema, infiltration, papules, vesicles; +++: bullous; ?: doubtful reaction, faint macular erythema only.

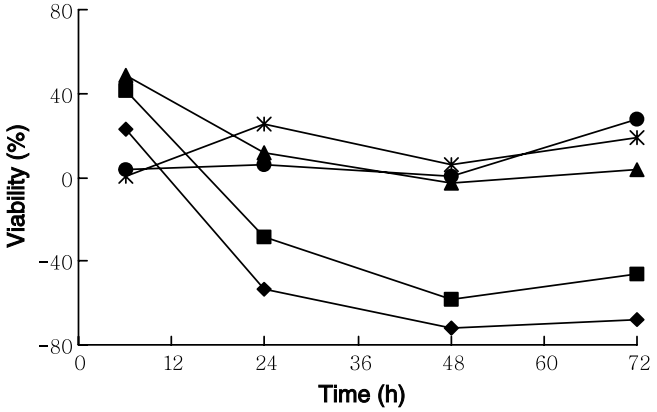

Fig. 6. Cytotoxicity on normal human fibroblast of the bacteriocin produced by *Lactococcus* sp. HY 449. (-♦-) 5000 AU/ml bacteriocin; (-■-) 1000 AU/ml bacteriocin; (-▲-) 500 AU/ml bacteriocin; (-●-) 100 AU/ml bacteriocin; (-✱-) 50 AU/ml of bacteriocin.

S. epidermidis, and *Strep. pyogenes* are also known to be the predominant bacteria causing infectious skin diseases such as furuncle and pyoderma (Leyden and Kligman, 1976).

Current concepts of cosmetology are based on the understanding of the complex biochemical mechanisms which causes the modification of mechanical, physical, and biochemical properties of the skin. The antimicrobial activities of many sources including chemicals and plant extracts have been studied for cosmetic uses, which could be an active cosmetic ingredient which developed mild fungicidal and bacteriostatic properties (Akiyama et al., 1990; Carson and Riley, 1994; Carson, 1998; Meisler and Mandelbaum, 1989).

To be appropriate for skin cosmetics, the active ingredient should be non-toxic, hypoallergenic, and non-irritant to the skin as possible. Moreover, the active ingredient is desired to be biodegradable to meet the growing demand. In general, bacteriocins are peptide or proteins, which exhibit inhibition against closely related strains. Numerous efforts have been made to test biopreservatives for the inhibition against the growth of food-borne pathogens and spoiling microorganisms (Nes and Holo, 2000). Therefore, bacteriocin was thought to be also used as an active

ingredient of cosmetics to control the growth of pathogenic bacteria and provide excellent protection against microbial inflammation on skin resulting in acne and other skin disorders.

The inhibitory effect of the bacteriocin produced by *Lactococcus* sp. HY 449 employed in this study was tested against skin-inflammatory bacteria. It was shown that the bacteriocin was able to inhibit the growth of selected bacteria efficiently. We chose *P. acnes* ATCC 6919 to further study the action mode of bacteriocin because the bacteria cause major skin troubles such as follicles and acne, and the degree of inhibition was significant at low doses. When cells were treated with bacteriocin, the release of cellular components may have been due to the membrane damage or cell lysis. Our results indicated that the ability of bacteriocin from *Latococcus* sp. HY449 to cause cell lysis of *P. acnes* ATCC 6919 was highly efficient in this aspect.

Indeed, when the nisin activity was determined during the storage at 35 °C for 48 h in various solutions, the level of approximately 400 IU/ml of nisin dropped to less than 5 IU/ml in 10% albumin solution (Rogers, 1991). The decrease in bacteriocin activity may be the result of interactions of bacteriocin with cosmetic compounds such as phospholipid or emulsifier. From the presented results, we suggest bacteriocin produced by *Lactococcus* sp. HY 449 be applied as an efficient antimicrobial agent as cosmetic ingredient.

Acknowledgements

The authors thank Korea Yakult Co. Ltd., for providing the strains of *Lactococcus* sp. HY449. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (02-PJ1-PG11-VN01-SV05-0003).

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