Article

Synergistic Antibacterial Effects of Probiotic Lactic Acid Bacteria with *Curcuma longa* Rhizome Extract as Synbiotic against *Cutibacterium acnes*

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Abstract: Acne is a chronic inflammatory skin disease induced by *Cutibacterium acnes*. Recently, the effects of probiotics, prebiotics and synbiotics have been researched for the treatment of skin diseases in humans. However, the synbiotic effect of probiotics and prebiotic *Curcuma longa* rhizome extract (CLE) on *C. acnes* remains ambiguous. Therefore, the aim of this study was to investigate the synergistic antibacterial activities of probiotic lactic acid bacteria (LAB) with CLE as a synbiotic against *C. acnes*. Agar well diffusion assays were performed to determine the inhibitory effects of each combination of one of five *Lactobacillus* sp. with CLE as synbiotics against *C. acnes* KCTC 3314. Among them, the comparison between the average diameters of inhibition zones showed that the synbiotic combination of *Lactobacillus acidophilus* A001F8 and CLE significantly increased the inhibition zone diameters against *C. acnes*, compared to the use of *Lactobacillus acidophilus* A001F8 or CLE alone ($p < 0.05$). In conclusion, the synbiotics of probiotic LAB and CLE showed synergistic antibacterial effects against *C. acnes*, suggesting therapeutic potential for this synbiotic combination in the development of cosmetics or medicine against *C. acnes*.

Keywords: *Cutibacterium acnes*; antibacterial activity; *Curcuma longa*; synbiotics; synergy; pre-and probiotics

1. Introduction

Acne vulgaris is a common skin disease resulting from sebum production in hair follicles on the face, neck and chest, induced by androgen, altered keratinization, inflammation and bacterial colonization. Among them, *Cutibacterium acnes* is known as a major cause [1].

Probiotics are live bacteria that have beneficial effects on the host; they have been proposed as an alternative to antibiotics, as the indiscriminate use of the latter can cause multidrug resistance or other side effects [2,3]. The direct antibacterial effects of probiotics against pathogens may be observed by inhibiting the growth of pathogens by the production of antimicrobial peptides and colonization resistance [4]. Therefore, probiotics have potential for use as cosmetic or medicinal ingredients against *C. acnes*.

Prebiotics are defined as nondigestible food ingredients, such as oligosaccharides, inulin and lactulose, which promote the growth and survival of probiotics; they are found in vegetables and fruit [5,6]. In human skin, prebiotic substances can be selectively applied to modulate microbial systems to achieve beneficial effects [7]. In order to develop natural prebiotics, various medicinal plants have been investigated. Among them, turmeric (*Curcuma longa* rhizome) is well known for its anti-inflammatory, antimicrobial and antioxidant activities [8]. A previous study reported that *Curcuma longa* rhizome extract (CLE) reduced the levels of reactive oxygen species (ROS) and cytokines produced by *C. acnes* [9]. However, few studies have focused on CLE as a prebiotic, and none has reported synergistic effects of combinations of probiotics and CLE as synbiotics on *C. acnes*.

Therefore, in this study, the synergistic effects of probiotics and prebiotics against *C. acnes* were investigated. First, the antibacterial effects on *C. acnes* of five potential probiotic strains were assessed. Then, the extracts of twelve natural medicinal plants that have prebiotic potential were screened for their antibacterial activities against *C. acnes* using antibacterial assays. It was found that only CLE displayed inhibition zones. Finally, each of the five lactic acid bacteria (LAB) species was applied with CLE to examine synergistic antibacterial effects on *C. acnes*.

2. Materials and Methods

2.1. Preparation of Plant Extracts

Eleven methanolic plant extracts (*Cornus officinalis*, *Allium cepa*, *Cudrania tricuspidata*, *Spinacia oleracea*, *Rubus coreanus*, *Daucus carota* var. *sativa*, *Chrysanthemum coronarium* var. *spatiosum*, *Brassica oleracea* var. *capitata*, *Mollugo verticillata* and *Schisandra chinensis*) and one ethanolic medicinal plant extract (*Curcuma longa* L.) rhizome used in this study were purchased from the Korea Plant Extract Bank, Korea Research Institute of Bioscience and Biotechnology (KRIBB; Daejeon, South Korea). All plant extracts were dissolved in dimethylsulfoxide (DMSO; Sigma, St Louis, MO, USA) to 10% of stock concentration and kept at -20°C , and then 0.01% of test concentration [10] in phosphate buffered saline (PBS) solution was used for antibacterial test.

2.2. Bacterial Species and Growth Conditions

The species of probiotic lactic acid bacteria (LAB) used in this study were *Lactobacillus acidophilus* A001F8, *Lactobacillus rhamnosus* A001G8, *Lactobacillus paracasei* A002C5, *Lactobacillus plantarum* A003A7 and *Lactobacillus casei* A003D4, all of which were isolated from fermented dairy food. These species were identified by 16Sr RNA gene sequencing. *C. acnes* KCTC 3314 was obtained from the Korean Collection for Type Cultures (KCTC; Daejeon, South Korea). The *Lactobacillus* species were grown in De Man, Rogosa, Sharpe broth (MRS broth; Difco, Detroit, MI, USA). *C. acnes* was grown at 37°C for 72 h in brain heart infusion broth (BHI broth; Difco, Detroit, MI, USA) and BHI agar under anaerobic conditions using GasPak pouch (BD GasPak™ EZ, Franklin Lakes, NJ, USA).

2.3. Antibacterial Activity by Agar Well Diffusion Assay

An agar well diffusion assay was performed to determine the antibacterial effects of the plant extracts, LAB with and without CLE against *C. acnes*, respectively. Vancomycin supplement for microbiology (Sigma; St Louis, MO, USA) was diluted in distilled water (DW) and used as a positive control. Each of the MRS broths was cultured with LAB with 0.01% DMSO as a control, and LAB with 0.01% CLE at 37°C for 24 h. First, the inocula of *C. acnes* (150 μL) were spread using sterile cotton swabs on BHI agar plates. Agar wells (8 mm) were punched with sterile pipette tips. Then, 100 μL plant extracts and LAB with and without 0.01% CLE were added to the agar well. All plates were incubated for 72 h at 37°C under anaerobic conditions using GasPak (BD GasPak™ EZ, Franklin Lakes, NJ, USA). The tests were performed in three independent experiments, and results are presented as the average values of the diameters of the inhibition zones [11].

2.4. Determination of Growth Curves of Probiotics with and without CLE

Lactobacillus sp. (*L. acidophilus* A001F8, *L. rhamnosus* A001G8, *L. paracasei* A002C5, *L. plantarum* A003A7 and *L. casei* A003D4) with and without 0.01% CLE were cultured at 37°C for 18 h. The absorbance was measured by a UV-spectrophotometer (Optizen POP, Mecasys, Korea) at 600 nm (OD600) every 2 h for 18 h. To ensure accuracy, each sample was ten-fold serially diluted with PBS.

2.5. Statistical Analysis

All data are shown as mean $\pm$ standard deviation (SD) for the quantitative variables. Frequencies and graphs are given for qualitative variables. A Student's *t*-test was performed using SPSS version

23 statistics software to determine statistically significant differences between the measurements of LAB alone and LAB + CLE. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Screening of Antibacterial Activities of Plant Extracts

An agar well diffusion assay was conducted to assess the antibacterial activities of twelve plant extracts against *C. acnes*. Antibacterial activities were recorded as the average of the diameters of the inhibition zones. As shown in Table 1, vancomycin (30 µg/well), used as a positive control, showed a 13.6 ± 0.29 -mm inhibition zone against *C. acnes*. With the exception of CLE, none of the plant extracts displayed inhibition zones. CLE showed a 10.8 ± 0.29 -mm inhibition zone. These results indicated that CLE has antibacterial activity.

Table 1. The inhibitory effects of plant extracts against *C. acnes*.

Name of Plant	Extracted Part	Inhibition Zone (mm)
		<i>Cutibacterium acnes</i>
<i>Cornus officinalis</i>	Berry	-
<i>Allium cepa</i>	Whole plant	-
<i>Curcuma longa</i> L.	Rhizome	10.8 ± 0.29
<i>Cudrania tricuspidata</i>	Root	-
<i>Spinacia oleracea</i>	Whole plant	-
<i>Rubus coreanus</i>	Leaves, Stem	-
<i>Daucus carota</i> var. <i>sativa</i>	Whole plant	-
<i>Chrysanthemum coronarium</i> var. <i>spatiosum</i>	Whole plant	-
<i>Brassica oleracea</i> var. <i>capitata</i>	Whole plant	-
<i>Zingiber officinale</i>	Root	-
<i>Mollugo verticillata</i>	Whole plant	-
<i>Schisandra chinensis</i>	Leaves	-
Vancomycin (Positive control)		13.6 ± 2.9

3.2. Synergistic Antibacterial Effects of *Lactobacillus* sp. with and without CLE against *C. acnes*

As shown in Figure 1, the antibacterial properties against *C. acnes* of each LAB species alone and with CLE were compared on five sets using agar well diffusion assay. Among them, *L. acidophilus* A001F8 showed the highest synergetic antibacterial activity, and the average value of the inhibition zone diameters of *L. acidophilus* A001F8 + CLE (13.8 ± 0.29) was significantly increased compared to *L. acidophilus* A001F8 alone (12.3 ± 0.57) ($p < 0.05$). Although for other LAB (*L. rhamnosus* A001G8, *L. paracasei* A002C5, *L. plantarum* A003A7 and *L. casei* A003D4) there was no statistically significant difference, the average values of the inhibition zones of LAB + CLE were larger than those of LAB alone.

Figure 1. The synergistic antibacterial effects of *Lactobacillus* sp. with and without CLE against *C. acnes*. Data were analyzed by Student's *t*-test and expressed as the means $\pm$ SD of three independent experiments (* $p < 0.05$).

3.3. Effect of CLE on the Growth of Five *Lactobacillus* sp.

The growth curves of five *Lactobacillus* sp. (*L. acidophilus* A001F8, *L. rhamnosus* A001G8, *L. paracasei* A002C5, *L. plantarum* A003A7 and *L. casei* A003D4) with and without CLE showed no significant differences for each set, implying that CLE did not stimulate growth of *Lactobacillus* sp. (Figure 2).

Figure 2. The growth curve of *L. acidophilus* A001F8 (A), *L. rhamnosus* A001G8 (B), *L. paracasei* A002C5 (C), *L. plantarum* A003A7 (D) and *L. casei* A003D4 (E), with and without CLE. There were no statistically significant differences in growth between LAB and LAB + CLE (Student's *t*-test; $p > 0.05$).

4. Discussion

Probiotics have the potential to prevent infections from pathogenic bacteria and promote the colonization of a healthy microbiome, thereby serving as potential alternative dermatological treatments [12]. Moreover, the use of synbiotics, i.e., combinations of probiotics and prebiotics, has been proposed to treat several skin conditions such as atopic dermatitis, acne, rosacea and wounds [11,13]. In this study, the synergistic antibacterial effects of probiotic LAB with CLE were evaluated against *C. acnes* using the same agar well diffusion assay applied in previous experiments [11,14]. First, a total of twelve candidate plant extracts that have prebiotic potential were selected based on a literature review [6,15]. These extracts were then subjected to screening for their antibacterial activities against *C. acnes*. The CLE was finally selected as the most effective substance in terms of its synergistic effect with probiotics to inhibit the growth of *C. acnes*.

Five combinations of LAB and CLE displayed more positive synergistic effects against the growth of *C. acnes* than LAB or CLE alone in the agar well diffusion assay. In this study, there were no

significant differences in the growth activities of five *Lactobacillus* sp. when CLE was added at a relatively low concentration of 0.01%, but a recent study reported that a 2% turmeric extract promoted the growth of *L. rhamnosus* GG (LGG) and *Bifidobacterium animalis* BB12 [16]. The use of different strains or concentrations of CLE could be the cause of the disagreement between our results and previous data.

According to previous studies, LAB are capable of inhibiting pathogen growth through the antibiofilm effects of biosurfactants or the antimicrobial effects of substances like bacteriocin [17,18]. Also, the bacteriocin produced by *Lactococcus* sp. HY 449 was reported to have a bacteriolytic action on the cell wall and membrane of *C. acnes* [19]. Moreover, prebiotics such as oligosaccharides (OS), inulin and lactulose are known to enhance the secretion and activity of bacteriocin produced by LAB [20,21]. Although the mechanisms of the enhancing action remain obscure, our results presented a novel synergistic anti-*C. acnes* effect of CLE in combination with probiotic LAB. Future studies are needed to investigate the metabolites produced by the synbiotics. Based on the above findings, cosmetics containing a combination of *L. acidophilus* A001F8 with CLE could have the potential to alleviate acne. Moreover, the systemic supplementation with these synbiotics could a positive effect on acne, according to a previous study [22].

In conclusion, these results suggest that *L. acidophilus* A001F8 with prebiotic CLE has a synergistic effect of antibacterial activity against *C. acnes*, and thus, that prebiotic CLE can be utilized for the development of cosmetics and medicines for the treatment of acne.

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