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Antagonistic Effects of *Lactobacillus plantarum* on *Candida albicans* in ME-180 Cervical Carcinoma Cell Culture

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Abstract

Background: *Candida albicans* is an yeast species that colonizes the vaginal and oral mucosa of healthy women. However, it exhibits pathogenicity when the balance between yeast and mucous membranes and host defense mechanisms is disrupted.
Objectives: To develop an auxiliary treatment for vaginitis, we evaluated the inhibitory effects of a probiotic bacterial strain isolated from kimchi on *C. albicans*.
Methods: *Lactobacillus plantarum*, which exhibits potent inhibitory activity against pathogenic bacteria and is resistant to broad-spectrum antibiotics, was isolated from commercially kimchi in Korea, and its antagonistic effects on *C. albicans* were examined in a mixed culture with ME-180 cervical carcinoma cells.
Results: *Candida albicans* caused extensive damage in ME-180 cells. In ME-180 cells inoculated with *L. plantarum* and then with *C. albicans*, the extent of cell damage increased as the concentration of the *C. albicans* culture increased. However, in ME-180 cells inoculated with *L. plantarum* at 10^6 CFU/mL or at a higher concentration, the extent of cell damage increased substantially with the concentration of *C. albicans*, indicating that *L. plantarum* inhibited the growth of *C. albicans*.
Conclusions: *Lactobacillus plantarum* did not directly inhibit the growth of *C. albicans* but may have inhibited biofilm development at an early stage, thereby preventing the growth and mucosal adhesion of *C. albicans*. Further investigation of the safety, side effects, and metabolism of *L. plantarum* and its potential infectivity in animals is required before the *L. plantarum* isolate can be used to treat vaginitis.

Keywords: *Lactobacillus plantarum*, *Candida albicans*, Probiotics, Cervical Cancer, Vaginitis

1. Background

Candidiasis is a fungal infection caused by *Candida* spp. that affects the mucosa and internal organs in humans (1). *Candida albicans* is an important yeast species, that colonizes the vaginal and oral mucosa of apparently healthy women. However, it can become pathogenic if and when the balance between yeast and mucous membranes and host defense mechanisms is disrupted (2-4). The World Health Organization and Food and Agriculture Organization define probiotics as microorganisms that exert beneficial effects on host health when present in adequate quantities, particularly by facilitating the maintenance of gastrointestinal health and digestion (5). Most probiotics are a part of the human mucosal microbiota, and are effective in preventing and treating atopy, eczema, dermatitis, and diarrhea as well as in treating inflammatory enteritis.

Probiotics also play an important role in maintaining the vaginal environment in healthy women. Meanwhile, the biofilm, in which microorganisms aggregate, exhibits self-protection, and the microorganisms communicate with each other via antibiotic resistance and exhibit social behavior through horizontal gene transfer and quorum sensing mechanisms. Probiotic strains secrete antagonists such as surfactants, bacteriocins, exopolysaccharides, organic acids, lactic acid, fatty acids, enzymes, and hydrogen peroxide, and reduce the biofilm biomass by changing the pH and initiating nutrient competition, which prevents biofilm formation by pathogens (6-10).

In a previous study, 140 probiotic strains were isolated from 35 types of Korean kimchi using 16S rRNA sequencing. Among them, *Lactobacillus plantarum* strains exhibiting antimicrobial activity and broad antibiotic resistance

were selected (11). The growth of the microorganisms exhibiting highest pathogenicity, including *C. albicans*, was found to be almost completely inhibited in the mixed culture containing the probiotic strains and six pathogenic microorganisms (12). In the hydrogen peroxide production test (unpublished), 43 of 140 probiotic strains (30.7%) isolated from kimchi produced H_2O_2, among which 25 of 53 *L. plantarum* strains (47.2%) produced H_2O_2. This was attributed to direct inhibition via lactic acid, hydrogen peroxide, and bacteriocin production and low pH induced by probiotics (12). In a previous study, 140 probiotic strains were isolated from commercially available kimchi in Korea; these bacteria were phylogenetically identified based on their 16S rRNA gene sequences and examined for resistance to 18 antibiotics (11). Examination of the inhibitory effects of the probiotics in ME-180 cervical carcinoma cells revealed that the growth of *C. albicans* was inhibited in ME-180 cultures initially inoculated with *L. plantarum* at relatively high concentrations, regardless of the concentration of the *C. albicans* culture (12).

2. Objectives

In our previous study, probiotics exhibiting resistance to broad-spectrum antibiotics and high levels of inhibitory activity against pathogenic bacteria were isolated. In this study, the inhibitory effects of *L. plantarum* on *C. albicans* were examined in ME-180 cervical carcinoma cell cultures to determine the potential of *L. plantarum* as an auxiliary treatment agent for vaginitis.

3. Methods

3.1. Bacterial Strains and Cells

The *C. albicans* strain KCTC 7752 was obtained from the Korean Collection for Type Cultures. The *L. plantarum* strain isolated from commercially available kimchi (in Korea) was stored at 4 °C in the laboratory (11). Human cervical cancer ME-180 cells (KCLB30033) were used for the cell culture experiments.

3.2. ME-180 Cell Culture and Inoculation with Lactobacillus plantarum and Candida albicans

Cell culture was performed as described previously (13, 14). RPMI 1640 (Welgene, Daegu, Korea) supplemented with 10% fetal bovine serum (Welgene, Korea) was used as the culture medium. ME-180 cells (KCLB 30033, Korean Cell Line Bank, Korea) were inoculated with *L. plantarum* at 10^4, 10^6, and 10^8 colony-forming units per milliliter (CFU/mL). Following this, the ME-180 cells were seeded into a 24-well plate (SPL Life Sciences, Gyeonggi-do, Korea) at a density of 2×10^5 cells/mL, followed by overnight incubation at 37°C in a 5% CO_2 incubator. The culture medium was replaced with fresh medium the following day, and *C. albicans* was used to inoculate the cells (added at 1 mL/well) at 10^4, 10^6, and 10^8 CFU/mL.

ME-180 cells were inoculated with both strains simultaneously, following which the cells were cultured for 5 h at 37°C in a 5% CO_2 incubator. The culture medium was removed, and the ME-180 cells were washed twice with Dulbecco's phosphate-buffered saline (DPBS; Welgene), followed by the addition of the culture medium (1 mL/well) and overnight incubation at 37°C in a 5% CO_2 incubator. The next day, the culture medium was removed, and the cells were washed twice with DPBS, dehydrated, and subjected to Gram staining. The cells were stained with crystal violet for 1 min, washed twice with DPBS, decolorized with alcohol for 1 min, and washed twice with DPBS. Next, the cells were stained with Safranin O for 1 min, washed twice with DPBS, dehydrated, and examined under a microscope (Olympus CK2 inverted microscope, Olympus Corp., NY, USA).

ME-180 cells were also alternately inoculated with the two strains. First, the cells were inoculated with one of the two strains at 10^4, 10^6, and 10^8 CFU/mL, and the plate was incubated at 37°C in a 5% CO_2 incubator for 5 h. The culture medium was removed, and the plate was washed twice with DPBS. Next, ME-180 cells were inoculated with the second strain at 10^6 and 10^8 CFU/mL and cultured at 37°C in a 5% CO_2 incubator for 5 h. The culture medium was removed, and the ME-180 cells were washed twice with DPBS (1 mL/well), followed by overnight incubation with the basal medium at 37°C and 5% CO_2. The ME-180 cells were stained as described above and examined under a microscope.

4. Results

Figure 1 shows the results obtained upon the inoculation of ME-180 cells with *L. plantarum* and *C. albicans*. Inoculation with *L. plantarum* at 10^4, 10^6, and 10^8 CFU/mL did not affect the growth of ME-180 cells, nor did it cause any cell damage. Inoculation with *C. albicans* at 10^4 CFU/mL did not affect the growth of ME-180 cells. However, significant necrosis was observed in ME-180 cells inoculated with *C. albicans* at 10^6 and 10^8 CFU/mL. In contrast, low levels of apop-

tosis were observed in ME-180 cells simultaneously inoculated with 10^4 CFU/mL of *C. albicans* and *L. plantarum* at all culture concentrations. A higher level of apoptosis was observed in ME-180 cells inoculated with 10^6 CFU/mL of *C. albicans*, and the extent of apoptosis was not significantly affected by the concentration of the *L. plantarum* culture.

Extensive damage was observed in ME-180 cells inoculated with 10^8 CFU/mL of *C. albicans*, regardless of the concentration of the *L. plantarum* culture (Figure 2). The same results were obtained for ME-180 cells that were first inoculated with *C. albicans* and then with *L. plantarum* at different culture concentrations. Although inoculation with 10^4 CFU/mL of *C. albicans* did not inhibit the growth of ME-180 cells, low levels of cell damage were observed upon inoculation with 10^6 CFU/mL of *C. albicans*, and the growth of *C. albicans* was notable. There were no significant differences in the levels of ME-180 cell damage depending on the *L. plantarum* culture concentration. In contrast, ME-180 cells inoculated with 10^8 CFU/mL of *C. albicans* showed extensive cell damage, regardless of the *L. plantarum* culture concentration (Figure 3).

Different results were obtained when the ME-180 cells were first inoculated with *L. plantarum* and then with *C. albicans* at different culture concentrations, as shown in Figure 4. The extent of damage in ME-180 cells increased as the concentration of *C. albicans* increased in the cultures inoculated with 10^4 CFU/mL of *L. plantarum*. Low levels of cell damage were observed in the ME-180 cultures inoculated with 10^6 CFU/mL of *L. plantarum*, irrespective of the *C. albicans* culture concentration. Meanwhile, cell damage was noticeably reduced in the ME-180 cell cultures inoculated with 10^8 CFU/mL of *L. plantarum*, irrespective of the *C. albicans* culture concentration. Figure 5 shows the results for ME-180 cells simultaneously inoculated with 108 CFU/mL of *C. albicans* and 10^8 CFU/mL of *L. plantarum*, as well as for those inoculated with 10^8 CFU/mL of *C. albicans* or *L. plantarum* first, followed by inoculation with the other microorganism at the same concentration later. The extent of cell damage was the greatest in the second condition, followed by those in the first and third conditions.

5. Discussion

Vaginitis is typically classified as bacterial vaginosis and candidal vaginitis. Candidal vaginitis, which is caused by *C. albicans*, is characterized by itching and the secretion of a thick, white vaginal fluid. An estimated 70 - 75% of women experience candidal vaginitis at least once in their lifetime, and 40 - 50% experience at least two recurrences within a year (15, 16). Santos et al. (17) reported that the secretion of anti-inflammatory cytokines and interleukin 8 and the activity of nuclear factor kappa B reduced in HeLa cervical carcinoma cells inoculated with *L. plantarum* and *L. fermentum* before and after inoculation with *C. albicans*. Kang et al. (18) reported that *L. plantarum* and *L. fermentum* attached to HT-29 human colorectal adenocarcinoma cells and inhibited the growth of *C. albicans*. Matsuda et al. (19) reported that *L. gasseri* and *L. crispatus* reduced the adhesion of *C. albicans* to HeLa cells.

In this study, *L. plantarum* was added to an ME-180 cell culture to examine its inhibitory effect on *C. albicans*. No damage was observed in the ME-180 cell cultures inoculated only with *L. plantarum*, whereas inoculation with *C. albicans* resulted in extensive damage to ME-180 cells. In contrast, the levels of damage to ME-180 cells increased significantly as the *C. albicans* concentration increased in ME-180 cultures simultaneously inoculated with *L. plantarum* and *C. albicans*, as well as in those that were first inoculated with *C. albicans* and then with *L. plantarum*. The same results were obtained when ME-180 cells were first inoculated with *L. plantarum* and then with *C. albicans*. However, the level of cell damage decreased noticeably in ME-180 cultures inoculated with *L. plantarum* at concentrations of 10^6 CFU/mL or higher, depending on the concentration of the *C. albicans* culture.

The inhibitory effect of *L. plantarum* on *C. albicans* in the ME-180 cell culture was not direct (e.g., via hydrogen peroxide, bacteriocin, lactic acid, and organic acids). Rather, the effect was presumed to be mediated by a reduction in the growth and mucosal adhesion ability of *C. albicans* by inhibition at the early stages of biofilm development. *Lactobacillus plantarum*, which exerted strong inhibitory effects on *C. albicans*, can be used as an auxiliary treatment agent for female vaginitis after safety studies have been conducted for assessing its metabolic activities and it has been confirmed to be non-infectious in animals.

5.1. Conclusions

The antagonistic effect of *L. plantarum* on *C. albicans* was observed in ME-180 cell cultures inoculated first with *L. plantarum*, and the effect increased as the concentration of the *L. plantarum* culture increased. However, almost no antagonistic effect was observed in ME-180 cell cultures simultaneously inoculated with *L. plantarum* and *C. albicans*, or in those inoculated with *C. albicans* first, regardless of the concentration of the *L. plantarum* culture. These findings indicate that *L. plantarum* may not have antagonized *C.*

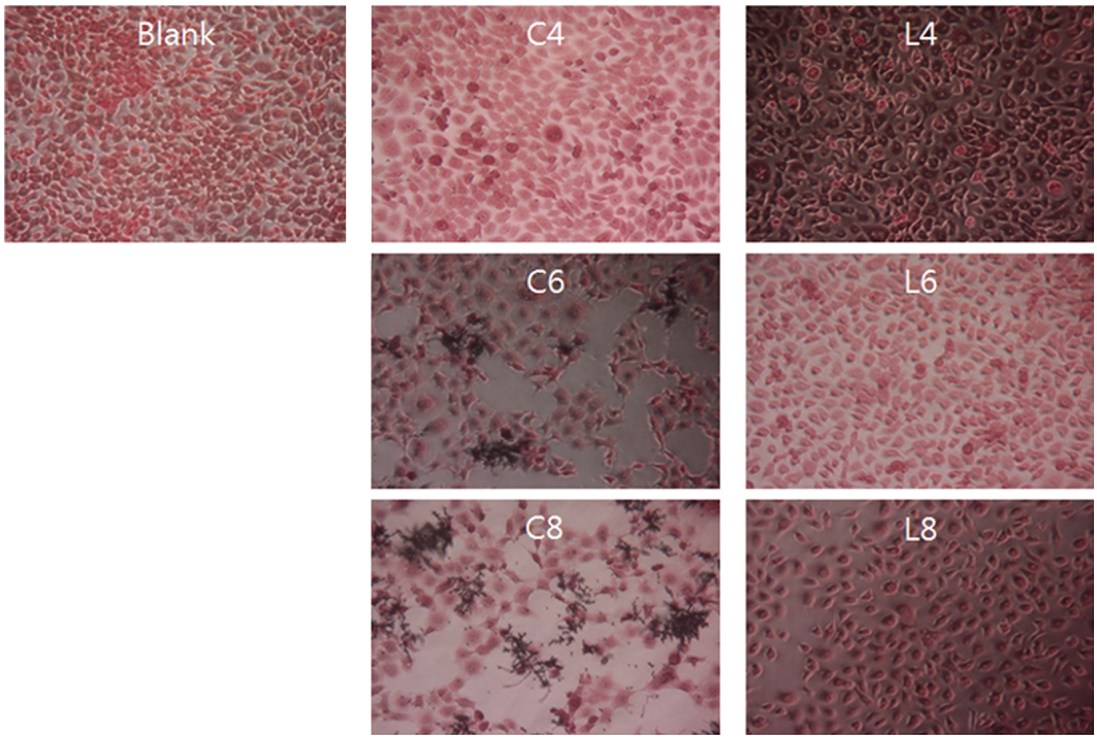

Figure 1. Morphology of M-180 cells inoculated with *Candida albicans* and *Lactobacillus plantarum*. Blank, uninoculated M-180 cells; C, *C. albicans*; L, *L. plantarum*. The numbers indicate 10^4, 10^6, and 10^8 CFU/mL, respectively.

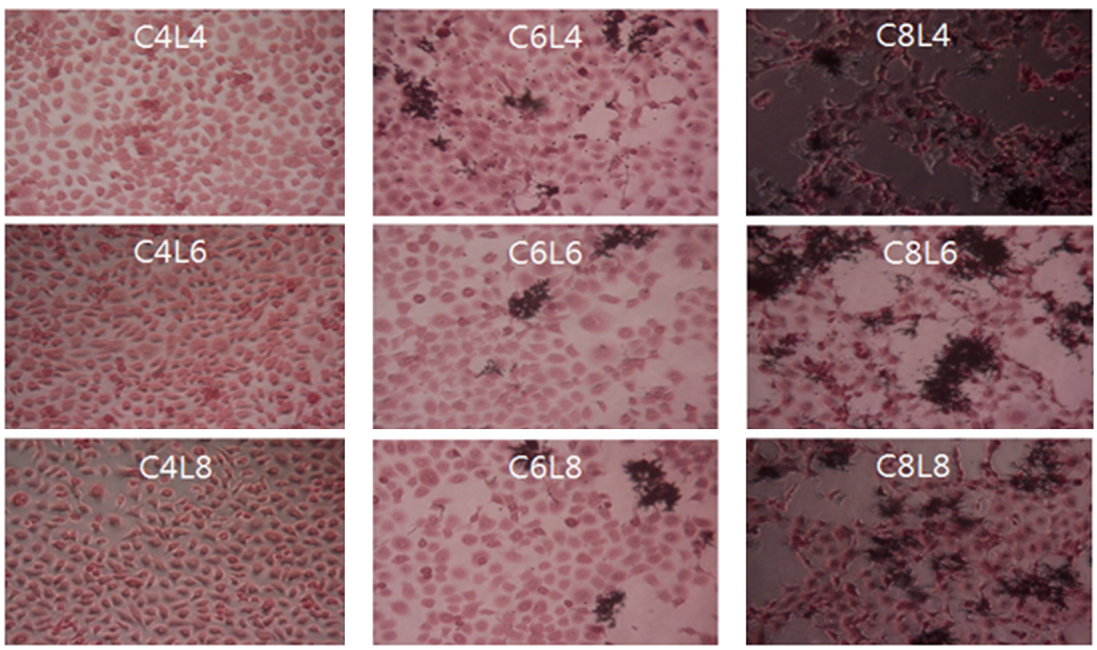

Figure 2. Morphology of ME-180 cells inoculated with *Candida albicans* and *Lactobacillus plantarum*. ME-180 cells were inoculated for 5 h, washed, and cultured for an additional 24 h. C, *C. albicans*; L, *L. plantarum*. The numbers indicate 10^4, 10^6, and 10^8 CFU/mL, respectively.

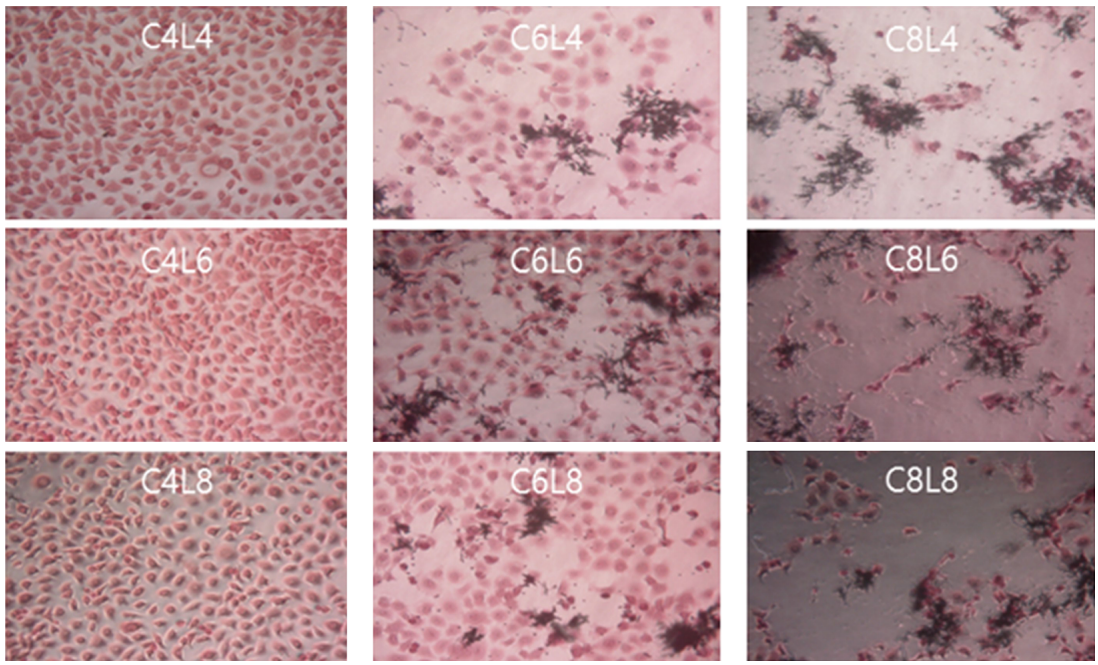

Figure 3. Morphology of ME-180 cells inoculated with *Candida albicans* and *Lactobacillus plantarum*. ME-180 cells were cultured with *C. albicans* for 5 h, then inoculated with *L. plantarum*, cultured for 5 h, washed, and cultured for an additional 24 h. C, *C. albicans*; L, *L. plantarum*. The numbers indicate 10^4, 10^6, and 10^8 CFU/mL, respectively.

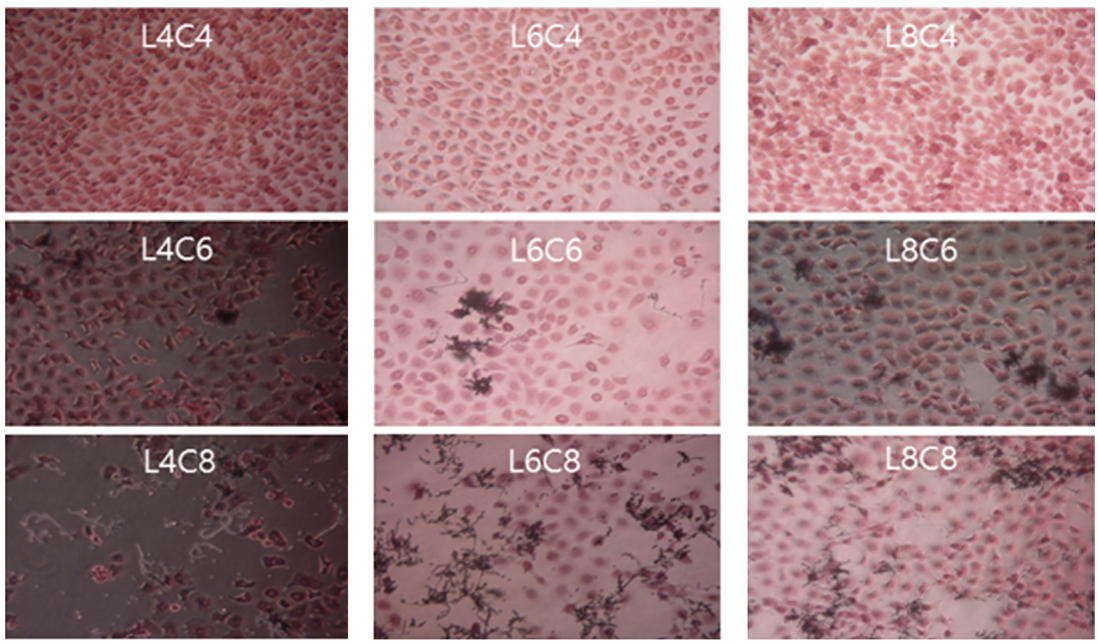

Figure 4. Morphology of ME-180 cells inoculated with *Candida albicans* and *Lactobacillus plantarum*. ME-180 cells were cultured with *L. plantarum* for 5 h, then inoculated with *C. albicans*, cultured for 5 h, washed, and cultured for an additional 24 h. C, *C. albicans*; L, *L. plantarum*. The numbers indicate 10^4, 10^6, and 10^8 CFU/mL, respectively.

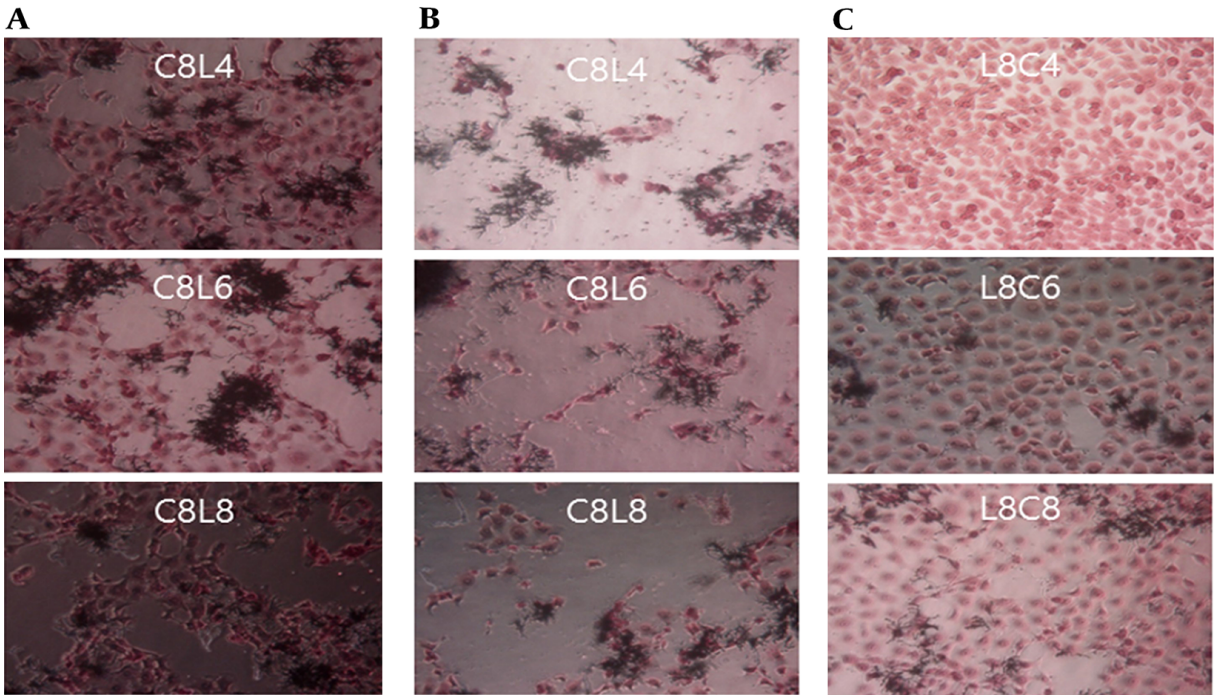

Figure 5. Antagonistic effects of *Lactobacillus plantarum* on *Candida albicans* in ME-180 cell cultures, depending on the order of inoculation. A. Simultaneous inoculation. B. Inoculation with *C. albicans* first. C. Inoculation with *L. plantarum* first. C, *C. albicans*; L, *L. plantarum*. The numbers indicate 10^4, 10^6, and 10^8 CFU/mL, respectively.

albicans directly by inhibiting its hydrogen peroxide, bacteriocin, lactic acid, and low pH. Rather, it may have inhibited biofilm development at an early stage, which subsequently inhibited the growth and mucosal adhesion ability of *C. albicans* in the ME-180 cell culture. Further studies should be conducted on the safety, side effects, and metabolism of *L. plantarum*, and it should be confirmed to be non-infectious in animals before the *L. plantarum* isolate derived from kimchi can be used as an auxiliary treatment agent for vaginitis.

Footnotes

Authors' Contribution: Study concept and design and Analysis and interpretation of data: YL, YY, and GK. Acquisition of data, drafting of the manuscript, and statistical analysis: YL, and YY. Critical revision of the manuscript for important intellectual content: YL and GK. Administrative, technical, and material support, and study supervision: GK.

Conflict of Interests: The authors declare that there is no conflict of interest regarding the publication of this article.

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