Antibacterial and Antifungal Activity of Lactobacillus Plantarum Isolated from Green Tea

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Abstract

**Background:** Balanced skin microflora is crucial for maintaining healthy normal skin function; however, changes in skin microflora are associated with skin diseases such as acne vulgaris, dandruff, and candidiasis. *Lactobacilli* are probiotics that possess antibacterial activity against pathogenic bacteria. In the present study, we assessed the potential antagonistic activity of *Lactobacillus plantarum* APsulloc 331261 and 331266, which were isolated from green tea (*Camellia sinensis*), in inhibiting the growth of bacteria (*Cutibacterium acnes*) and fungi (*Candida albicans, Malassezia globosa, and Malassezia restricta*) related to skin diseases.

**Results:** The antibiotic susceptibility of *C. acnes, C. albicans, M. globosa, and M. restricta* was tested using agar overlay and co-culture transwell methods to determine the antibacterial and antifungal activity of *L. plantarum* APsulloc 331261 and 331266. Results of the agar overlay method revealed that the two *L. plantarum* strains, APsulloc 331261 and 331266, inhibited the growth of *C. acnes* and *C. albicans*, respectively (zone diameters of inhibition: 20.0 ± 2.0 to 27.0 ± 3.6 mm; R values: 4.3 ± 1.8 to 5.5 ± 1.7). Moreover, the conditioned media (cell-free culture supernatants) derived from *L. plantarum* APsulloc 331261 and 331266 inhibited the growth of *C. acnes, C. albicans, M. globosa, and M. restricta*.

**Conclusions:** These results suggest that *L. plantarum* APsulloc 331261 and 331266 could be used as candidate probiotics to control the balance of skin microflora.

**Background**

Probiotics are gaining immense attention in scientific research and are widely consumed worldwide due to their beneficial effects on health [1, 2]. Lactic acid bacteria (LAB) produce lactic acid as a major product through carbohydrate fermentation, and *Lactobacillus* forms the major part of this group, as it is abundantly present in the intestines of vertebrates [3]. *Lactobacillus plantarum* is considered one of the most widely used probiotics in the food and pharmaceutical industries [4]. It undergoes unique metabolic processes to produce numerous vitamins and host immunomodulatory substances [5, 6]. *L. plantarum* produces various antibacterial compounds such as hydrogen peroxide, acids, and bacteriocin [7]. Bacteriocins are peptides that inhibit or kill the bacterial strains; however, in general, they are harmless to the human body and surrounding environment [8, 9]. Bacteriocins are less toxic than chemically synthesised antibiotics because they are resistant to proteases in the gastrointestinal system [10, 11].

The skin possesses diverse flora, which act as a defence mechanism against pathogenic invasions by producing protein complex antibiotics [12, 13]. Disruptions in the balance between the host and microorganism can lead to skin diseases or infections [13]. *Cutibacterium acnes*, a gram-positive and aerotolerant bacterium, is the primary cause of acne [14]. The onset of acne occurs in the sebaceous gland of the hair follicle, and various factors contribute to the secretion of the sebaceous glands and cause abnormal keratinisation in the hair follicles, resulting in clogging of the hair follicles and acne symptoms. Furthermore, it triggers inflammation with redness and oedema, further aggravating acne
symptoms [15, 16]. *Malassezia*, the main cause of dandruff, is a normal skin flora found in approximately 75–98% of healthy adults [17]. In Korea, *Malassezia restricta* and *Malassezia globosa* are commonly linked to dandruff [18]. Although *Malassezia* does not produce its own essential fatty acids for survival, it comprises eight lipases with three types of phospholipases[19]. The sebum produced in the scalp is decomposed by lipase to create oleic acid. Oleic acid triggers dandruff by rapidly dividing the skin cells in humans who are more sensitive than normal individuals, after passing the outer layers of the skin[20]. In candidiasis, *Candida* infects the skin, fingernails, mucous membranes, and other parts. *Candida* is a commensal fungus normally present in humans; however, when the immune system of the body is compromised or the epidermis is damaged by trauma, it causes infections [21–23]. *Candida albicans* causes 70–80% of all *Candida* infections, and tinea pedis is a candidiasis that occurs on the fingertips [24]. *C. albicans* is also associated with dandruff [25].

To treat skin diseases occurring due to imbalanced skin microbiome, antibiotics and antifungals are usually administered either orally or topically to suppress the bacterial and fungal growth; however, these may have adverse effects, such as dose-dependent toxicity in patients, and prolonged use may lead to the emergence of resistant pathogenic strains [17, 26, 27]. Colonisation of multi-drug-resistant bacteria transfers the antibiotic resistance genes to commensals or potential pathogens, thereby delaying the effect of antibiotics due to persistence of the resistance genes in the microbiota [28]. Topical or oral application of probiotics, such as *Lactobacillus*, could improve skin health by reducing skin colonisation or inflammation [29–31]; therefore, it is necessary to develop novel strains of probiotics with antimicrobial properties that are safe and sustainable.

*L. plantarum* strains APsulloc 331261 and 331266 isolated from green tea (*Camellia sinensis*) have been sequenced and annotated using whole-genome sequence analysis, and their safety and benefits have also been described [32]. *L. plantarum* APsulloc 331261 alleviated gastric inflammation in an alcohol-induced gastric ulcer murine model [33]. Moreover, it markedly increased the abundance of various intestinal beneficial bacteria such as *Bifidobacterium* spp. and *Clostridium butyricum* [33]. Furthermore, *L. plantarum* APsulloc 331261-derived extracellular vesicles exerted anti-inflammatory effects on human skin by inducing macrophage polarisation [34].

In the present study, we examined the antibacterial and antifungal activity of *L. plantarum* APsulloc 331261 and 331266 against four strains (*M. globosa*, *M. restricta*, *C. albicans*, and *C. acnes*). Bacteria or fungi were incubated with *L. plantarum* APsulloc 331261 and 331266 using a plate agar overlay assay. We further validated the growth inhibition effects of *L. plantarum* APsulloc 331261 and 331266 against bacteria and fungi using a co-culture transwell assay.

**Results**

**L. plantarum** APsulloc 331261 and 331266 inhibit the growth of *Cutibacterium acnes* and *Candida albicans***
The results of the agar overlay assay used to determine bacterial and fungal growth inhibition are summarised in Tables 2 and 3. *L. plantarum* strains APSulloc 331261 and 331266 significantly inhibited the growth of *C. acnes* or *C. albicans*. After 24 h of incubation, *L. plantarum–C. acnes* and *C. albicans* overlays revealed markedly strong zones of bacterial and fungal inhibition, respectively, around the *L. plantarum* APSulloc 331261 and 331266 colonies (Fig. 1). *L. plantarum* APSulloc 331261 inhibited the growth of *C. acnes* and *C. albicans* with ZDIs of 22.0 ± 1.7 and 23.0 ± 1.0 mm, respectively. In addition, *C. acnes* and *C. albicans* exhibited high sensitivity to *L. plantarum* APSulloc 331266 (ZDIs of 20.0 ± 2.0 and 27.0 ± 3.6 mm, respectively; Table 2). Furthermore, the ‘R’ value revealed the antibacterial and antifungal activity of *L. plantarum* APSulloc 331261 and 331266. The action of *L. plantarum* APSulloc 331261 presented ‘R’ values of 5.5 ± 1.3 and 23.0 ± 1.0 mm, whereas *L. plantarum* APSulloc 331266 had ‘R’ values of 4.3 ± 1.8 and 5.5 ± 1.7 mm against *C. acnes* and *C. albicans*, respectively (Table 3). No significant differences in growth inhibition were found between *L. plantarum* APSulloc 331261 and 331266.

For a more detailed evaluation regarding the growth inhibition of *C. acnes* and *C. albicans* in a co-culture system with *L. plantarum*, we conducted growth assays using a transwell chamber with a 0.4-µm porous membrane. These experiments revealed that *C. acnes* and *C. albicans* growth decreased in the presence of *L. plantarum* APSulloc 331261 and 331266 (Fig. 2A and B). APSulloc 331261 reduced the growth of *C. acnes* and *C. albicans* by 61.2% and 77.7%, respectively, compared to the control. APSulloc 331266 decreased the growth of *C. acnes* and *C. albicans* by 70.2% and 70.1%, respectively, compared to the control. Despite the physical separation of the microorganisms, bacterial and fungal growth retardation may be caused by nutrient competition and diffusible inhibitors (e.g., lactic acid and bacteriocin) produced by *L. plantarum* APSulloc 331261 and 331266. Moreover, the growth inhibition assays using conditioned media (CM) derived from *L. plantarum* APSulloc 331261 and 331266 revealed that the growth of *C. acnes* and *C. albicans* was suppressed by *L. plantarum* culture supernatants in a dose-dependent manner (Fig. 2C and D). *L. plantarum* APSulloc 331261 and 331266 completely inhibited the growth of *C. acnes* in 100%-CM, while the two strains caused 35.4% and 40.0% growth inhibition in 50%-CM, respectively, compared to the control. Furthermore, *L. plantarum* APSulloc 331261 and 331266 significantly inhibited the growth of *C. albicans* by 68.1% and 75.8% in 100%-CM and 37.3% and 61.9% in 50%-CM, respectively, compared to the control.

**L. plantarum** APSulloc 331261 and 331266 inhibit the growth of *Malassezia globosa* and *Malassezia restricta*

The growth of dandruff-producing fungi *M. globosa* and *M. restricta* was observed in the absence or presence of *L. plantarum* APSulloc 331261 and 331266. Interestingly, the marked antifungal activity of *L. plantarum* APSulloc 331261 and 331266 was detected in a transwell co-cultured with fungus. A significant decrease in the growth of *M. globosa* and *M. restricta* was observed (Fig. 3A and B). APSulloc 331261 reduced the growth of *M. globosa* and *M. restricta* by 71.1% and 72.3%, respectively, compared to the control. APSulloc 331266 decreased the growth of *M. globosa* and *M. restricta* by 77.6% and 75.9%, respectively, compared to the control. Moreover, CM derived from *L. plantarum* APSulloc 331261 and
331266 showed antifungal activity against *M. globosa* and *M. restricta* in a dose-dependent manner (Fig. 3C and D). *L. plantarum* APsulloc 331261 and 331266 significantly inhibited the growth of *M. globosa* by 90.9% and 93.7% in 100%-CM and 80.5% and 87.5% in 50%-CM, respectively, compared to the control. Moreover, *L. plantarum* APsulloc 331261 and 331266 significantly inhibited the growth of *M. restricta* by 96.9% and 94.9% in 100%-CM and 89.3% and 89.7% in 50%-CM, respectively, compared to the control.

In general, these viability assay results provide strong evidence that *L. plantarum* strains APsulloc 331261 and 331266 can exert antibacterial and antifungal effects.

**Discussion**

Probiotics have recently become a popular microbiome therapy, and researchers are interested in exploring the mechanisms through which probiotic microorganisms interact with the body [35]. In addition to the commercially available probiotic strains, LAB from various natural sources are being isolated and screened to discover better probiotic strains [36]. In the present study, we examined the antibacterial (*C. acnes*) and antifungal (*C. albicans*, *M. globosa*, and *M. restricta*) activity of *L. plantarum* strains APsulloc 331261 and 331266 isolated from green tea, which have been previously described [32].

Probiotic lactobacilli can be considered important alternatives to antibiotics because their various mechanisms of antagonism against pathogenic bacteria have been widely studied [37–39]. Hydrogen peroxides, bacteriocins, and short-chain fatty acids, such as acetic, propionic, and butyric acids, are antimicrobials produced by probiotic lactobacilli, and they change the redox potential of the intestinal environment and restrict the supply of vital nutrients to the pathogens [40]. The skin comprises diverse microorganisms, and the sebaceous glands, which are densely found across the face, scalp, and chest, promote the growth of lipophilic microorganisms, such as *C. acnes*, *C. albicans*, *M. globosa*, and *M. restricta*, by producing sebum [41]. In the present study, *L. plantarum* APsulloc 331261 and 331266 exhibited excellent antibacterial and antifungal activity in both the transwell co-culture (inhibition rate: *C. acnes*, 61.2% and 70.7%; *C. albicans*, 77.7% and 70.1%; *M. globosa*, 71.1% and 77.6%; *M. restricta*, 72.3 and 75.9%, respectively) and agar overlay assays (ZDI: *C. acnes*, 22.0 ± 1.7 and 20.0 ± 2.0 mm; *C. albicans*, 23.0 ± 1.0 and 27.0 ± 3.6 mm, respectively); the ‘R’ values for *C. acnes* were 5.5 ± 1.3 and 4.3 ± 1.8 mm, while for *C. albicans*, the ‘R’ values were 4.7 ± 0.3 and 5.5 ± 1.7 mm, respectively. Manzoor et al. reported that *L. plantarum*, *L. salivarius*, and *L. fermentum* isolated from fermented fruits and vegetables had an excellent antibacterial spectrum (ZDI: 26–28 mm) against pathogenic bacteria [42]. Moreover, Mashak reported that *L. plantarum* isolated from Kashk-e Zard and Tarkhineh exhibited antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella enterica serovar Typhimurium* with ZDIs of 21–30, 20–26, 19–26, and 20–24 mm, respectively [43]. According to Halder et al. [44], the four Lactobacillus species (*L. animalis*, *L. plantarum*, *L. acidophilus*, and *L. rhamnosus*) exhibited excellent antibacterial activity, with the highest activity shown by *L. plantarum*, and all ZDI and R values indicated high inhibitory capacity.
Probiotics and prebiotics have been widely evaluated for the treatment and prevention of infectious diseases due to their presence in the microbiome of several parts of the human body. In particular, studies in the field of skin diseases, such as acne vulgaris, tinea pedis, dandruff, and candidiasis, have revealed the effect of probiotics following topical application or ingestion [45]. Probiotics or prebiotic cosmetics have been recently developed, which rebalance the composition of skin microflora by inhibiting the growth of harmful pathogens while promoting the growth of beneficial bacteria among the resident microorganisms [46]. Kang et al. demonstrated the therapeutic effect of a lotion prepared as a concentrated powder (CBT SL-5) from Enterococcus faecalis SL-5-conditioned media on C. acnes-infected patients [47]. Muizzuddin et al. reported that L. plantarum extract (1% and 5%) reduced the size of acne lesions in clinical studies [30]. Jung et al. demonstrated that probiotic capsules (L. acidophilus, L. bulgaricus, and Bifidobacterium bifidum) significantly improved acne vulgaris by exerting a synergistic anti-inflammatory effect with systemic antibiotics and reduced the potential side effects of chronic antibiotic use [48]. Recently, L. plantarum IS-10506 isolated from dadih reduced clinical symptoms in children with mild and moderate atopic dermatitis [49]. De Seta et al. reported that L. plantarum P17630 prevented Candida vaginitis infection recurrence in patients with vulvovaginal candidiasis [50]. Reygagne et al. suggested the beneficial effects of Lactobacillus paracasei NCC2461 ST11 on patients with moderate to severe dandruff, wherein the clinical efficacy (free and adherent dandruff, erythema, and global clinical score) improved significantly and decreased the number of M. restricta and M. globosa in the scalp microbiota [51].

Although the results from in vitro studies should be drawn carefully, our findings further support the hypothesis that probiotic L. plantarum strains APsulloc 331261 and 331266 may ameliorate homoeostasis and microbial profile in the skin.

Conclusion

The present in vitro study revealed that L. plantarum strains APsulloc 331261 and 331266 isolated from green tea could inhibit the growth of bacteria (C. acnes) and fungi (C. albicans, M. globosa, and M. restricta). The antibacterial and antifungal activity of L. plantarum APsulloc 331261 and 331266 was tested using two different in vitro methods. Future studies are warranted to investigate the effectiveness of L. plantarum APsulloc 331261 and 331266 as alternative therapeutics for eliminating antibiotic resistance and treating infection.

Methods

Fungal and Bacterial Strains and Culture Conditions

L. plantarum APsulloc 331261 and 331266 isolated from green tea (Osulloc farm, Jeju island, South Korea) were provided by AMOREPACIFIC (Yongin, Korea, KCCM11179P and KCCM11180P), and their safety and probiotic properties have been previously described [32]. L. plantarum APsulloc 331261 and 331266 were cultured at 37 °C for 24 h in MRS broth (Becton, Dickinson and Company; Sparks, MD, USA).
The fungal and bacterial strains used in this study were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and are listed in Table 1. *C. albicans* was cultured in yeast malt broth (Becton, Dickinson and Company) at 37 °C, on a 200 rpm shaking incubator for 24 h. *M. globosa* and *M. restricta* were cultured in modified Leeming & Notman agar (MLNA; ATCC medium 2737) without agar at 30 °C, on a 200 rpm shaking incubator for 24 h. *C. acnes* was cultured in modified reinforced *Clostridium* medium (ATCC medium 2107) under anaerobic conditions at 37 °C for 72 h. All fungal and bacterial strains were adjusted to an OD$_{600nm}$ of 0.5 before use.

### Growth inhibition using plate agar overlay assay

The effects of the presence of *L. plantarum* APsulloc 331261 and 331266 on the growth of *C. acnes* and *C. albicans* were assessed on agar plates as previously described [52]. For the plate agar overlay assays, *L. plantarum* APsulloc 331261 and 331266 (4 µL of OD$_{600nm}$ 0.5) were spotted on 1.5% MRS agar plates. After incubation for 2 days at 37 °C, the plates were overlaid with *C. acnes* and *C. albicans* suspension (OD$_{600nm}$ 0.5) in 0.7% MRS soft agar that was prepared after cooling to 40 °C in an autoclaved agar medium. Following solidification, the plates were incubated at 37 °C for 1 day in aerobic (*C. albicans*) or anaerobic (*C. acnes*) conditions. The zone diameter of inhibition (ZDI) value was determined, following the method reported by Shokryazdan et al. [53] (ZDI <10 mm, weak inhibition; ZDI 10–20 mm, intermediate inhibition; ZDI >20 mm, strong inhibition). Moreover, ‘R’ (width of clear zone) values were measured and interpreted, following the method devised by Kohler et al. [54] as follows: $R = \frac{d_{\text{Inhibition}}}{d_{\text{Spot}}}$ (where ‘d$_{\text{Inhibition}}$’ is the diameter of clear zone around ‘d$_{\text{Spot}}$’ and ‘d$_{\text{Spot}}$’ refers to the diameter of the *Lactobacillus* spot grown over the MRS agar plate; R < 2 mm, no inhibition; R = 2–5 mm, low inhibition; R > 6 mm, high inhibition).

### Bacterial and fungal viability assays

The fungal and bacterial viability during co-culture with *L. plantarum* APsulloc 331261 and 331266 was examined using 6-well transwell chamber with a 0.4-µm porous membrane (Co-star, Corning, NY, USA). *C. acnes* and *C. albicans* (0.2% of OD$_{600nm}$ 0.5 with MRS in the well) were incubated in the presence or absence of *L. plantarum* APsulloc 331261 and 331266 (0.2% of OD$_{600nm}$ 0.5 with MRS) in the inserts of transwells or *L. plantarum* APsulloc 331261- or APsulloc 331266-CM (100%, 75%, 50%, and 25% diluted with MRS) at 37 °C for 24 h. *M. globosa* and *M. restricta* (0.2% of OD$_{600nm}$ 0.5 with MLNA in the well) were incubated in the presence or absence of *L. plantarum* APsulloc 331261 and 331266 (0.2% of OD$_{600nm}$ 0.5 with MRS) in the inserts of transwells or *L. plantarum* APsulloc 331261- and APsulloc 331266-CM (100%, 75%, 50%, and 25% diluted with MRS) at 37 °C for 24 h. After 24 h of incubation, the viability of *C. acnes*, *C. albicans*, *M. globosa*, and *M. restricta* was measured at OD$_{600nm}$ using a microplate reader (SpectraMax i3x; Molecular Devices, San Jose, CA, USA).
Statistical analysis

Data are presented as the mean ± standard deviation (SD). The control and test groups were compared using a paired \( t \)-test. Multiple comparisons were evaluated using one-way analysis of variance, followed by post-hoc \( t \)-tests. \( P \)-values < 0.05 were considered statistically significant.

Abbreviations

ATCC: American Type Culture Collection

CM: Conditioned media

LAB: Lactic acid bacteria

MLNA: Modified Leeming & Notman agar

SD: Standard deviation

ZDI: Zone diameter of inhibition

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request. All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

B. J. K. and J.N. designed the research; J.N. and S. Y. K. conducted the research; B. J. K., J.N., S. Y. K., J. O. L., Y. J. K., E.L., M.C. and M.P. analysed data; S. Y. K., J. O. L, Y. J. K., and E.L. prepared the figures; B. J. K. and J.N. wrote the paper. B.J.K. had primary responsibility for the final content. All authors have read and approved the final manuscript.

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Tables
Table 1
Fungal and bacterial strains used in the present study

| Strain                  | ATCC no.      |
|-------------------------|---------------|
| *Candida albicans*      | ATCC 90028    |
| *Cutibacterium acnes*   | ATCC 6919     |
| *Malassezia globosa*    | MYA-4612      |
| *Malassezia restricta*  | MYA-4611      |

Table 2
Antibacterial and antifungal activity of the isolated *Lactobacillus plantarum* APsulloc 331261 and 331266, in terms of ZDI, following the agar overlay method

| *L. plantarum* isolates | ZDI (mm) ± SD |
|-------------------------|---------------|
|                         | *C. acnes*    | *C. albicans* |
| APsulloc 331261         | 22.0 ± 1.7    | 23.0 ± 1.0    |
| APsulloc 331266         | 20.0 ± 2.0    | 27.0 ± 3.6    |

Table 3
Antibacterial and antifungal activity of the isolated *Lactobacillus plantarum* APsulloc 331261 and 331266, in terms of 'R' values, following the agar overlay method

| *L. plantarum* isolates | R value (mm) ± SD |
|-------------------------|------------------|
|                         | *C. acnes* | *C. albicans* |
| APsulloc 331261         | 5.5 ± 1.3 | 4.7 ± 0.3    |
| APsulloc 331266         | 4.3 ± 1.8 | 5.5 ± 1.7    |

Figures
Agar overlay assay demonstrates the antibacterial and antifungal activity of the isolated Lactobacillus plantarum APsulloc 331261 and 331266 against Cutibacterium acnes and Candida albicans. The zone diameter of inhibition (ZDI) and 'R' values were observed around L. plantarum APsulloc 331261 and 331266 strains (grown on the plates as spot forms) against C. acnes and C. albicans.
Figure 2

Inhibition of Cutibacterium acnes and Candida albicans growth by co-culture with L. plantarum APsulloc 331261 and 331266. Transwell chamber with a 0.4-μm porous membrane was used to determine the antibacterial and antifungal effects of APsulloc 331261 and 331266 on Cu. acnes (A) and C. albicans (B). Conditioned media from APsulloc 331261 and 331266 were collected after 24 h of growth and diluted with OD600nm of 0.5 using MRS medium. Various concentrations of the conditioned media were added to the inserts of transwells, following which Cu. acnes (C) and C. albicans (D) were inoculated. After incubation at 37 ° for 24 h, the cell densities were determined at OD600nm. All data represent the mean ± SD. W** P < 0.01 vs. MRS.
Figure 3

Inhibition of Malassezia globosa and Malassezia restricta growth by co-culture with Lactobacillus plantarum APsulloc 331261 and 331266. Transwell chamber with a 0.4-μm porous membrane was used to determine the antibacterial and antifungal effects of APsulloc 331261 and 331266 on M. globosa (A) and M. restricta (B). Conditioned media from APsulloc 331261 and 331266 were collected after 24 h of growth and diluted with OD600nm of 0.5 using MRS medium. Various concentrations of the conditioned media were added to the transwell inserts, following which M. globosa (C) and M. restricta (D) were inoculated. After incubation at 37 °C for 24 h, the cell densities were determined at OD600nm. All data represent the mean ± SD. * P < 0.05 and ** P < 0.01 vs. MRS.