Journal of Dental Sciences 19 (2024) 2163–2171

Available online at www.sciencedirect.com

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Original Article

Aggregation and adhesion ability of various probiotic strains and *Candida* species: An *in vitro* study

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Received 8 March 2024; Final revision received 13 March 2024
Available online 22 March 2024

KEYWORDS
Adhesion;
Aggregation;
Candida;
Postbiotics;
Probiotics

Abstract *Background/purpose:* The ability of probiotics to inhibit *Candida* adhesion is a crucial characteristic that prevents *Candida* colonization and infection progression. This study aimed to explore aggregation, adhesion, and cell surface characterization of probiotic and *Candida* strains and to evaluate the effect of probiotics and their cell-free supernatants (CFSs) as postbiotics on *Candida* adhesion to human oral keratinocytes.
Materials and methods: Eight probiotic strains and five reference *Candida* strains were tested for autoaggregation, coaggregation, adhesin on human oral keratinocytes (H357), and cell surface properties. The anti-*Candida* adhesion activities of probiotic strains and CFSs were investigated.
Results: The results showed that most probiotics exhibited high adhesion to H357 cells, specifically oral probiotic *Lacticaseibacillus rhamnosus* SD4, *Limosilactobacillus fermentum* SD7, and *L. rhamnosus* SD11, and adhesion ability of probiotic strains was strongly related to their autoaggregation, cell surface charges, and hydrophobicity. *Candida* strains also revealed a high level of adhesion to H357 cells. *Candida albicans* and *Candida glabrata* showed significantly higher adhesion abilities than others. After a combination of *Candida* with probiotics or their CFSs, *Candida* adhesion was significantly reduced. The anti-*Candida* adhesion property of probiotics was strongly related to their autoaggregation, coaggregation, and adhesion abilities.

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https://doi.org/10.1016/j.jds.2024.03.016

Conclusion: This study demonstrated that oral probiotic strains may be useful probiotics for preventing and treating oral candidiasis due to their high ability of aggregation, adhesion, and anti-*Candida* adhesion to H357 cells.

Introduction

Probiotics have received attention for preventing and treating *Candida* infection due to an increase in antifungal drug resistance and toxicity. Probiotic *Lactobacillus* strains have been reported *in vitro* and *in vivo* to be beneficial for candidiasis management, including oral candidiasis.[1,2]

Oral candidiasis is an opportunistic fungal infection of the oral cavity caused by *Candida* species. Although *Candida albicans* is the predominant species in oral candidiasis patients, a number of non-*albicans Candida* (NAC) species including *Candida glabrata* (up to 20.5%), *Candida tropicalis* (up to 12.9%), *Candida dubliniensis* (up to 10.9%), and *Candida krusei* (up to 3.4%) have also been isolated.[3–5]

The anti-adhesion property of pathogens, which involves adhesion and aggregation abilities, is a desirable factor for probiotics.[6] Autoaggregation is the gathering of the same microorganism strains, which can create a barrier to prevent pathogen adhesion. Coaggregation is the gathering of different microorganism strains, which can inhibit the dissemination of the pathogen to adhesion sites on host mucosal tissues.[7] Previous studies have reported that the coaggregation ability of probiotic *Lactobacillus* strains with *Candida* species varied depending on the strain, however, most studies have only examined intestinal and vaginal probiotic *Lactobacillus* strains.[7–9] There have been very few studies relating to oral probiotic strains.[10] Regarding anti-*Candida* adhesion, several previous studies have also shown that probiotic *Lactobacillus* can inhibit the *C. albicans* adherence on the vaginal and intestinal mucosal surfaces.[11–14] A few studies have examined the anti-adhesion of *C. albicans* on oral epithelial cells.[15]

Besides the beneficial effects of probiotic strains, the secreted metabolic products from probiotic strains, known as postbiotics, have also been recently proposed as beneficial effects. It has been suggested that using postbiotics may be an alternative therapeutic strategy without the complications associated with administering live probiotic bacteria.[16] Previous studies reported that postbiotics could reduce the adhesion of *C. albicans* on vaginal epithelial cells by *Lactobacillus* CFSs, and biosurfactants.[13,14,17,18] However, research on the effect of probiotics and their postbiotics on *Candida* adherence to oral epithelial cells is limited.

Probiotic *Lactobacillus* strains are derived from various sources including dairy products and human-origins such as breast milk, feces, the gut, vagina, and the oral cavity. Commercial probiotics using *L. rhamnosus* GG (originating from the feces of healthy adults) and *L. casei* Shirota (originating from cheese) strains have been extensively utilized over an extended period for health promotion purposes. *Lactobacillus paracasei* CNCM I-1572, commercially known as *L. casei* DG® and isolated from human feces, is a probiotic strain that can restore the balance of gut microbiota and reduce intestinal inflammation.[19] Similarly, *Lactobacillus reuteri* ATCC PTA 6475, isolated from human breast milk, shows potential in preserving the health of gut microbiota, reducing inflammation, and decreasing bone loss in older women with low bone mineral density.[20] Our previous studies have demonstrated that oral probiotic *Lactobacillus* strains, *L. paracasei* SD1, *L. rhamnosus* SD4, *L. fermentum* SD7, and *L. rhamnosus* SD11, exhibited the ability of coaggregation, adhesion, and anti-adhesion of pathogenic bacteria to human oral keratinocytes.[21,22] However, the impact of these commercially available probiotic strains and our probiotic strains on the anti-adhesion of the *Candida* species is currently limited.

This *in vitro* study aimed to explore the aggregation and adhesion abilities, and the cell surface characterization of probiotic and *Candida* strains, and to evaluate the effect of probiotics and postbiotics on the adhesion of *Candida* to H357 cells as a model for human oral keratinocytes.

Materials and methods

Microbial strains and culture conditions

Four strains of oral probiotics, *L. paracasei* SD1 (SD1), *L. rhamnosus* SD4 (SD4), *L. fermentum* SD7 (SD7), and *L. rhamnosus* SD11 (SD11) were derived from our collection. *L. casei* Shirota (LC; Yakult, Bangkok, Thailand) and *L. paracasei* CNCM I-1572 (LP; Sofar SpA., Milan, Italy) were isolated from commercial products and confirmed by matrix-assisted laser desorption ionization time-of flight (MALDI-TOF) mass spectrophotometry (Bruker, Billerica, MA, USA). *L. rhamnosus* ATCC 53103 (LGG) and *L. reuteri* ATCC PTA 6475 (LR) were used as reference strains. Five reference *Candida* strains, *C. albicans* ATCC 90028 (Ca), *C. dubliniensis* MYA 577 (Cd), *C. glabrata* ATCC 66032 (Cg), *C. krusei* ATCC 6258 (Ck), and *C. tropicalis* ATCC 13803 (Ct), were included in this study.

Probiotic strains were grown in de Man Rogosa Sharpe (MRS; Difco, Detroit, MI, USA) broth and *Candida* strains were cultured in Sabouraud dextrose broth (Difco). All strains were incubated at 37 °C for 24 h, harvested by centrifugation at 3000 rpm for 10 min, washed twice with phosphate-buffered saline (PBS), and adjusted to a 600nm optical density (OD) at 0.5 (approximately 10^7 CFU/mL for *Lactobacillus* and 10^6 CFU/mL for *Candida*).

Autoaggregation and coaggregation

The aggregation abilities of probiotic and *Candida* strains were determined using a spectrophotometric assay modified by Jørgensen et al.[10] For autoaggregation, 4 mL of probiotic or *Candida* suspension was vortexed for 10 s and

incubated at 37 °C for 1, 2, and 4 h without agitation. The absorbance of suspensions was measured at OD_{600nm} values and the autoaggregation percentage (%) was calculated as $[1 - (A_h/A_0)] \times 100$; where A_h represents suspension absorbance at different time points (1, 2, and 4 h) and A_0 represents initial absorbance. For coaggregation between probiotics and *Candida*, 2 mL of individual probiotic and *Candida* strains were mixed and incubated at 37 °C for 4 h without agitation. The percentage of coaggregation was expressed as $[1 - (A_{Mix}/((A_L + A_C)/2)] \times 100$; where A_L and A_C are the suspension absorbances of the *Lactobacillus* or *Candida* strain alone, and A_{Mix} is the absorbance of the mixed solutions.

Cell surface characterization

The cell surface charges and hydrophobicity of probiotic and *Candida* strains were examined using a modification of Silva-Diaz et al.[23] Three milliliters of the individual tested strains and 1 mL of each solvent (chloroform, ethyl acetate, and xylene) were mixed for 30 s and left for 30 min. Then the absorbance of the aqueous phase was measured at OD_{600nm}. The percentage of hydrophobicity or charged surfaces was calculated according to the formula: $(\%) = (A - A_0/A) \times 100$ (A and A_0 represent the absorbance before and after mixing, respectively) and categorized as low (0–35%), moderate (36–70%), or high (71–100%).

Adhesion ability

The human oral keratinocyte H357 cells preparation and adhesion experiment were performed according to Piwat et al.[21] For the adhesion experiment, 1 mL of individual probiotic or *Candida* suspension was added into an individual well containing confluent monolayers of H357 cells and incubated at 37 °C with 5% CO_2 for 90 min. The H357 monolayers were washed with PBS to remove non-adhered probiotic or fungal cells. The keratinocytes were trypsinized by 1 mL of 0.25% trypsin–EDTA. The total number of adherent *Candida* and probiotics on H357 cells were examined by serial dilutions, which were then cultured on Sabouraud dextrose agar (Difco) for fungal growth and MRS agar for probiotic growth. Adhesion ability was expressed as percentages using the formula: $(\%) = (A_0/A) \times 100$; where A and A_0 are log 10 numbers of microbial strains (CFU/mL) prior and post-adhesion.

Anti-*Candida* adhesion of probiotic *Lactobacillus* strains and their cell-free supernatants

Probiotic *Lactobacillus* CFSs preparation was a modified method of Rossoni et al.[24] One milliliter of each probiotic suspension ($OD_{600\ nm}$ of 0.5) was added to 6 mL of MRS broth and incubated at 37 °C for 48 h. The supernatants were collected by centrifugation at 4200 rpm for 10 min at 4 °C and adjusted to neutral pH (pH 7). All supernatants were filtered with a 0.45 μm pore size membrane (Sartorius AG, Göttingen, Germany). All neutral-CFSs were freshly prepared and validated by viable counts before each experiment.

The effects of probiotics and their neutral-CFSs on *Candida* adherence were tested as described previously with minor modifications.[22] An equal volume (0.5 mL) of probiotics or neutral-CFSs and *Candida* strains were added into an individual well containing confluent monolayers of H357 cells and incubated at 37 °C for 2 h. The ability of probiotic cells or neutral-CFSs to inhibit *Candida* adhesion was calculated as the percentage of reduction in the *Candida* adhesion compared with those that did adhere to H357 cells in the absence of the probiotic cells or neutral-CFSs.

Statistical analysis

All assays were performed as three independent experiments. The Kruskal–Wallis test was used to analyze the differences in aggregation, surface characterization, adhesion ability, and anti-*Candida* adhesion of probiotics. The Mann–Whitney *U* test was performed to compare the anti-*Candida* adhesion of probiotic strains and neutral-CFSs. The correlation coefficients between aggregation, adhesion, and the cell surface properties of probiotics were evaluated by Spearman's rho test. The data analysis was performed using IBM SPSS Statistics version 29.0.0.0 (241) (IBM, Armonk, NY, USA) and significant differences were considered as $P < 0.05$.

Results

Autoaggregation and coaggregation

The autoaggregation activity of all probiotic and *Candida* strains significantly increased over time, and a 4-h incubation period was used in this study. Generally, most *Candida* strains had a higher auto-aggregation ability than probiotics. At 4 h, *C. albicans* had the highest autoaggregation (98.65%), while *C. glabrata* had the lowest ability (65.65%). Among the probiotics, *L. fermentum* SD7 showed the highest autoaggregation (63.24%), whereas *L. casei* Shirota showed the lowest activity (22.45%) (Fig. 1a).

Coaggregation depended on the autoaggregation activity of individual probiotic and *Candida* strains. There was a significant positive correlation between autoaggregation and coaggregation ability ($r_s = 0.632$, $P < 0.001$). *L. fermentum* SD7 had the highest coaggregation activity with all *Candida* (56.28–71.11%), whereas the remaining strains showed moderate coaggregation to all *Candida* (40.58–65.03%) (Fig. 1b). These results were confirmed by microscopic appearances, and examples of coaggregation were observed with a light microscope as shown in Fig. 2.

Cell surface characterization

The percentages of probiotic and *Candida* affinities for chloroform, ethyl acetate, and xylene are demonstrated in Fig. 3a-c. There were great variations in cell surface charges and hydrophobicity in both probiotic and *Candida* strains. For probiotic strains, *L. fermentum* SD7 had the highest affinity for all solvents (chloroform 93.84%, ethyl

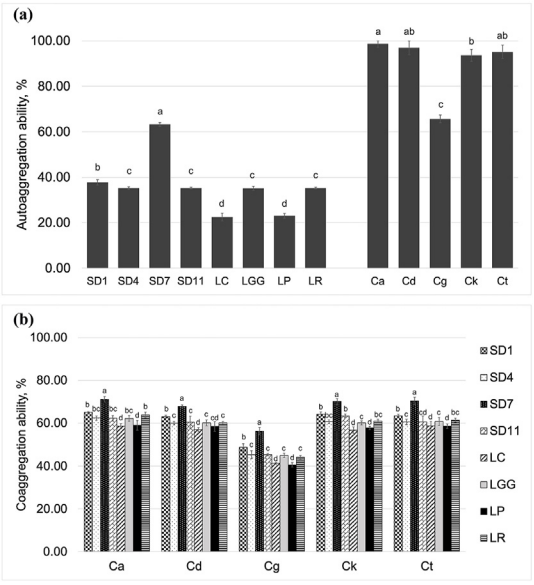

Figure 1 Autoaggregation (a) and coaggregation (b) of probiotic and *Candida* strains. The data were shown as median values and interquartile ranges. Different lowercase letters were statistically significant differences within probiotic or *Candida* strains ($P < 0.05$). *L. paracasei* SD1 (SD1); *L. rhamnosus* SD4 (SD4); *L. fermentum* SD7 (SD7); *L. rhamnosus* SD11 (SD11); *L. casei* Shirota (LC); *L. paracasei* CNCM I-1572 (LP); *L. rhamnosus* GG ATCC 53103 (LGG); *L. reuteri* ATCC PTA 6475 (LR); *C. albicans* ATCC 90028 (Ca); *C. dubliniensis* MYA 577 (Cd); *C. glabrata* ATCC 66032 (Cg); *C. krusei* ATCC 6258 (Ck); *C. tropicalis* ATCC 13803 (Ct).

acetate 75.29%, and xylene 83.47%), while the remaining strains had a high affinity for chloroform (71.46—86.32%) and a low affinity for ethyl acetate (14.85—30.50%), except *L. reuteri* ATCC PTA 6475 which had moderate ethyl acetate affinity (49.39%). The cell surface hydrophobicity of individual probiotic strains varied greatly, ranging from moderate to high (46.68—79.04%).

All *Candida* strains had a high affinity for chloroform (71.89—98.73%) and a moderate affinity for xylene (41.41—59.37%). *C. krusei* had the highest affinity for chloroform and xylene, whereas *C. albicans* had the lowest affinity for both solvents. Ethyl acetate affinity was moderate for *C. tropicalis* (42.05%), *C. dubliniensis* (38.50%), and *C. albicans* (38.47%), but low for the remaining strains (27.39—29.56%).

Adhesion ability

All probiotic and *Candida* strains were able to adhere to H357 cells as shown in Fig. 4. For probiotics, *L. rhamnosus* SD4, *L. fermentum* SD7, and *L. rhamnosus* SD11 showed high adhesion ability (83.44—84.08 %), followed by *L. reuteri* ATCC PTA 6475 (76.46%), *L. rhamnosus* GG (75.56%) and *L. paracasei* SD1 (75.06%), whereas *L. casei* Shirota and *L. paracasei* CNCM I-1572 showed moderate adhesion ability to H357 (67.30% and 68.14%, respectively). A positive correlation between adhesion or aggregation ability and cell surface characteristics of all probiotic strains was observed (Table 1).

All *Candida* strains showed a high adhesion ability to H357 cells (79.76—87.05%). *C. albicans* showed the highest adhesion ability (87.05%), but there was no statistically significant difference in the adhesion ability of *C. albicans* and *C. glabrata* (86.91%).

Anti-*Candida* adhesion of probiotic *Lactobacillus* cells and their cell-free supernatants

Both probiotic cells and their neutral-CFSs were able to reduce *Candida* adhesion on H357 cells depending on the probiotic and fungal strains tested. The reduction of *Candida* adhesion ranged from 28.65% to 70.62% by probiotic cells and 44.28%—74.55% by their neutral-CFSs (Fig. 5). Neutral-CFSs significantly decreased the adhesion of *Candida* more than probiotic cells ($P < 0.001$). The most potent anti-*Candida* adhesion properties of probiotic strains were observed for *L. fermentum* SD7 (48.02—70.62%) and *L. rhamnosus* SD4 (46.18—69.05%) (Fig. 5a), while those of neutral-CFSs were observed for *L. rhamnosus* SD4 (52.58—74.55%) and *L. paracasei* SD1 (50.00—73.15%) (Fig. 5b). *L. casei* Shirota and *L. paracasei* CNCM I-1572 as well as their neutral-CFSs reduced *Candida* adhesion to a lesser extent. Additionally, anti-*Candida* adhesion by probiotic *Lactobacillus* strains significantly correlated with the ability of *Lactobacillus* to autoaggregate, coaggregate, or adhere to H357 (Table 2).

For *Candida*, probiotic *Lactobacillus* cells were able to reduce the adhesion of *C. albicans* the most (43.48—70.62%), followed by *C. glabrata*, *C. krusei*, *C.*

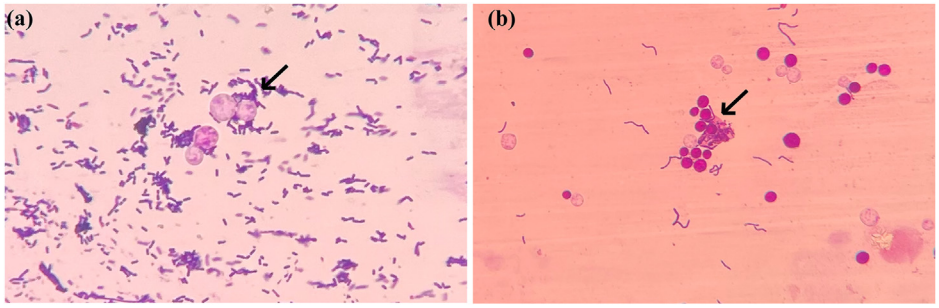

Figure 2 Coaggregation between *L. fermentum* SD7 and *C. albicans* (a) or *L. paracasei* SD1 and *C. albicans* (b) under a light microscope at 40x magnification.

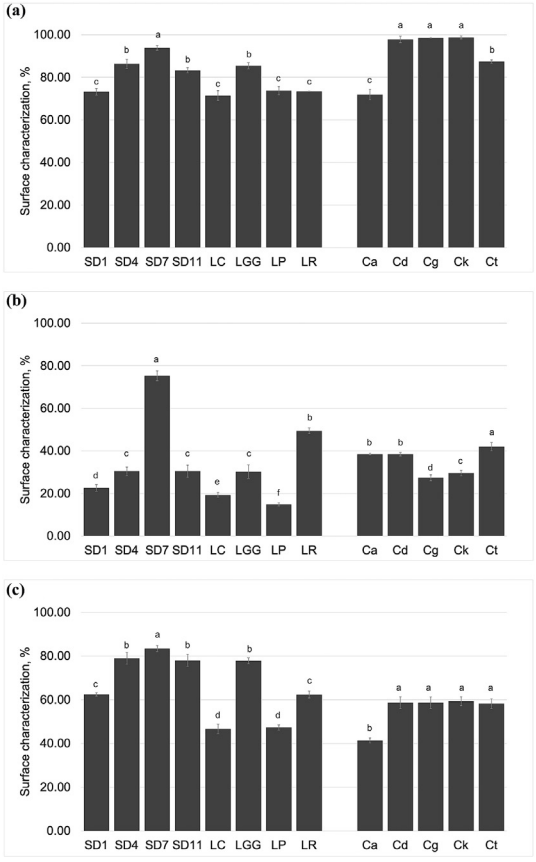

Figure 3 Cell surface characterization of probiotic and *Candida* by different solvents using chloroform (a), ethyl acetate (b), and xylene (c). The data were shown as median values and interquartile ranges. Different lowercase letters showed statistically significant differences within probiotic or *Candida* strains ($P < 0.05$). *L. paracasei* SD1 (SD1); *L. rhamnosus* SD4 (SD4); *L. fermentum* SD7 (SD7); *L. rhamnosus* SD11 (SD11); *L. casei* Shirota (LC); *L. paracasei* CNCM I-1572 (LP); *L. rhamnosus* GG ATCC 53103 (LGG); *L. reuteri* ATCC PTA 6475 (LR); *C. albicans* ATCC 90028 (Ca); *C. dubliniensis* MYA 577 (Cd); *C. glabrata* ATCC 66032 (Cg); *C. krusei* ATCC 6258 (Ck); *C. tropicalis* ATCC 13803 (Ct).

tropicalis, and *C. dubliniensis*, whereas neutral-CFSs were able to reduce the adhesion of *C. albicans* the most (68.34–74.55%), followed by *C. krusei*, *C. glabrata*, *C. tropicalis*, and *C. dubliniensis*.

Discussion

Probiotic *Lactobacillus* strains could inhibit *Candida* adhesion pathogenesis via aggregation activity and competition of *Candida* adhesion. Previous studies focused on the effect of probiotics on *C. albicans* adherence to intestinal or vaginal mucosa.

Aggregation ability is a desirable characteristic of probiotics in preventing pathogens from surface colonization. In this study, all tested strains had a different level of aggregation ability and there was a strong positive correlation between autoaggregation and coaggregation. *L. fermentum* SD7 had the highest autoaggregation and coaggregation with all *Candida* strains, while *L. casei* Shirota and *L. paracasei* CNCM I-1572 with the lowest autoaggregation showed significantly lower coaggregation activities. This result was similar to our prior study, which found that *L. fermentum* SD7 had the highest level of autoaggregation and coaggregation to various pathogenic bacteria.[22] Additionally, Collado et al.[25] examined the aggregation ability of various commercial lactic acid bacteria. Their findings revealed that *L. fermentum* ME-3 showed the highest autoaggregation and also higher coaggregation ability, whereas *L. casei* Shirota presented lower autoaggregation and coaggregation with all enteric pathogens. For *Candida*, most *Candida* strains exhibited high levels of autoaggregation and coaggregation except for *C. glabrata*, which demonstrated lower autoaggregation and coaggregation activity. This finding is in agreement with a previous study, which found that *C. glabrata* had the lowest degree of autoaggregation and coaggregation with yeast probiotic *Saccharomyces boulardii*, whereas *C. albicans and C. krusei* had the highest degree of aggregation activity.[26]

Adhesion not only aids *Lactobacillus* in protecting the mucosal epithelium but it is also an important virulence factor of pathogens. According to the results, all probiotic *Lactobacillus* and *Candida* strains had high adhesion abilities to human oral keratinocytes H357 cells. Regarding pathogen adhesion, *C. albicans* and *C. glabrata* both displayed the highest levels of adhesion to the H357 monolayer. Previous studies found no significant differences in the adherence ability between *C. albicans* and *C. glabrata* in human buccal epithelial cells and the vaginal epithelial cell line VK2/E6E7.[27,28] However, previous studies presented that *C. albicans* tend to be more adherent to buccal epithelial cells than NAC isolates.[29,30]

For *Lactobacillus* adhesion, our findings corroborated the results of our previous study, which discovered that *L. rhamnosus* SD4, *L. fermentum* SD7, and *L. rhamnosus* SD11 had the highest adhesion ability.[22] *L. casei* Shirota and *L. paracasei* CNCM I-1572 had the lowest adhesion ability. Earlier studies on the adhesion ability of various probiotic *Lactobacillus* strains reported that *L. casei* Shirota had lower adhesion ability on Caco-2 cells than other probiotics.[31,32] Moreover, Haukioja et al.[33] found that the adhesion of *L. casei* Shirota and *L. paracasei* 12.11a on human buccal epithelial cells was significantly lower than *L. rhamnosus* GG and *L. rhamnosus* 5.1a. Our results showed that *L. paracasei* SD1 exhibited a higher level of adhesion than *L. paracasei* CNCM I-1572. It is possible that *L. paracasei* SD1, originating from the human oral cavity, was better able to adhere to human oral mucosa than the non-oral origin *L. paracasei* CNCM I-1572 strain.[34,35] Furthermore, our results found that autoaggregation of all tested *Lactobacillus* strains positively correlated with adhesion ability ($r_s = 0.614$, $P < 0.001$) (data not shown).

A number of studies have shown that bacterial cell surfaces have a role in aggregation and adherence to epithelial cells.[21,22,36] Xylene, chloroform, and ethyl acetate solvents were used to assess the hydrophobicity, electron donor, and electron acceptor characteristics of the microbial cell surface, respectively. The results found that the

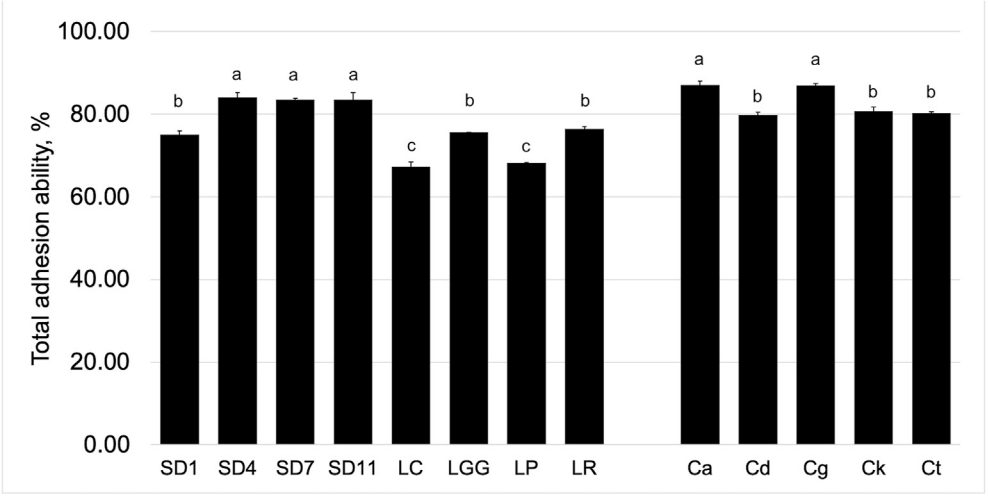

Figure 4 The total adhesion ability of probiotic and *Candida* strains on H357 cells. The data were shown as median values and interquartile ranges. Different lowercase letters showed statistically significant differences within probiotic or *Candida* strains ($P < 0.05$). *L. paracasei* SD1 (SD1); *L. rhamnosus* SD4 (SD4); *L. fermentum* SD7 (SD7); *L. rhamnosus* SD11 (SD11); *L. casei* Shirota (LC); *L. paracasei* CNCM I-1572 (LP); *L. rhamnosus* GG ATCC 53103 (LGG); *L. reuteri* ATCC PTA 6475 (LR); *C. albicans* ATCC 90028 (Ca); *C. dubliniensis* MYA 577 (Cd); *C. glabrata* ATCC 66032 (Cg); *C. krusei* ATCC 6258 (Ck); *C. tropicalis* ATCC 13803 (Ct).

Table 1 Correlation coefficient of Spearman (r_s) between cell surface charges and autoaggregation, coaggregation, or adhesion of all probiotic strains.

Assay	Adhesion to solvent		
	Chloroform	Ethyl acetate	Xylene
Autoaggregation	0.648 ($P < 0.001$)	0.545 ($P < 0.001$)	0.756 ($P < 0.001$)
Coaggregation	0.238 ($P < 0.001$)	0.336 ($P < 0.001$)	0.281 ($P < 0.001$)
Adhesion	0.794 ($P < 0.001$)	0.715 ($P < 0.001$)	0.891 ($P < 0.001$)

hydrophobicity of probiotics had a strong positive correlation to the autoaggregation and adhesion ability. Thus, most probiotic strains with higher adhesion to xylene (higher hydrophobicity) showed high autoaggregation and adhesion abilities, while *L. casei* Shirota and *L. paracasei* CNCM I-1572 with lower adhesion to xylene (lower hydrophobicity) showed low autoaggregation and adhesion abilities. Moreover, the results indicated that all tested probiotics and *Candida* strains had a higher affinity for chloroform, an acidic solvent, than for the ethyl acetate solvent, which implies that all tested probiotics and fungal yeast cells are strong electron donors (negative charge). This may be due to the existence of a peptidoglycan and glucan—chitin complex of bacterial and fungal cell walls, respectively.

Candida adhesion to H357 cells decreased after being combined with probiotic *Lactobacillus*. This reduction in *Candida* adhesion was associated with the aggregation and adhesion abilities of probiotics. Four oral probiotics (*L. paracasei* SD1, *L. rhamnosus* SD4, *L. fermentum* SD7, and *L. rhamnosus* SD11) and two commercial strains (*L. rhamnosus* GG and *L. reuteri* ATCC PTA 6475) with high aggregation and adhesion ability had high activity for anti-*Candida* adhesion, while *L. casei* Shirota and *L. paracasei* CNCM I-1572 with low aggregation and adhesion ability appeared to be low in activity for anti-*Candida* adhesion. This finding implies that the important mechanisms of probiotic *Lactobacillus* for protection against *Candida* colonization are aggregation ability and competition for adherence sites in the oral epithelium.

The effect of neutral-CFSs on anti-*Candida* adherence might be due to secreted exometabolites including hydrogen peroxide, proteins, and antimicrobial compounds. It is speculated that these exometabolites may affect *Candida* growth and surface energies and modulate the mucosal immune response to prevent cells from proliferating and adhesion.[11,37] Furthermore, probiotic *Lactobacillus* strains can secrete biosurfactants, which are amphiphilic surface-active compounds that can reduce surface and interfacial tension. Consequently, they can decrease the adhesion of pathogens including *Candida* strains.[18,38,39] In this study, *Lactobacillus* neutral-CFSs as postbiotics showed more antagonistic effects on *Candida* adherence than live probiotics. This characteristic is extremely interesting because products that use postbiotics instead of live probiotics are more stable, have fewer complications associated with administering live probiotics, and provide more benefits to their consumers.

Moreover, our results found that probiotic *Lactobacillus* and their CFSs were strongly effective against the adhesion of *C. albicans*, *C. glabrata*, and *C. krusei*. This is particularly significant given that (i) *C. albicans* is the most common cause of *Candida* infection, (ii) *C. glabrata* has the highest prevalence of NAC species isolated from oral candidiasis, and (iii) *C. krusei* is highly resistant to fluconazole associated with an increased incidence of invasive candidiasis, candidemia, and mortality.[4,40] Therefore, these probiotics and postbiotics may be useful for oral candidiasis management.

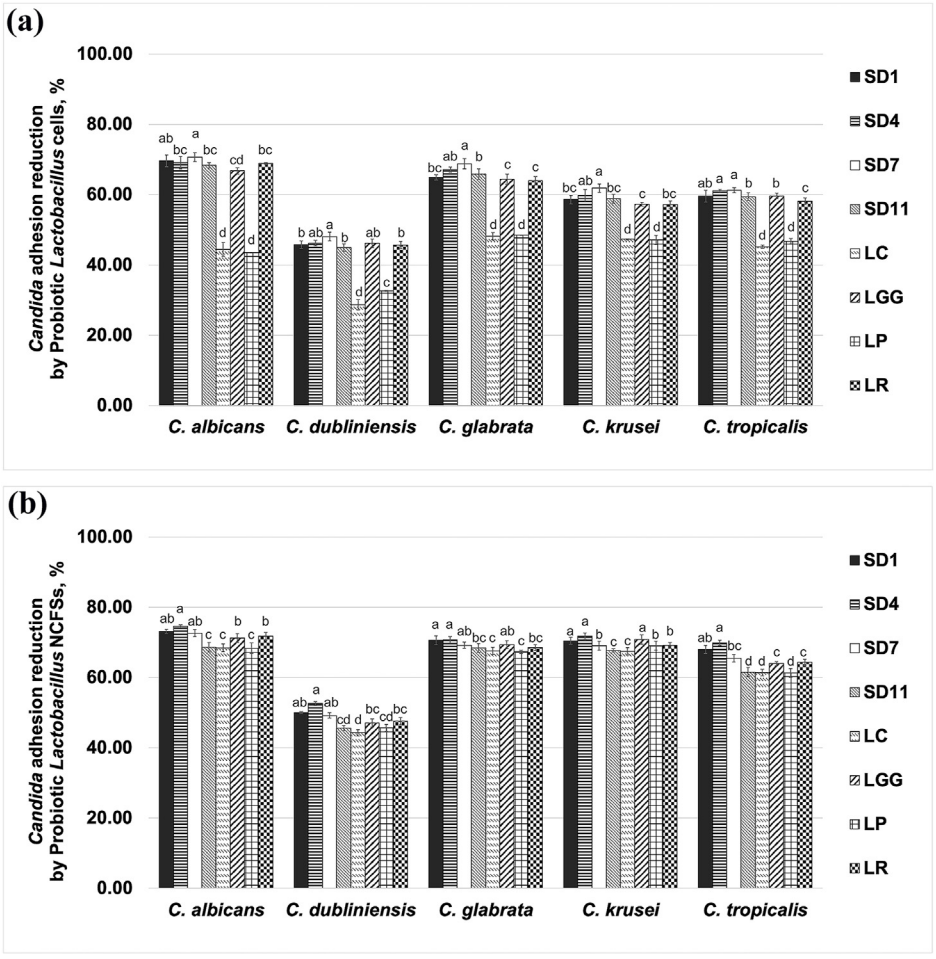

Figure 5 *Anti-Candida* adhesion to H357 cells by probiotic cells (a) and their neutral cell-free supernatants (NCFSs) (b). The data were shown as median values and interquartile ranges. Different lowercase letters showed statistically significant differences within *Candida* strains ($P < 0.05$). *L. paracasei* SD1 (SD1); *L. rhamnosus* SD4 (SD4); *L. fermentum* SD7 (SD7); *L. rhamnosus* SD11 (SD11); *L. casei* Shirota (LC); *L. paracasei* CNCM I-1572 (LP); *L. rhamnosus* GG ATCC 53103 (LGG); *L. reuteri* ATCC PTA 6475 (LR).

Table 2 Correlation coefficient of Spearman (r_s) between anti-*Candida* adhesion and autoaggregation, coaggregation, or adhesion ability of all probiotic strains.

Assay	Anti-*Candida* adhesion on H357 cells
Autoaggregation	0.572 ($P < 0.001$)
Coaggregation	0.251 ($P < 0.001$)
Adhesion	0.551 ($P < 0.001$)

The mechanisms of probiotic *Lactobacillus* strains and CFSs on *Candida* adhesion are still unclear. Further investigations are necessary for a thorough understanding of the mechanisms of adhesion inhibition such as identifying the effective composition of exometabolites or testing in appropriate animal models. Furthermore, it will be critical to investigate the impact of probiotics on the immune system at the oral mucosa in order to determine how these changes in immune responses on the susceptibility to oral candidiasis.

In conclusion, the current *in vitro* study demonstrated that four oral probiotic *Lactobacillus* strains, *L. paracasei* SD1, *L. rhamnosus* SD4, *L. fermentum* SD7, and *L. rhamnosus* SD11, showed a high level of aggregation and adhesion ability relating to cell surface properties compared to commercial probiotic strains (*L. rhamnosus* GG and *L. reuteri* ATCC PTA 6475). Our results indicated that oral probiotic *Lactobacillus* strains and their neutral-CFSs exhibited strong anti-*Candida* adhesion, and that they may be useful for probiotics preventing and treating oral candidiasis.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

This study was supported by a research grant for a graduate student's thesis from the Graduate School, Prince of Songkla University, Hat Yai, Songkhla, Thailand. The authors would like to acknowledge the Oral Microbiology Laboratory, Faculty of Dentistry, Prince of Songkla University for facilitating all equipment.

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