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Factors Influencing Aggregation and Adhesion of Oral *Lactobacillus gasseri*

Lata PLANSANGKET[1,2], Rawee TEANPAISAN[2,3] and Supatcharin PIWAT[1,2,*]

[1]*Department of Preventive Dentistry, Faculty of Dentistry, Prince of Songkla University, Songkhla 90112, Thailand*
[2]*Common Oral Diseases and Epidemiology Research Center, Faculty of Dentistry, Prince of Songkla University, Songkhla 90112, Thailand*
[3]*Department of Stomatology, Faculty of Dentistry, Prince of Songkla University, Songkhla 90112, Thailand*

([*]Corresponding author's e-mail: supacharin.p@psu.ac.th)

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Abstract

Lactobacillus gasseri is one of the *Lactobacillus* species, which has been considered as an oral probiotic. The probiotic characters, e.g., the aggregation and adhesion abilities, may be affected by the changes in the oral environment. This study aimed to investigate the effect of cell surface components, some enzymes, pH, sugars, and ions on the aggregation (auto- and coaggregation) abilities of oral *L. gasseri* and on the ability to adhere to oral epithelial cells. The control group contained the bacteria in pH 7.0 solution. The results indicated that the cell surface-related protein components were essential for the aggregation and internalized adhering abilities of *L. gasseri*. For environmental factors, amylase, calcium ion, and magnesium ion significantly increased both autoaggregation and coaggregation ability, while these abilities were significantly decreased by lactose. The coaggregation between *L. gasseri* and *S. mutans* ATCC 25175^{TM} was significantly increased by pH 4.0, whereas glucose, sucrose, trypsin, and lysozyme significantly decreased the coaggregation. The adhesion was significantly increased by pH 4.0, pH 8.0, calcium ion, and magnesium ion, whereas enzymes and sugars did not affect this ability. These traits could be used for the preliminary screening of the potential candidates of probiotics with possible anti-caries properties.

Keywords: Aggregation, Adhesion, *Lactobacillus gasseri*, *Strptococcus mutans*, Environmental factors

Introduction

Lactobacillus gasseri constitutes a major part of the homofermentative *Lactobacillus* species that are found in the oral cavity, gastrointestinal tract, and vagina [1]. *L. gasseri* has been identified from different sites in the oral cavity such as saliva, dental plaque, and periodontal tissue [2,3]. *L. gasseri* has been proposed as one of the predominant species commonly found in newborns and is one of the early colonizers in the gastrointestinal tract and existing throughout adulthood [4].

L. gasseri shows various health benefits through its antimicrobial activity, bacteriocin production, and immunomodulation of the innate and systemic immune responses [1]. Studies on the probiotic characteristics of *L. gasseri* suggested that the strain also exhibits antagonistic effects against gastrointestinal pathogens belonging to the genera *Clostridium*, *Cronobacter*, *Helicobacter*, and *Campylobacter* [5-7]. Moreover, *L. gasseri* has been reported to have protective effects against oral pathogens in *in vitro* and also in *in vivo* studies. Kobayashi *et al*. demonstrated that *L. gasseri* SBT2055 reduced alveolar bone loss, detachment, and disorganization of the periodontal ligament, and this strain was effective in preventing *Porphyromonas gingivalis*-accelerated periodontal disease [8]. Besides,

another study reported that *L.gasseri* inhibited the growth of *Streptococcus mutans*, *Streptococcus sobrinus, Actinomyces naeslundii, Actinomyces oris, Candida albicans,* and *Fusobacterium nucleatum* [9].

The important characteristics of bacteria for colonization and existence in the oral cavity are the ability to adhere to host tissues and to aggregate to form biofilms or to take the advantage of the ecosystem [10]. These phenotypic traits are widely used for screening potentially probiotic strains. Auto aggregation and adhesion to host cells of probiotic strains appear to be necessary for forming a barrier. Coaggregation of probiotic strains with the pathogens is an important ability for displacing pathogens from the oral cavity and human gut [11-13]. Thus, potentially probiotic bacteria with these abilities may have the possibility to colonize, function stably in the host, and prevent the colonization of the pathogenic microorganisms.

A study of adhesion and aggregation abilities and the surface charges of various *Lactobacillus* species derived from the human oral cavity reported that *L. gasseri* strains showed high externalization and aggregation (auto- and coaggregation) abilities [11]. Variations of the adherence ability among species and strains may be related to the diversity of the response to stress factors in the host environment such as acid-base condition, enzyme, and substrate; these adaptations may help bacteria survive in the environmental conditions encountered in the oral cavity [14]. Moreover, different agents or environments may be able to block the adhesion and aggregation of bacteria [15,16]. The oral environment, which is constantly changing, may affect these binding properties of the oral bacterial cell surface. To gain an understanding of how bacteria aggregate or adhere to surfaces in such an environment, in vitro experiments research was conducted. The overall aim of the present study was to investigate the effect of cell surface components, some enzymes, pH, sugars, and ions on the aggregation (auto- and coaggregation) abilities of oral *L. gasseri* and on the adhesion ability to oral epithelial cells.

Materials and methods

Bacterial strains and culture conditions

Seventeen strains of oral *Lactobacillus gasseri* were selected from the culture collection of the Department of Stomatology, Faculty of Dentistry, Prince of Songkla University, Thailand. The strains had initially been identified to species level from the previous study of Piwat *et al*. [17]. The prevalence of *L. gasseri* in our previous study was relatively low. However, most the strains come from low-caries children [17]. Before experimental use, the bacteria were grown on Man Rogosa Sharpe agar (MRS, Difco™, USA) in an anaerobic condition (80 % N_2, 10 % H_2, and 10 % CO_2) at 37 °C for 18 - 24 h, and then transferred to Man Rogosa Sharpe broth (MRS, Himedia®, India) for an additional 18 - 24 h incubation.

The cariogenic pathogen, *S. mutans* ATCC 25175™, was used in the aggregation assay. The strain was cultured on a blood agar plate (BBL™, USA) for 18 - 24 h, and transferred to Brain Heart Infusion broth (BHI, Bacto™, USA) in an aerobic condition (5 % CO_2) at 37 °C for an additional 18 - 24 h incubation.

Factors affecting adhesion and aggregation ability of bacterial cells

The effect of the factors was determined according to the method of Lang *et al.* [18] with some modifications. Overnight cultures of *L. gasseri* and *S. mutans* ATCC 25175™ were harvested by centrifugation at 3,000 rpm for 10 min and washed twice with phosphate-buffered saline (PBS) pH 7.0. The cell pellets were treated in different solutions as follows: (i) chemical agents for cell surface component test: 10 mM Metaperiodate in 0.1 M citrate-phosphate buffer (pH 4.5), 5 M LiCl in distilled water, and Proteinase K in 50 mM Tris-HCl buffer (pH 7.5), (ii) enzyme solution (1 mg/mL): Enzyme amylase in 15 mM PBS (pH 7.0), lysozyme in Tris EDTA sucrose buffer (pH 8.0), trypsin in 50 mM Tris-HCl buffer (pH 8.0), pepsin in 10 mM citrate-phosphate buffer (pH 4.5), and lipase in 50 mM Tris-HCl buffer (pH 8.0), (iii) PBS buffer adjusted to pH (4.0, 8.0), (iv) 50 mM sugar (glucose, sucrose and lactose), (v) 10 mM calcium or magnesium. The bacteria in PBS solution (pH 7.0) were used in the control group.

Treated bacterial suspensions were mixed by vortexing. The optical density was measured at 600 nm ($OD_{600\ nm}$) using a spectrophotometer (Ultrospec 2000 ^{TM}) to give viable counts of approximately 10^{10} CFU/mL ($OD_{600\ nm}$ = 0.5) for aggregation assay and 10^8 CFU/mL ($OD_{600\ nm}$ = 0.25) for adhesion assay.

Autoaggregation and coaggregation assays

Autoaggregation assay was performed according to Kos *et al*. [19] with some modification. The treated bacterial suspensions (4 mL) were mixed by vortexing (Vortex-Genie 2^{TM}) for 10 s and auto aggregation was determined after 24 h of incubation at 37 °C. One milliliter of the upper layer of each tube was carefully removed after 24 h. The absorbance of the supernatant was measured at 600 nm. The percent of autoaggregation was expressed as follows:

$$\% \text{ of autoaggregation} = (1\text{-}A_{time} / A_{initial}) \times 100 \quad (1)$$

where A_{time} is the optical density of the upper layer of the suspensions at 24 h after incubation, $A_{initial}$ is the optical density of the suspensions at baseline.

Coaggregation assay between *L. gasseri* and *S. mutans* ATCC 25175^{TM} was performed according to Piwat *et al.* (2015) [12]. Equal volumes (2 mL) of the treated *L. gasseri* and *S. mutans* ATCC 25175^{TM} suspensions were mixed and vortexed for 10 s. After incubation at 37 °C for 24 h to allow coaggregation to occur, 1 mL of the upper layer of the supernatant was carefully removed. Absorbance was measured at 600 nm. The coaggregation percentage was expressed as:

$$\% \text{ of coaggregation} = [(A_{L.gasseri} + A_{S.\ mutans}) / 2] - A_{mix}] / [(A_{L.gasseri} + A_{S.\ mutans}) / 2] \times 100 \quad (2)$$

where $A_{L.gasseri}$ is the optical density of *L. gasseri* suspensions at initial time, $A_{S.\ mutans}$ is the optical density of *S. mutans* ATCC 25175^{TM} suspensions at initial time, A_{mix} is the optical density of the mixed *L. gasseri* and *S. mutans* ATCC 25175^{TM} suspensions at 24 h after incubation.

Adhesion to keratinocyte cells

The adhesion activity of *L. gasseri* was assessed according to Kintarak *et al*. [20], using H357 keratinocyte cells as an oral squamous carcinoma cell line. The treated *L. gasseri* suspensions were added to H357 keratinocyte cells in Dulbecco's modified Eagle's medium (DMEM) on the 24-well plates. After incubation for 1 h at 37 °C in anaerobic condition, each well of the plates was washed twice with PBS (pH 7.0)to remove free and nonattached bacterial cells.The adherent bacteria both extracellular (Externalization) and intracellular bacteria (Internalization) were counted as total adhesion.

For internalization assay, the treated *L. gasseri* and H357 monolayer cells in 24-well plates were prepared using the same protocol as above. Then, 1 mL of DMEM containing gentamicin (100 µg/mL) was added to each well to kill extracellular bacteria and incubated for 2 h at 37 °C under an anaerobic condition. After that, each well of the plates was washed twice with PBS (pH 7.0).

To determine the bacterial cell number, trypsin-EDTA was added to H357 monolayer cells and incubated at 37 °C for 10 min. Then Triton X-100 was added to each well for keratinocyte cell lysis. After incubation for 15 min at 37 °C, a ten-fold serial dilution of the suspension was plated onto MRS plates and incubated under anaerobic conditions at 37 °C for 48 h to measure the number of viable bacterial colonies. The percent adhesion was expressed as follows:

$$\text{Externalization (CFU/mL)} = \text{Total adhesion (CFU/mL)} - \text{Internalization (CFU/mL)}$$
$$\% \text{ Total adhesion or internalization or Externalization} = (A/A_0) \times 100 \quad (3)$$

where A_0 = number of bacterial cells (CFU/mL) at the beginning, A = number of bacterial cells (CFU/mL) adhered totally or internalization or externalization

Statistical analysis

All experiments were independently conducted twice. The results were expressed as means ± standard deviation and median of the percentage of aggregation or adhesion. The Mann-Whitney U test was applied to compare data from the control group and treated groups. The software package used for the analyses was the SPSS statistical program (SPSS Inc., Chicago, IL). Differences were considered significant when p-value < 0.05.

Results and discussion

Chemical treatments for cell surface component of *L. gasseri*

Cell surface component is an important factor for the aggregation and adhesion of bacterial cells and may have strain-specific properties. The cell surface structure of lactobacilli contains a variety of components, including peptidoglycan, teichoic acids, exopolysaccharides, and proteins, which function through different mechanisms. Loss of specific cell surface elements can reduce these abilities [14].

In the present study, the oral *L. gasseri* strains showed 30 - 40 % of the ability to internalization in keratinocyte cells. There are little data in the literature on *Lactobacillus* internalization; moreover, the study about the internalization of *L. gasseri* to keratinocyte cells is still limited. Nevertheless, the adhesion and invasion capacity to epithelial cells of the *Lactobacillus* strains from the vagina have been studied by Santos *et al.* [21]. They showed that *L. gasseri* was able to internalize in HeLa cells. Microscopy observations of HeLa cell monolayers revealed that cell monolayers incubated with the *Lactobacillus* strains remained intact and showed no visible changes when compared to the non-inoculated control. As well, none of the *Lactobacillus* strains induced apoptosis in the HeLa cells [21]. In the present study, oral *L. gasseri* was subjected to chemical treatments, including proteinase K, LiCl, and metaperiodate, to characterize the cell surface components responsible for its aggregation and adhesion abilities. Proteinase K is a non-specific serine protease with a very high specific activity for general digestion of proteins. LiCl solution was used to remove the crystalline surface layer (S-layer) protein from the bacterial cell wall, while the contribution of carbohydrate molecule was examined by oxidizing cell surface carbohydrates with metaperiodate solution.

The results (**Table 1**) indicate that treatment with proteinase K had a highly significant effect on the aggregation abilities of *L. gasseri* as compared to the untreated samples. In the absence of surface-related proteins, the autoaggregation and coaggregation abilities were reduced from 70.09 and 50.94 % to 49.13 and 41.59 %, respectively. *L. gasseri* treated with LiCl and metaperiodate showed no significant change in the ability for aggregation and adhesion as compared with controls. For adhesion ability, treatment of the cells with proteinase K significantly affected only the internalization, while no effect was seen on total adhesion or externalization abilities. The results suggested that the cell surface-related protein components are essential for the aggregation and internalized adhering abilities of the strains. Therefore, if bacterial cells are under conditions that affect the protein on the cell surface, they may show a reduction in properties.

Table 1 Effect of chemical treatments for cell surface component on the aggregation and adhesion abilities of *L. gasseri*[a].

Factors	% Aggregation ability			% Adhesion ability[c]		
	Autoaggregation *L. gasseri*	**Autoaggregation *S. mutans***	**Coaggregation[b]**	**Total adhesion**	**Internalization**	**Externalization**
Control	70.09 ± 9.50 (69.76)	35.75 (33.10)	50.94 ± 7.15 (52.81)	67.73 ± 11.22 (68.83)	40.72 ± 11.82 (37.83)	27.01 ± 8.30 (26.19)
Proteinase K	49.13 ± 7.82 (47.38**)	37.41 (35.70)	41.59 ± 6.36 (41.85**)	64.43 ± 5.52 (64.30)	33.36 ± 6.63 (32.94*)	31.07 ± 5.48 (32.37)
LiCl	64.89 ± 6.82 (63.24)	41.10 (41.30)	47.06 ± 6.81 (46.61)	61.32 ± 8.92 (61.74)	35.78 ± 8.05 (36.55)	25.52 ± 6.18 (27.58)
Metaperiodate	66.80 ± 7.38 (66.17)	45.35 (44.40)	50.13 ± 8.84 (48.29)	62.46 ± 10.08 (62.34)	32.97 ± 14.46 (32.48)	29.49 ± 8.20 (28.29)

[a]Data are expressed as mean ± SD (median)
[b]Coaggregation between *L. gasseri* strains and *S. mutans* ATCC 25175^{TM}
[c]Adhesion ability for *L. gasseri* strains with oral squamous carcinoma cell line, H357 keratinocyte cells, in Dulbecco's modified Eagle's medium
*Significant differences at p-value < 0.05 between control and tested group
**Significant differences at p-value < 0.001 between control and tested group

Effect of enzymatic treatments

Probiotic bacteria should remain either in the oral cavity or GI tract to display the properties of probiotics. Some oral and GI tract enzymes were used to investigate the effect on bacterial cells (**Table 2**). The results showed that the aggregations were affected by some enzymatic treatments while the adhesion was not affected. The oral enzymes amylase, which is the main enzyme in saliva that breaks carbohydrates (starches) down to maltose, and lysozyme, a prominent antimicrobial protein of human saliva, were used for testing. The amylase enzyme significantly increased both autoaggregation and coaggregation capabilities compared to buffer controls. As a result of the antibacterial activity, lysozyme can cause the degradation of the peptidoglycan-containing layer of bacterial cell wall, ultimately resulting in bacterial lysis [22]. The lysis of Gram-positive bacteria with lysozyme is species and strain specific [23,24]. According to Chassy *et al*., the study showed each strain of *S. mutans* and *Lactobacillus* spp. had different percent of lysis when treated with lysozyme; this may be due to the cell wall components having strain-dependent differences [24]. In this study, lysozyme completely inhibited both autoaggregation of *S. mutans* ATCC 25175^{TM} and coaggregation between *L. gasseri* and *S. mutans* ATCC 25175^{TM}. It is suggested that lysozyme inhibits the aggregation of *S. mutans*, whether autoaggregation or coaggregation with other bacteria. This corresponds with previous studies, in which lysozyme activity in the oral cavity has mainly been characterized against oral streptococci especially *S. mutans* [25-27]. For GI tract enzymes, only trypsin treatment significantly decreased the coaggregation of *L. gasseri* and *S. mutans* compared to the control group. This effect may be due to the digestion of proteins on the cell surface.

It can be concluded from our result that *L. gasseri* can survive and exhibit aggregation and adhesion ability under the various oral and GI tract enzymes conditions, excluding coaggregation with *S. mutans* after lysozyme treatment. Tolerance to these digestive enzymes is possibly considered as an important property for the development into probiotics.

Effect of pH, sugars, and ions

The aggregation and adhesion abilities of *L. gasseri* strains after treatment with pH, sugars, and ions are shown in **Table 3**. Concerning the aggregation ability, the present study indicated that calcium ion and magnesium ion significantly increased both autoaggregation of *L. gasseri* and coaggregation between *L. gasseri* and *S. mutans* ATCC 25175^{TM}, while pH 4.0 increased only the coaggregation ability. Among the effect of sugars on aggregation abilities, lactose was the only one that significantly reduced both

autoaggregation and coaggregation, while glucose and sucrose significantly decreased the coaggregation ability. It was noted that some factors, such as pH 4.0, glucose, and sucrose, had a significant effect on coaggregation between the bacterial cells, whereas the autoaggregation of *L. gasseri* was not affected. It may be the influence of autoaggregation of *S. mutans* that increased from 35.75 to 72.19 % at pH 4.0 and decreased after treatment with glucose and sucrose from 35.75 to 26.95 and 12.32 %, respectively.

Regarding adhesion ability, total adhesion and internalization were significantly increased by pH 4.0, pH 8.0, and calcium ion while magnesium ion significantly increased only the internalization. The results showed that neither kind of sugar significantly affected adhesion.

Lactobacillus generally survives well under acidic conditions [28-30]. Therefore, it is not surprising that *L. gasseri* can aggregate and adhere at low pH conditions. But it is noteworthy from this experiment, that *L. gasseri* also increased its adhesion ability in basic conditions (at pH 8.0). That may be the reason for the high prevalence of *L. gasseri* in periodontal tissue [3] which has a pH range between 6.5 - 8.5 [31].

Calcium and magnesium ions are inorganic substances that can be found in the food. Moreover, these ions are components of the inorganic part of human saliva and are involved in the formation of teeth [32]. It has previously been demonstrated that these ions are also important for adhesion and aggregation properties [33-35]. The results in this study imply that the aggregation and adhesion abilities of *L. gasseri* can be induced and also increased when in an environment that contains calcium and magnesium ions such as in saliva or in calcium-rich food or dairy products.

Regarding the selected sugar, glucose sucrose and lactose are the main sugars obtained from food. Glucose and sucrose are commonly added to many processed foods, while lactose is found naturally in milk and dairy products [36]. The findings of the present study indicated that the sugars especially lactose had the effect of decreasing the aggregation of *L. gasseri* and *S. mutans* ATCC 25175^{TM}. Many studies have demonstrated that lactose inhibited aggregation between the cells of both gram-positive and gram-negative bacteria via the lectin-carbohydrate interaction mechanism of cell surface elements [37-41]. In addition, other studies have suggested that *L. gasseri* was not adapted to grow well in a milk environment [42,43], which may explain the phenomenon above.

Table 2 The effect of the treatment with enzymes on aggregation and adhesion abilities[a].

Factors	% Aggregation ability			% Adhesion ability[c]		
	Autoaggregation *L. gasseri*	**Autoaggregation *S. mutans***	**Coaggregation[b]**	**Total adhesion**	**Internalization**	**Externalization**
Control	70.09 ± 9.50 (69.76)	35.75 (33.10)	50.94 ± 7.15 (52.81)	67.73 ± 11.22 (68.83)	40.72 ± 11.82 (37.83)	27.01 ± 8.30 (26.19)
Enzymes						
Amylase	78.03 ± 9.01 (80.42*)	31.17 (31.50)	74.60 ± 11.77 (78.27**)	65.99 ± 7.86 (65.66)	40.12 ± 8.40 (39.15)	25.86 ± 9.12 (27.39)
Lysozyme	71.00 ± 13.83 (72.92)	0.00 (0.00)	0.00 (0.00**)	65.33 ± 8.98 (65.48)	40.99 ± 6.59 (41.22)	24.33 ± 7.29 (24.58)
Trypsin	64.67 ± 8.39 (62.18)	39.33 (38.80)	46.35 ± 6.14 (46.01*)	67.47 ± 6.88 (70.21)	39.84 ± 8.78 (36.36)	27.63 ± 7.25 (29.00)
Pepsin	66.68 ± 8.07 (67.00)	43.33 (42.90)	48.95 ± 6.93 (49.04)	63.65 ± 5.01 (64.03)	36.59 ± 7.66 (35.11)	27.06 ± 5.67 (28.43)
Lipase	66.78 ± 8.22 (67.24)	40.71 (40.70)	50.13 ± 6.94 (48.65)	63.03 ± 5.97 (62.53)	36.75 ± 6.89 (36.21)	26.27 ± 5.86 (27.02)

[a]Data are expressed as mean ± SD (median)
[b]Coaggregation between *L. gasseri* strains and *S. mutans* ATCC 25175^{TM}
[c]Adhesion ability for *L. gasseri* strains with oral squamous carcinoma cell line, H357 keratinocyte cells, in Dulbecco's modified Eagle's medium
*Significant differences at p-value < 0.05 between control and tested group
**Significant differences at p-value < 0.001 between control and tested group

Table 3 Effect of pH, sugars and ions on the aggregation and adhesion abilities[a].

Factors	% Aggregation ability			% Adhesion ability[c]		
	Autoaggregation *L. gasseri*	**Autoaggregation *S. mutans***	**Coaggregation[b]**	**Total adhesion**	**Internalization**	**Externalization**
Control	70.09 ± 9.50 (69.76)	35.75 (33.10)	50.94 ± 7.15 (52.81)	67.73 ± 11.22 (68.83)	40.72 ± 11.82 (37.83)	27.01 ± 8.30 (26.19)
pH						
pH 4.0	75.97 ± 9.40 (76.73)	72.19 (73.98)	63.39 ± 11.87 (58.31*)	83.26 ± 6.49 (84.28**)	53.27 ± 7.35 (54.13**)	29.99 ± 9.77 (30.17)
pH 8.0	70.54 ± 9.86 (71.26)	47.58 (47.75)	51.61 ± 5.54 (51.20)	78.78 ± 5.63 (78.24*)	50.53 ± 7.75 (48.92*)	28.25 ± 8.76 (28.71)
Sugars						
Glucose	66.67 ± 14.08 (66.67)	26.95 (28.55)	35.27 ± 11.72 (34.83**)	67.83 ± 11.00 (66.54)	41.76 ± 9.17 (39.94)	26.07 ± 9.58 (26.87)
Sucrose	67.77 ± 15.00 (71.86)	12.32 (12.37)	35.70 ± 15.83 (32.74*)	66.53 ± 10.32 (65.60)	41.91 ± 11.98 (40.26)	24.62 ± 7.33 (26.03)
Lactose	57.12 ± 16.34 (58.56*)	18.44 (18.03)	31.60 ± 10.37 (36.56**)	67.34 ± 9.21 (68.25)	41.81 ± 9.99 (40.62)	25.53 ± 8.35 (24.81)
Ions						
Calcium	84.55 ± 6.81 (83.70**)	43.07 (44.90)	57.64 ± 8.06 (60.21*)	83.62 ± 5.56 (82.10**)	54.79 ± 9.85 (53.24**)	28.83 ± 10.00 (30.47)
Magnesium	82.52 ± 9.31 (81.74*)	32.06 (32.20)	55.95 ± 6.52 (57.53*)	74.52 ± 6.97 (76.23)	47.23 ± 8.03 (47.52*)	27.29 ± 7.80 (27.44)

[a]Data are expressed as mean ± SD (median)
[b]Coaggregation between *L. gasseri* strains and *S. mutans* ATCC 25175^{TM}
[c]Adhesion ability for *L. gasseri* strains with oral squamous carcinoma cell line, H357 keratinocyte cells, in Dulbecco's modified Eagle's medium
*Significant differences at p-value < 0.05 between control and tested group
**Significant differences at p-value < 0.001 between control and tested group

Conclusions

Probiotic strains with good abilities to aggregate and adhere to oral epithelial cells could be better suited to colonize the oral cavity. However, aggregation and adhesion abilities are dependent on environmental factors. Moreover, the cell surface properties of bacteria are thought to play an important role in these abilities. For this, *L. gasseri* used the cell surface-related protein components for the aggregation and internalized adhering abilities. *L. gasseri* can survive and exhibit aggregation and adhesion ability under various environmental conditions. These traits could be used for the preliminary screening of other potential probiotic candidates with possible anti-caries properties.

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