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Original Article

Characterization of adhesion, anti-adhesion, co-aggregation, and hydrophobicity of *Helicobacter pylori* and probiotic strains

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الملخص

أهداف البحث: توصيف قدرة الالتصاق لتسع سلالات من الملوية البوابية وثمانية بروبيوتيك في خلايا الخلايا الكيراتينية الفموية البشرية (خلايا اتش٣٥٧) مقارنة بالخلايا المعوية (خلايا كاكو-٢ و خلايا هيك-٦). بعد ذلك، تم فحص القدرات المضادة للالتصاق والتجميع المشترك لسلالات الكائنات الحية المجهرية المختارة على سلالات الملوية البوابية.

طرق البحث: تسع سلالات من الملوية البوابية تضمنت الملوية البوابية أ،ت،س،س،٤٣٥٠٤ (سلالة من النوع)، وتم عزل ٨ سلالات إكلينيكية من عينات فموية لثلاثة مرضى (واحد غير مصاب، ومريض التهاب معدي، ومريض سرطان معدي). تم استخدام سلالات الكائنات الحية المجهرية الثمانية المختارة على النحو التالي: لاكتوباسيلس باراكاسي (اس دي1) و لاكتوباسيلس رامنوساس (اس دي4) و لاكتوباسيلس رامنوساس (اس دي11) و ليموسيلاكتوباسيلوس فيرمنتوم (اس دي7) و لاكتوباسيلس رامنوساس (ج ج) و ليموزيلاكتياز5؛ تم فحص قدرات التصاق الملوية البوابية وسلالات البروبيوتيك المضادة للالتصاق في خلايا اتش٣٥٧ وخلايا كاكو-٢ و خلايا هيك-٦. تم فحص التجميع المشترك عند مختلف الأس الهيدروجيني، والكراهية للماء والمستقبلات السطحية لخطوط الخلايا لسلالات الملوية البوابية.

النتائج: جميع سلالات الكائنات الحية المجهرية والملوية البوابية يمكن أن تلتصق بـ خلايا اتش٣٥٧ أفضل بكثير من خلايا كاكو-٢ و خلايا هيك-٦. أظهرت ثلاث سلالات بروبيوتيك (اس دي7 و اس دي4 و اس دي11) التصاق أعلى بكثير من سلالات أخرى. من بين سلالات الملوية البوابية السريرية، كان لعزلات مريض سرطان المعدة أعلى قدرة على الالتصاق لجميع سلالات الخلايا المختبرة. سلالات البروبيوتيك التي أظهرت قدرة عالية على الالتصاق، يمكن أن توفر مقاومة عالية للالتصاق وتجمع مشترك ضد سلالات الملوية البوابية. يمكن أن تشجع الظروف الحمضية تكتل البروبيوتيك لسلالات الملوية البوابية.

الاستنتاجات: تقدم هذه الدراسة معلومات عن قدرات التصاق الملوية البوابية وسلالات الكائنات الحية المجهرية في الغشاء المخاطي للفم مقارنة بالغشاء المخاطي المعوي. قد تكون سلالات معينة من الكائنات الحية المجهرية مفيدة في القضاء الناجح على عدوى الملوية البوابية عن طريق منع الالتصاق والتجميع المشترك.

الكلمات المفتاحية: المضادة للالتصاق؛ تجميع مشترك؛ الملوية البوابية؛ كره الماء؛ بروبيوتيك

Abstract

Objectives: To characterize the adhesion ability of nine *Helicobacter pylori* strains and eight probiotics in human oral keratinocyte cells (H357 cells) in comparison to intestinal cells (Caco-2 and HIEC-6 cells). Subsequently, the anti-adhesion and co-aggregation abilities of the selected probiotic strains on *H. pylori* strains were investigated.

Methods: Nine *H. pylori* strains, including *H. pylori* ATCC43504 (type strain), and 8 clinical strains, were

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isolated from oral samples of three patients (one non-disease, one gastritis patient, and one gastric cancer patient). Eight selected probiotic strains were used, as follows: *Lacticaseibacillus paracasei* SD1, *Lacticaseibacillus rhamnosus* SD4, *L. rhamnosus* SD11, *Limosilactobacillus fermentum* SD7, *L. rhamnosus* GG, *Limosilactobacillus reuteri* ATCC-PTA6475, *Lacticaseibacillus casei* Shirota, and *L. paracasei* CNCM I-1572. The adhesion and anti-adhesion abilities of *H. pylori* and the probiotic strains were investigated in H357, Caco-2, and HIEC-6 cells. Co-aggregation at various pHs, hydrophobicity, and surface receptors of the cell lines for *H. pylori* strains were examined.

Results: All probiotic and *H. pylori* strains adhered to H357 significantly better than Caco-2, and HIEC-6 cells. Three probiotic strains (SD7, SD4, SD11) showed significantly higher adhesion than others. Of the clinical *H. pylori* strains, isolates from a gastric cancer patient had the highest adhesion ability to all of the cell lines tested. Probiotic strains that exhibited high adhesion ability provided high anti-adhesion and co-aggregation against *H. pylori* strains. Acidic conditions encouraged the co-aggregation of probiotics to *H. pylori* strains.

Conclusion: This study provides information relating to the adhesion abilities of clinical *H. pylori* and probiotic strains to the oral mucosa when compared to the intestinal mucosa. Certain probiotic strains may be useful for the successful eradication of *H. pylori* infection *via* anti-adhesion and co-aggregation.

Keywords: Anti-adhesion; Co-aggregation; *Helicobacter pylori*; Hydrophobicity; Probiotic

Introduction

The human oral cavity is the initial part of the body that connects to the digestive tract. Recent studies have found that the oral microbiome plays a crucial role in both oral and systemic health.[1] The oral microbiota can cause not only oral diseases but also systemic diseases.[2] *Helicobacter pylori* belongs to the oral microbiome and is found mostly in dental plaque.[3] Previous research has demonstrated that *H. pylori* exerts numerous pathological effects on gastritis, peptic ulcers, and gastric cancer.[4,5] Furthermore, some studies have reported that the use of steroids and the presence of psychological illness may increase the risk of *H. pylori* gastritis.[6–8] Recent reports showed that oral *H. pylori* and stomach infection presented a high recurrence rate of gastritis (13.2%–18.4%).[9] Such findings may relate to the potential adherence of *H. pylori* strains to the oral epithelial cells since adhesion is the first step for all microorganisms to colonize in hosts and results in commensalism or infection. However, such information is limited and most studies have investigated the adhesion ability of *H. pylori* strains to gastric cells[10–13] or CaCo-2 cells.[12]

Some evidence has been reported that probiotics could be used as an adjunctive treatment for eradicating *H. pylori* infection in the gastric mucosa.[14,15] Thus, there has been increasing levels of interest in probiotics as an alternative way to improve health against infection, largely due to an increase in the failure rate associated with antibiotic treatment.[16] Such failure links to the spreading of antibiotic resistance[17] and patient compliance affected by antibiotic-associated adverse events. Some studies found that probiotics could disturb the adherence of *H. pylori* strains *via* co-aggregation[18] or compete with pathogens for host surface receptors, thus resulting in the inhibition of *H. pylori* adhesion to gastric epithelial cells.[19] We hypothesized that the anti-adhesion ability of probiotics against *H. pylori* strains in the oral cavity may help prevent a host from pathogenic infection at the initial step.

In this study, we aimed to characterize the adhesion ability of eight probiotics and nine *H. pylori* strains in human oral keratinocyte cells (H357 cells) compared to intestinal cells (Caco-2 and HIEC-6 cells). In addition, we investigated the anti-adhesion and co-aggregation abilities of selected probiotic strains on *H. pylori* strains.

Materials and Methods

Bacterial strains and culture conditions

The eight selected probiotic strains used in this study were *L. paracasei* SD1, *L. rhamnosus* SD4, *L. rhamnosus* SD11 and *L. fermentum* SD7 from a culture collection held by the Faculty of Dentistry, Prince of Songkla University; *L. rhamnosus* GG and *L. reuteri* ATCC PTA6475, which were purchased from the American Type Culture Collection; and *L. casei* Shirota and *L. paracasei* CNCM I-1572 which were isolated from commercial products.

The nine *H. pylori* strains were *H. pylori* ATCC43504 (type strain) and eight clinical strains isolated from the oral samples of three patients (H01–H03 were three isolates from one non-disease subject; G01-G03 were isolated from one gastritis patient; and C01–C02 were two isolates from one gastric cancer patient). This research study received ethical approval (REC.63-540-10-1). All clinical isolates were identified based on their typical morphology on the selected medium for *H. pylori* (Campylo-Thioglycollate medium) (Himedia, India) and gave a positive reaction for catalase, oxidase, and urease tests. Subsequently, all strains were confirmed by PCR with specific primers for *H. pylori*.[20]

Probiotics and pathogens were cultured in De Man, Rogosa and Sharpe (MRS) agar and brain heart infusion (BHI) agar, respectively, at 37 °C for 24–48 h in anaerobic conditions. The probiotic and *H. pylori* strains were adjusted to 10^8 CFU/mL for experimental use.

Cell lines and culture conditions

Human oral squamous cell carcinoma (H357 cells), human colorectal adenocarcinoma cells (Caco-2), and human normal intestinal epithelial cell line-6 cells (HIEC-6 cells) were used in this study. All cells were cultured in Dulbecco's modified Eagle's medium (DMEM)[21,22] at 37 °C in 5% CO_2.

Total adhesion of probiotic and H. pylori strains

The total adhesion assay was examined using the modified method described by Sophatha and Teanpaisan.[23] An individual tested strain (probiotics or pathogen) was added into each well of cells for 1 h. The cells were washed to remove unbound bacteria, and trypsin–EDTA was added to release the bacterial cells. Then, we counted the number bacterial adhered onto the agar plate. Total adhesion was expressed as a percentage.[22]

Anti-adhesion of probiotics to H. pylori strains

Anti-adhesion ability was evaluated according to the competitive adhesion of probiotics against *H. pylori*, as defined previously.[24] Equal volumes (1 mL) of the tested probiotic and *H. pylori* strain was added simultaneously into wells containing the tested cell lines and incubated for 1 h. After washing to remove non-bound bacteria, the adhered *H. pylori* were counted on a selective medium supplemented with 5% whole blood. Then, we calculated anti-adhesion (%) of the probiotic to *H. pylori*.

Co-aggregation assays of probiotic and H. pylori strains at various pH levels

Probiotics and *H. pylori* were adjusted with a buffer solution at various pH levels. Cell suspensions of each probiotic strain were mixed along with the pathogen and incubated at 37 °C for 24 h. Co-aggregation assays were then performed according to Sophatha et al.[22] The upper suspensions were measured for absorbance at OD_{600nm}.

Hydrophobicity of probiotics and H. pylori strains

Each bacterial suspension was mixed with xylene (ratio 3:1, mL). After incubation for half an hour, the aqueous phase was separated and measured at OD_{600nm}. Hydrophobicity was calculated and categorized according to Sophatha et al.[22]

Surface receptors of cell lines involved in the adhesion of H. pylori strains

The surface receptors of cells were determined by treating the cells with a final concentration of 1 mg/mL of proteinase K, lipase, and sodium metaperiodate.[25] The reaction was incubated at 37 °C for 20 min. The adhesion ability of the pathogen to the pretreatment cells was evaluated, using the methods described above.

Statistical analysis

All data were tested for normality test; all data were found to be non-parametric. Data presented as medians (min, max). Differences in adhesion, co-aggregation, and hydrophobicity between groups were evaluated by the Kruskal–Wallis test. Differences in anti-adhesion ability and surface receptors between the groups were analyzed by the Mann–Whitney U test. Statistical analysis was carried out with STATA version 14.0 software.

Results

Total adhesion of probiotic and H. pylori strains

The probiotic strains showed differences in adhesion ability, as did the *H. pylori* strains. This indicated that all probiotic and *H. pylori* stains could adhere to H357 in a manner that was significantly better than other cells (Table 1). Analysis demonstrated that among the probiotic strains (SD7, SD4 and SD11) showed significantly higher levels of adhesion than the others; *L. casei* Shirota had the lowest adhesion ability.

These data revealed a distinction in adhesion ability among clinical *H. pylori* stains. The isolates from a gastric cancer patient had the highest adhesion ability to all tested cell lines; this was followed by the clinical isolate from a non-diseased subject and a gastritis patient, respectively (Table 1).

Table 1: Adhesion ability of probiotic- and *H. pylori* strains to H357, Caco-2 and HIEC-6 cells.

Strains	Total adhesion (%)		
	H357	CaCo2	HIEC
Probiotic strains:			
L. fermentum SD7	90.7 (89.9–91.6)^{A,a}	86.5 (86.0–86.9) ^{AB,a}	74.0 (71.8–74.0)^{B,a}
L. rhamnosus SD4	89.9 (87.6–89.9)^{A,a}	83.1 (81.0–84.0) ^{AB,a}	71.6 (71.6–74.4)^{B,a}
L. rhamnosus SD11	88.8 (87.4–89.9)^{A,a}	82.0 (81.0–84.0) ^{AB,a}	71.9 (70.6–77.6)^{B,a}
L. paracasei SD1	74.5 (73.0–74.5)^{A,b}	72.6 (71.0–74.2)^{A,b}	62.8 (60.8–67.9)^{B,b}
L. rhamnosus LGG	73.6 (73.0–74.5)^{A,b}	75.7 (74.7–77.1)^{A,b}	62.8 (60.8–68.9)^{B,b}
L. paracasei CNCM I-1572	74.5 (73.0–74.5)^{A,b}	67.5 (62.8–68.8) ^{AB,b}	60.8 (59.5–62.8)^{B,b}
L. reuteri ATCC PTA 6475	73.5 (73.0–74.0)^{A,b}	67.5 (66.3–68.8) ^{AB,b}	61.8 (60.8–62.8)^{B,b}
L. casei Shirota	66.9 (64.9–68.3)^{A,c}	57.6 (57.0–58.2)^{B,c}	56.4 (54.3–58.5)^{B,c}
***H. pylori* strains:**			
ATCC 43504	81.7 (79.0–85.2)^{A,ab}	73.3 (72.8–73.9)^{B,ab}	61.3 (58.5–61.7)^{C,b}
H01–H03	71.3 (69.7–81.5)^{A,b}	64.0 (62.8–89.0)^{B,b}	57.3 (56.7–61.7)^{C,c}
G01-G03	69.0 (56.7–70.9)^{A,c}	63.3 (50.8–64.1)^{B,b}	56.9 (40.8–57.4)^{C,c}
C01–C02	87.6 (86.4–88.9)^{A,a}	76.0 (75.1–77.1)^{B,a}	64.5 (63.8–65.3)^{C,a}

Different capital letters indicate statistically significant different adhesion between the cell lines (H357, Caco-2 and HIEC-6 cells) in the same bacterial strain; lowercase letters indicate statistically significant different adhesion between the probiotic strains or *H. pylori* strains in the same tested cell line; data analyzed by Kruskal–Wallis test at $p < 0.05$.

Table 2: Anti-adhesion (%) of probiotic strains against clinical *H. pylori* with different adhesion ability in tested cell lines.

Strains	Clinical strains of *H. pylori* with	
	>70% adhesion	<70% adhesion
H357 cell:		
L. fermentum SD7	65.5 (64.3–68.8)^{A,a}	66.8 (63.9–79.2)^{A,a}
L. rhamnosus SD11	55.9 (52.2–65.1)^{B,b}	64.5 (54.9–72.8)^{A,a}
L. rhamnosus SD4	57.9 (55.8–63.3)^{B,b}	61.5 (54.8–67.2)^{A,ab}
L. paracasei SD1	52.6 (49.3–54.7)^{B,c}	52.9 (52.2–60.4)^{A,c}
L. rhamnosus LGG	50.6 (45.5–54.0)^{B,c}	54.4 (51.7–65.5)^{A,c}
L. paracasei CNCM I-1572	52.2 (49.9–56.1)^{B,c}	53.3 (51.0–62.8)^{A,c}
L. reuteri ATCC PTA 6475	51.7 (49.9–55.6)^{B,c}	60.4 (53.1–68.4)^{A,b}
L. casei Shirota	37.0 (28.3–46.9)^{B,d}	44.9 (40.2–60.4)^{A,d}
Caco-2 cells:		
L. fermentum SD7	69.8 (67.6–71.5)^{B,a}	73.9 (70.0–85.8)^{A,a}
L. rhamnosus SD11	56.4 (48.5–52.5)^{B,b}	66.8 (58.8–81.5)^{A,b}
L. rhamnosus SD4	55.3 (48.5–60.1)^{B,b}	64.1 (60.6–76.4)^{A,bc}
L. paracasei SD1	49.4 (47.5–51.6)^{B,c}	60.6 (42.2–69.3)^{A,c}
L. rhamnosus LGG	50.4 (46.3–51.7)^{B,c}	65.2 (48.8–74.0)^{A,bc}
L. paracasei CNCM I-1572	50.4 (47.2–53.1)^{B,c}	64.4 (42.2–69.3)^{A,bc}
L. reuteri ATCC PTA 6475	50.4 (47.2–51.7)^{B,c}	65.1 (42.2–69.3)^{A,bc}
L. casei Shirota	38.4 (35.5–40.7)^{B,d}	48.6 (31.3–59.4)^{A,d}
HIEC-6 cells:		
L. fermentum SD7		73.7 (56.4–90.5)^{a}
L. rhamnosus SD11		64.3 (40.8–79.0)^{b}
L. rhamnosus SD4		57.8 (38.8–81.7)^{bc}
L. paracasei SD1		52.9 (42.0–73.2)^{c}
L. rhamnosus LGG		51.6 (38.8–76.7)^{c}
L. paracasei CNCM I-1572		53.3 (36.1–76.6)^{c}
L. reuteri ATCC PTA 6475		55.8 (41.3–77.6)^{c}
L. casei Shirota		29.2 (22.7–55.8)^{d}

Different capitals indicate statistically significant differences in adhesion (row); data was analyzed by the Mann Whitney U test. Lowercase letters indicate statistically significant differences between individual bacteria (column); the data was analyzed by the Kruskal–Wallis test at $p < 0.05$.

Anti-adhesion properties of probiotics against H. pylori strains

When considering the anti-adhesion properties of probiotics against clinical *H. pylori* strains, we found that such ability depended on the probiotics and clinical *H. pylori* strains involved. The clinical strains were classified based on their adhesion ability as strains with adhesion of >70% or as strains with <70% adhesion. Analysis demonstrated that probiotics could provide significantly higher anti-adhesion against the clinical *H. pylori* strains with low adhesion (<70% adhesion) compared to the clinical *H. pylori* strains with high adhesion (>70% adhesion) (Table 2). However, there was a variation of anti-adhesion ability among the probiotic strains. *L. fermentum* SD7 had the highest anti-adhesion compared to the others, while *L. casei* Shirota showed the lowest anti-adhesion. Similar results were observed for all of the tested cell lines (Table 2).

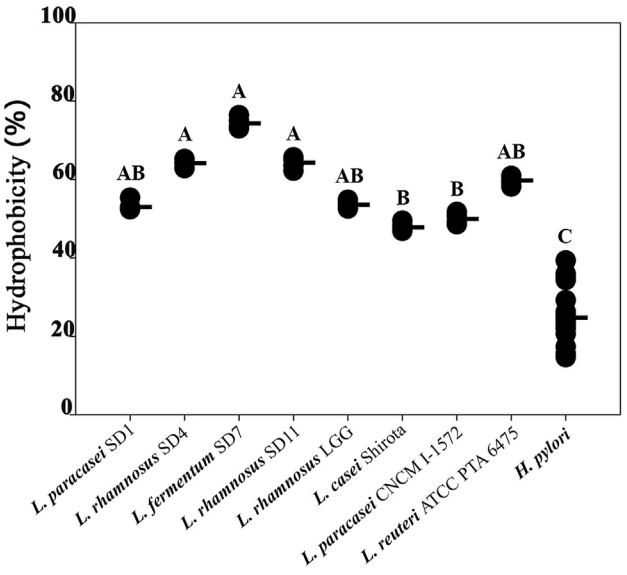

Figure 1: Hydrophobicity of selected probiotics and *H. pylori* strains was assessed using xylene. Different letters indicate statistical significance at $p<0.05$ by the Kruskal-Wallis test.

Co-aggregation assays of probiotic and H. pylori strains at various pH levels

Next, we investigated the co-aggregation ability of probiotics and *H. pylori* strains at various pH levels (3, 5, 7, and 8). Analysis revealed that co-aggregation ability depended on the pH and probiotic strains. An acidic pH (3 and 5) led to

Table 3: Median (max-min) of co-aggregation ability between probiotics and *H. pylori* strains at various pH.

Strains	pHs			
	pH 3	pH 5	pH 7	pH 8
L. fermentum SD7	67.0 (50.1–89.0)^{B,a}	79.6 (77.4–87.0)^{A,a}	62.8 (47.8–79.7)^{B,a}	47.6 (30.8–67.2)^{C,a}
L. rhamnosus SD11	58.9 (42.4–67.2)^{A,b}	59.5 (50.5–64.1)^{A,b}	40.3 (27.7–58.6)^{B,b}	37.9 (23.1–56.0)^{B,b}
L. rhamnosus SD4	55.9 (42.4–67.2)^{B,b}	59.8 (50.6–64.3)^{A,b}	39.8 (27.1–58.3)^{C,b}	35.8 (24.8–59.6)^{C,b}
L. paracasei SD1	52.7 (36.3–58.5)^{B,b}	60.2 (51.4–64.6)^{A,b}	40.4 (27.8–58.6)^{C,b}	37.8 (22.5–56.5)^{C,b}
L. rhamnosus LGG	57.2 (35.4–60.3)^{B,b}	60.1 (51.2–64.6)^{A,b}	40.5 (27.9–58.7)^{C,b}	38.1 (22.9–56.6)^{C,b}
L. paracasei CNCM I-1572	50.5 (35.4–60.3)^{B,bc}	59.5 (50.4–64.0)^{A,b}	41.1 (28.6–59.2)^{C,b}	38.2 (22.5–57.2)^{C,b}
L. reuteri ATCC PTA 6475	47.5 (35.1–65.1)^{B,c}	59.8 (50.8–64.3)^{A,b}	41.7 (29.4–59.6)^{C,b}	39.4 (24.5–57.5)^{C,b}
L. casei Shirota	46.4 (34.2–66.3)^{B,c}	56.3 (47.8–61.4)^{A,c}	39.0 (26.6–57.1)^{C,b}	35.4 (21.3–53.9)^{C,b}

Different capital letters indicate statistically significant differences of co-aggregation ability between probiotics and *H. pylori* strains compared at various pH (3, 5, 7, 8); lowercase letters indicate statistically significant different co-aggregation ability between probiotics and *H. pylori* strains compared at the same pH; data was analyzed by Kruskal–Wallis test at $p < 0.05$.

Table 4: Surface receptors of H357, Caco-2 and HIEC-6 cells for *Helicobacter pylori* strains.

Treatments	Total adhesion, %		
	H357	Caco-2	HIEC-6
Untreated (Control)	72.8 (58.4−89.4)	64.3 (51.1−77.5)	59.9 (41.4−65.6)
Proteinase K	66.2 (53.1−68.4)[a]	54.4 (47.1−58.1)[a]	50.5 (33.9−54.6)[a]
Lipase	71.9 (56.9−88.9)	63.4 (51.9−77.2)	56.1 (41.6−60.1)[a]
Sodium metaperiodate	68.4 (54.1−71.9)[a]	57.5 (51.2−66.8)[a]	59.1 (41.4−66.6)

[a] Indicate statistically significant differences of adhesion of *H. pylori* strains to cell lines after treating compared to untreated; data was analyzed by the Kruskal−Wallis test at $p < 0.05$.

higher levels of co-aggregation than neutral and basic conditions. The highest co-aggregation ability was detected in *L. fermentum* SD7 (Table 3).

Hydrophobicity of probiotics and H. pylori strains

Analysis revealed different levels of hydrophobicity among the probiotic strains (Figure 1). The hydrophobicity of *L. fermentum* SD7 (74.3%) was high, whereas the hydrophobicity of the remaining strains was moderate ranging from 47.8 to 64.3%. We also detected a variation in hydrophobicity among the nine pathogen strains. The hydrophobicity of *H. pylori* ATCC43504 and the two clinical isolates from a gastric cancer patient were moderate (35.2−39.8%), while that of the remaining strains were low (15.42−25.1%).

Surface receptors of cell lines involved in the adhesion of H. pylori strains

Analysis showed that the adhesion of *H. pylori* strains to H357 and Caco-2 cells were significantly reduced after treating these cell lines with proteinase K and sodium metaperiodate. However, the adhesion of pathogens to HIEC-6 cells significantly decreased after treatment with proteinase K and lipase (Table 4). This indicated that the surface receptors on H357 and Caco-2 cells were glycoproteins, while those on HIEC-6 cells were lipoproteins.

Discussion

Antibiotics are currently used to treat *H. pylori* infection; however, there is a mounting body of evidence relating to drug resistance and side effects.[26] Therefore, probiotics have recently gained a significant attention as a new tactic to enhance the therapeutic effect.[27] Some reports have shown that probiotic strains could be used as adjunctive therapy to improve and eradicate *H. pylori* infection in the stomach.[14,16] Literature reviews have reported that probiotics possess different mechanisms to eradicate or limit the growth of *H. pylori*; for example, reducing *H. pylori* survival in acid conditions by the production of lactic acid to inhibit *H. pylori* urease, thus causing the death of *H. pylori* by the production of bacteriocins, organic acids and biosurfactants.[16] Of these mechanisms, the anti-adhesion and coaggregation of probiotics against *H. pylori* strains have been considered to be crucial properties for preventing attachment, since the attachment of a pathogen is the first step in the continued colonization of the human gastric mucosa. In fact, the oral mucosa should be considered as the first place of initial attachment, subsequently leading to colonization in other parts of the digestive system.

We characterized the adhesion ability of *H. pylori* and probiotic strains in various cell line models, including H357 cells (representing oral mucosa cells) along with Caco-2 and HIEC-6 cells (representing the intestinal tract). This is the first study to investigate the adhesion ability of *H. pylori* using oral mucosa cells. In addition, we included clinical *H. pylori* strains derived from different disease statuses (non-disease, gastritis and gastric cancer). The present study found that there are differences of adhesion ability between clinical *H. pylori* strains and probiotics. We demonstrated that individual probiotic and *H. pylori* strains showed the highest adhesion ability to oral epithelial cells (H357 cells), followed by intestinal cells (Caco-2 and HIEC-6 cells).

Surprisingly, significant variation of adhesion ability and hydrophobicity were detected among *H. pylori* strains even though they were from the same species. We found that hydrophobicity in *H. pylori* strains was relatively low compared to probiotic strains, although, their adhesion abilities were high. This finding may be explained by the fact that *H. pylori* strains can firmly adhere to gastric epithelial cells *via* a group of outer membrane proteins (OMPs) such as BabA and SabA.[10,28] Patients with gastric cancer are known to have higher levels of BabA expression than normal subjects.[29,30] Adhesion of *H. pylori* to the gastric mucosa could trigger the expression of several virulence genes.[10] Similarly, in this study, we found that *H. pylori* strains isolated from patients with gastric cancer had relatively higher adhesion ability than the others. A number of studies have investigated the relationship between the colonization of *H. pylori* in the mouth and stomach, and suggested that the mouth is a potential reservoir for *H. pylori* and a possible route for transmission to other sites.[31−33] In this study, we observed high adhesion ability of *H. pylori* strains to oral epithelial cells (H357 cells), especially strains from patients with gastric cancer, thus supporting the presence of *H. pylori* in the mouth. However, the mechanisms and consequences of *H. pylori* adhesion to oral mucosa cells are still unclear and need to be clarified. It is important to note that surface receptors on H357 and Caco-2 cells for *H. pylori* strains were different from that of HIEC-6 cells. Our findings suggested that the surface receptors on H357 and Caco-2 cells appeared to be glycoproteins, while those on HIEC-6 cells were lipoproteins.

Anti-adhesion and co-aggregation have been considered as beneficial properties for probiotic strains due to their ability to inhibit *H. pylori* colonization to host mucosa cells. Previous research has shown that the efficacy of probiotics is

strain specific and that different strains can provide different benefits to the host. This is consistent with the findings of our previous study in which three probiotic strains (SD7, SD4, SD11) had high levels of adhesion to H357 and Caco-2 cells; this correlated to the hydrophobicity of individual strains.[22] These strains also provided high levels of anti-adhesion and co-aggregation for *H. pylori* strains. Similarly, previous research demonstrated that three probiotic strains (SD7, SD4, SD11) exhibited high anti-adhesion and co-aggregation properties for oral pathogens such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum,* and *Aggregatibacter actinomycetemcomitans*.[22] In addition, we found that the anti-adhesion ability of probiotic strains also depended on the adhesion ability of *H. pylori* strains. We found that *H. pylori* strains with the high adhesion ability (>70% adhesion) tended to resist the anti-adhesion of probiotic strains. Moreover, co-aggregation between probiotics and *H. pylori* strains were high in acidic conditions between pH 3−5. Therefore, acidic conditions may promote the co-aggregation of probiotics to *H. pylori* strains.

Conclusion

As adhesion to the cavity is the first step for *H. pylori* colonization, it follows that disruption at this stage could help to prevent *H. pylori* infection. This study provides information relating to the adhesion abilities of clinical *H. pylori* and probiotic strains to the oral mucosa compared to the intestinal mucosa. All probiotic and *H. pylori* strains could adhere to H357 significantly better than Caco-2 and HIEC-6 cells. Three probiotic strains (SD7, SD4, SD11) showed significantly higher levels of adhesion than the others. Of the clinical *H. pylori* strains, isolates from a patient with gastric cancer had the highest adhesion ability of all cell lines tested. Anti-adhesion and co-aggregation of probiotic strains are related to certain strains of probiotics and the clinical status of *H. pylori* strains (gastric cancer). Probiotic strains that exhibit high adhesion ability could provide high levels of anti-adhesion and co-aggregation against *H. pylori* strains. Acidic conditions could encourage the co-aggregation of probiotics to *H. pylori* strains. As the oral cavity is a reservoir for *H. pylori*, such information may be useful for the eradication of this bacterium from the oral cavity, thus leading to the successful prevention of transmission and recolonization of *H. pylori* to gastric organs.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The research study obtained ethical approval (No. REC.63-540-10-1, Approval Date: 3 September 2021) from the office of the Human Research Ethics Committee (HREC) at the Faculty of Medicine, Prince of Songkla University.

Authors' contribution

SS, MW, PR, NP were involved in the concept and design of this study, NJ performed the experiments, data analysis, and wrote the manuscript. SS, AK, MW, PR and NP collected samples from patients and revised the manuscript. RT was involved in the concept and design of the study, and helped to write and revise the study. RT also approved the final manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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