JARQ 60 (2), 167-174 (2026) https://www.jircas.go.jp

Characterization of Candidate Lactic Acid Bacteria with Dual Application for Biopreservation and Probiotics

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Abstract

Lactic acid bacteria (LAB) are used in the food industry for fermentation and preservation. Additionally, certain LAB provide beneficial activity to animal or human health. This study assessed five LAB strains, including *Latilactobacillus sakei* A60 and 4-44, *Lactiplantibacillus paraplantarum* D02, and *Latilactobacillus curvatus* 4-36 and 4-43, as new fermentation starters with both antimicrobial and probiotic activities. The strains showed growth at 5°C and over 6% (w/v) NaCl, which are the conditions expected in food processing and storage. Their antimicrobial activity against eight microorganisms commonly associated with food spoilage was examined at various growth temperatures. They exhibited antimicrobial activity at 25°C, 30°C, and 37°C and showed higher activity at 25°C than at 30°C and 37°C. All strains except D02 showed antimicrobial activity at 5°C. In terms of probiotic activities, all strains showed high or comparable mucin-binding activity, and strains A60 and 4-43 exhibited relatively higher tolerance to bile than that of *Lacticaseibacillus rhamnosus* GG, a potential probiotic bacterium. Strains A60, D02, and A-36 showed increased mucin adhesion via culturing with 6% (w/v) NaCl. These strains would be useful not only in food processing and storage but also in health-related applications, as they colonize the intestine and suppress the growth of pathogenic bacteria.

Discipline: Food
Additional key words: antimicrobial activity, mucin-binding activity

Introduction

Lactic acid bacteria (LAB) are traditionally used in food production. They produce various metabolites, such as lactic acid and diacetyl, which contribute to the flavor and taste of foods (Wang et al. 2021). Moreover, certain LAB strains produce antimicrobial peptides referred to as bacteriocins, which are ribosomally synthesized, thermo- and low-pH-stable, and degradable via proteases (Cotter et al. 2013). Although the activities of LAB bacteriocins are generally limited against Gram-positive bacteria that are phylogenetically similar to their producers, various peptides exerting activity against foodborne pathogens have been identified, such as *Listeria* and *Staphylococcus* (Jiang et al. 2022, Mechoud et al. 2017). The producer strains of bacteriocins are valuable and used as fermentation starters to prevent microbial contamination (Dicks et al. 2004, Yilmaz et al. 2022).

Recently, the applications of LAB have expanded from food production to health, based on the recognition of probiotics. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO 2002). The adhesion of bacterial cells to host mucosal surfaces is a critical process that enables the execution of probiotic functions, such as colonization of the gastrointestinal (GI) tract to provide antagonistic activity against pathogens (Lee et al. 2003) and stimulation of the immune system (Plant & Conway 2002). Additionally, the target molecule of cell adhesion is mucin, a sugar-linked protein complex and a component of the mucosal surface (Nishiyama et al. 2016). Currently, metagenomic studies have reported that LAB strains harboring biosynthetic bacteriocin gene clusters are isolated from the microflora of human GI tracts, suggesting that the antimicrobial activity of LAB bacteriocins may protect the GI tract from harmful bacteria and contribute to maintaining a

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Received 16 May 2025; accepted 25 August 2025; J-STAGE Advanced Epub 28 January 2026.
https://doi.org/10.6090/jarq.25J04

balance of microbiota (Garcia-Gutierrez et al. 2019, Walsh et al. 2015).

Novel LAB strains and their functions have been explored worldwide, leading to the expansion of their applications (Liu et al. 2021, Yue et al. 2021). A mixture of several bacterial starters is used for fermentation, as biopreservatives, and for their probiotic properties. This requires the evaluation of processing conditions, such as the optimum growth temperature for each strain and any undesirable effects they may have on each other. Selecting dual-functional LAB, which have both antimicrobial and mucin-binding activities, will save screening efforts for each strain with various applications and reduce production costs by simplifying food manufacturing processes. We screened for strains showing antimicrobial activity and evaluated their suitability in food production and biopreservation. Several indices of probiotics, including tolerance to GI stress and mucin-binding ability, were evaluated.

Materials and methods

1. Bacterial strains and growth conditions

Table 1 lists the strains used in this study. *Latilactobacillus sakei* A60 and 4-44, *Lactiplantibacillus paraplantarum* D02, and *Latilactobacillus curvatus* 4-36 and 4-43 were isolated from pickles and stored at −80°C in deMan, Rogosa, and Sharpe (MRS) broth (Oxoid, Basingstoke, United Kingdom) and 15% (v/v) glycerol (Nacalai Tesque, Kyoto, Japan). These strains were cultured in MRS broth at 30°C for 20 h before use as a pre-culture. *Latilactobacillus sakei* subsp. *sakei* JCM 1157^T, *Pediococcus pentosaceus* JCM 5885, *Leuconostoc mesenteroides* subsp. *mesenteroides* JCM 6124^T, and *Lactobacillus dextrinicus* JCM 5887^T cultured in MRS broth at 30°C for 18 h were used as indicator strains in antimicrobial activity assays. *Bacillus coagulans* JCM 2257^T was cultured in trypticase soy broth (BD, Sparks, MD, USA) containing 0.6% (w/v) yeast extract (BD) (Tryptic Soy Broth with Yeast Extract: TSB-YE) at 37°C for 24 h with agitation. *Bacillus subtilis* JCM 1465^T and *Bacillus circulans* JCM 2504^T were cultured in TYB-YE broth at 30°C for 24 h, also with agitation. *Listeria innocua* JCM 32814^T was cultured in TYB-YE broth at 37°C for 24 h. *Lacticaseibacillus rhamnosus* GG (ATCC 53103^T), a human-origin and potential probiotic bacterium (Capurso 2019) that can bind with mucin (Styriak et al. 2003), was cultured in MRS broth at 37°C for 24 h. *Lactiplantibacillus paraplantarum* JCM 12533^T and *Latilactobacillus curvatus* JCM 1096^T were used as

Table 1. Strains used in this study

Species	Strain No.	Source
Selected strains		
Latilactobacillus sakei	A60	NITE[a]
Latilactobacillus sakei	4-44	NITE
Lactiplantibacillus paraplantarum	D02	NITE
Latilactobacillus curvatus	4-36	NITE
Latilactobacillus curvatus	4-43	NITE
Reference strains		
Lacticaseibacillus rhamnosus	ATCC 53103^T	ATCC[b]
Lactiplantibacillus paraplantarum	JCM 12533^T	JCM[c]
Latilactobacillus curvatus	JCM 1096^T	JCM
Indicator strains		
Pediococcus pentosaceus	JCM 5885	JCM
Leuconostoc mesenteroides subsp. *mesenteroides*	JCM 6124^T	JCM
Lactobacillus dextrinicus	JCM 5887^T	JCM
Latilactobacillus sakei subsp. *sakei*	JCM 1157^T	JCM
Bacillus coagulans	JCM 2257^T	JCM
Bacillus subtilis	JCM 1465^T	JCM
Bacillus circulans	JCM 2504^T	JCM
Listeria innocua	JCM 32814^T	JCM

[a] NITE: National Institute of Technology and Evaluation, Shibuya, Japan
[b] ATCC: American Type Culture Collection, Manassas, Virginia, US
[c] JCM: Japanese Collection of Microorganisms, Wako, Japan

reference strains in a high-salt tolerance test. These two strains were cultured as described above for the same species.

2. Antimicrobial activity assay

The antimicrobial spectrum was tested against various indicator strains using the spot-on-lawn method (Ennahar et al. 2001). Furthermore, the relationships between antimicrobial activity and growth temperature were analyzed to evaluate the utility of the strains in food production and preservation. All the pre-cultured objective LAB strains were inoculated in 5 mL MRS broth (1%, v/v) and then cultured at 5°C for two weeks and at 25°C, 30°C, and 37°C for 20 h. The cell-free culture supernatants were obtained via filtration using 0.25 μm cellulose acetate filters (DISMIC; ADVANTEC, Tokyo, Japan). The antimicrobial activity assay was performed based on the spot-on-lawn method. Briefly, 10 μL of twofold-diluted culture supernatants were spotted onto 7 mL of Lactobacilli Agar AOAC (LAA; BD) or TSB-YE agar (1.5%, w/v), which was inoculated (1%, v/v) with *L. sakei* JCM 1157^T, *P. pentosaceus* JCM 5885, *L. mesenteroides* subsp. *mesenteroides* JCM 6124^T, and *L. dextrinicus* JCM 5887^T or *B. coagulans* JCM 2257^T, *B. subtilis* JCM 1465^T, *B. circulans* JCM 2504^T, and *L. innocua* JCM 32814^T, respectively. The growth inhibition zones of the indicator strains were evaluated after overnight incubation. The activity titer of each culture supernatant was expressed in arbitrary activity units (AUs) per mL, calculated using the reciprocal of the highest dilution that inhibited growth. Biological triplicates were created for all tests.

3. Growth characteristics at high salt concentration

Each broth with high salt concentrations (3, 6, 9, and 12%, w/v NaCl in MRS broth) was inoculated with 1% (v/v) of a fresh overnight broth culture and then incubated at 30°C. The growth of objective LAB strains for 1 d was determined by visual inspection.

4. Tolerance to GI conditions

Washed overnight, cultured cells were suspended in 0.85% (w/v) NaCl (pH 6.5), and the suspension was plated onto MRS agar plates. Next, the cells were resuspended in 0.85% (w/v) NaCl adjusted to pH 2.5 using 1 N HCl and incubated at 37°C for 1.5 h and then plated onto MRS agar plates (Kimoto-Nira et al. 2010). The number of surviving cells was determined via a plate count procedure on MRS agar. All plates were then incubated at 30°C for 1-2 d except for strain GG, which was cultured at 37°C. The tolerance to bile was determined as follows. MRS broth was dispensed in 4 mL volumes and sterilized by autoclaving at 121°C for 15 min, Bacto Oxgall (BD) was dissolved in distilled water and sterilized via autoclaving at 121°C for 15 min, and sterilized Oxgall solution was added to the broths to a final concentration of 0.3% (v/v), which is considered a suitable bile concentration in a medium for selecting probiotic bacteria for human use (Gilliland et al. 1984). Each broth was inoculated with 0.04 mL of a fresh overnight broth culture and then incubated at 37°C. After a 24 h incubation, bacterial growth was determined by measuring the absorbance of culture suspensions at a wavelength of 620 nm using a Spectronic 20 spectrophotometer (Bausch & Lomb, Rochester, NY, USA) against an uninoculated broth blank. Bile tolerance was calculated by dividing the experimental data by those of control values (growth without bile) (Kimoto-Nira et al. 2015a).

5. Microtiter plate mucin-binding assay

All strains cultured under different conditions were assayed for binding to mucin (partially purified type III porcine stomach, Sigma-Aldrich, Saint Quentin Fallavier, France) immobilized on 96-well immunoplates as described previously (Kimoto-Nira et al. 2015b). Microplates (Maxisorp Nunc, Roskilde, Denmark) were covered with approximately 0.5-1.0 mg/mL mucin in 50 mmol/L carbonate/bicarbonate buffer (pH 9.6) overnight at 4°C. After immobilization, the wells were washed three times with phosphate-buffered saline (PBS) and blocked for 1 h with PBS plus 1% (v/v) Tween 20 at 37°C. Subsequently, 100 μL of each strain suspended in 0.85% (w/v) NaCl and adjusted to an optical density at 620 nm of 1.0 was added to each well. The plates were incubated for 2 h at 37°C. Non-adherent cells were removed by washing three times with 200 μL of PBS plus 0.05% (v/v) Tween 20, and the plates were then dried at 55°C. Adherent cells were stained by adding 100 μL of crystal violet solution (1%, w/v crystal violet in 33%, v/v acetic acid (Narisawa et al. 2005)) to each well for 45 min. After two washes with PBS, 100 μL of 50 mmol/L citrate (pH 4.0) was added to each well, and the plates were incubated at room temperature for 45 min. The absorbance at 595 nm was measured using a plate reader (iMark, Bio-Rad, Irvine, CA, USA), indicating the level of adherent bacterial cells. Blank wells without bound mucin served as controls. *Lacticaseibacillus rhamnosus* GG was used as a positive control.

6. Statistical analysis

Data are expressed as the mean or the mean with standard deviation from two or three culture samples. Results of antimicrobial assays are representative of three

independent experiments. Statistical comparisons were analyzed using a Student's t-test to compare cells grown with and without 6% (w/v) NaCl. EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria), was used to calculate the difference in multiple comparisons by applying the Tukey-Kramer test (Kanda 2013). A *P*-value < 0.05 was considered significant. A *P*-value < 0.1 was considered a trend.

Results and Discussion

1. Antimicrobial activity and growth temperatures

Five LAB strains (*L. sakei*, *L. curvatus*, and *L. paraplantarum*) out of 81 strains from laboratory collection were pre-screened for antimicrobial activity against *L. sakei* JCM 1157^T via direct colony assays (Barefoot & Klaenhammer 1983) (data not shown) and were further analyzed. If LAB-derived antimicrobial compounds, such as bacteriocin, exert strong bactericidal or bacteriostatic effects against various foodborne pathogens, the supplementation of bacteriocin itself and the LAB into food production processes will be effective. In this study, we focused on antimicrobial activity at various growth temperatures, which are key conditions for food production and storage.

Initially, the growth of the five strains cultured at various temperatures was investigated (Table 2). All tested strains showed maximum growth at 30°C for culturing, except strains 4-43 and 4-44. Strains A60, 4-43, and 4-44 showed growth under 5°C for two weeks, which may be desirable in food preservation.

Next, the antimicrobial activity at different growth temperatures was evaluated (Table 2). The broadest spectrum was observed in strain *L. sakei* A60 cultured at 25°C. Although strain 4-44 was classified as *L. sakei*, similar to strain A60, its activity spectrum was limited to *L. sakei* JCM 1157^T and *B. circulans* JCM 2504^T alone. Pediocin-like bacteriocins (e.g., sakacins, which are typical bacteriocins produced by *L. sakei*) contain the YGNGVXC motif, which contributes to strong antagonistic effects against *Listeria* (Chikindas et al. 1993, Rodriguez et al. 2002). However, *L. sakei* 4-44

Table 2. Antimicrobial activity of the tested strains at different growth temperatures

Strain	Growth temperature (°C)[a]	OD_{620}	Indicator							
			JCM 5885	JCM 6124^T	JCM 5887^T	JCM 1157^T	JCM 2257^T	JCM 1465^T	JCM 2504^T	JCM 32814^T
A60	5	1.05	-[b]	200[c]	200	600	600	-	800	1200
	25	1.17	300	200	500	800	800	100	800	1200
	30	1.3	100	100	600	900	400	-	800	1200
	37	1.2	-	-	100	800	-	-	300	900
D02	5	0.44	-	-	-	-	-	-	-	-
	25	1.48	200	-	400	300	300	-	300	500
	30	1.5	200	-	100	200	300	-	-	500
	37	1.35	-	-	100	300	-	-	-	500
4-36	5	0.75	-	200	100	900	600	-	700	1200
	25	1.18	-	-	300	700	500	-	800	1100
	30	1.25	-	-	200	800	300	-	700	1000
	37	1.2	-	-	-	600	-	-	300	900
4-43	5	1.02	-	200	100	800	600	-	600	1100
	25	1.08	-	-	-	700	300	-	600	900
	30	1.1	-	-	-	600	200	-	500	900
	37	1.1	-	-	-	600	-	-	500	900
4-44	5	1.02	-	-	-	900	-	-	-	-
	25	1.35	-	-	-	1000	-	-	300	-
	30	1.31	-	-	-	900	-	-	300	-
	37	1.17	-	-	-	900	-	-	100	-

[a] All strains were cultivated at 5°C for 2 weeks, or 25°C, 30°C, and 37°C for 20 h.
[b] A hyphen indicates that no activity was observed.
[c] The activity titer of each culture supernatant was expressed in arbitrary activity units (AUs) per mL, which was calculated using the reciprocal of the highest dilution causing growth inhibition. Biological triplicates were created for all tests.

showed limited activity related to sakacin B but differed in its activity against *B. circulans* (Samelis 1994). *Lactiplantibacillus paraplantarum* strain D02 showed moderate activity against all strains except *L. mesenteroides* subsp. *mesenteroides* JCM 6124^T and *B. subtilis* JCM 1465^T. Only three studies have described the production of bacteriocins in *L. paraplantarum*, specifically paraplantaricins C7, TC318, and L-ZB1 (Gui et al. 2014, Hussein et al. 2020, Lee et al. 2007). The activity of the 4-36 and 4-43 strains of *L. curvatus* differed from that of *L. dextrinicus* JCM 5887^T. Their inhibitory effects against *L. innocua* JCM 32814^T suggested that strains 4-36 and 4-43 produce pediocin-like bacteriocins since *Latilactobacillus curvatus* strains are known to produce sakacins and curvacins.

The correlation between growth temperature and antimicrobial activity is a crucial factor in food processing. In this study, the maximum growth of several strains was observed at 30°C, but their maximum antimicrobial activity was observed at 25°C. Since the bacteriocin production is generally induced in the log phase of growth (Diep et al. 2000), this was considered to be the effect of a slightly elongated log phase. In addition, it has been reported that bacteriocin production can be influenced by growth conditions, such as media components, growth temperature, and pH. These effects vary among different strains (Yang et al. 2018). Further study is needed to clarify the relationship between growth conditions and antimicrobial activity in the tested strains for their application in food processing.

Regarding the utility in food preservation, strains A60, 4-36, and 4-43 showed a broad antimicrobial spectrum after two weeks of incubation at 5°C. Notably, the three strains retained their strong activity at 5°C against *L. innocua* JCM 32814^T, which is closely related to the foodborne pathogen *Listeria monocytogenes* (Coma et al. 2001). Although the molecular identification and further characterization of antimicrobial compounds produced by these five strains are necessary, variations in their antimicrobial spectra may contribute to the availability of many types of food materials, fermentation procedures, and the reduction of microbial contamination.

2. Growth in high salt concentrations

To evaluate the tolerance to high salt concentrations available for a specific food (i.e., pickles and fermented sausage), the growth of the tested strains in MRS broth containing NaCl at 3, 6, 9, and 12% (w/v) was determined and compared with that of their type strains. All tested strains grew in 3% (w/v) NaCl-containing broth, but not in 12% (w/v) NaCl. *Latilactobacillus sakei* A60 and 4-44 grew in MRS broth containing 6% NaCl, although the type strain *L. sakei* JCM 1157^T did not. Among the *L. sakei* strains, the highest salt tolerance was observed in strain 4-44, which tolerated up to 9% (w/v) NaCl. For *L. paraplantarum*, the growth of strain D02 was confirmed in 9% (w/v) NaCl-containing broth, whereas the type strain JCM 12533^T tolerated up to 6% (w/v) NaCl. *Latilactobacillus curvatus* 4-36 and 4-43 showed growth comparable to that of the strain JCM 1096^T type in MRS containing 6% (w/v) NaCl and potentially survived in broth containing 9% (w/v) NaCl.

3. Tolerance to GI environment

The tolerance of the tested strains to low pH and bile exposure was assessed to evaluate their potential as probiotics. The viable cell counts of the strains, with and without exposure to low pH, are shown in Table 3. Before treatment, the number of colonies of the tested strains was estimated to be 10^9 colony-forming units (CFU)/mL. After exposure to pH 2.5 for 1.5 h, the number of viable cells decreased; however, the cells were not entirely killed under these conditions. The delta log CFU/mL of the tested strains ranged from 1.82 to 3.79 and was 0.37 for the positive control strain, GG.

The growth characteristics of the strains in MRS broth with and without bile are presented in Table 4. Bile tolerances were calculated based on the growth in MRS broth without bile serving as the control. All tested strains showed tolerance against 0.3% (w/v) bile, ranging from 28.1 to 60.3%; the tolerance for strain GG was 41.5%.

Probiotics need to be resistant to the digestive process in the stomach (exposure to low pH) and in the intestine (exposure to bile). Furthermore, many bacterial species require intestinal attachment for colonization of the GI tract. The antimicrobial activity is retained when bacteria colonize and grow in the intestine, where pathogen growth is inhibited via mechanisms such as competitive adhesion to intestinal cells, competition for

Table 3. Tolerance to low pH of the tested strains

	Viable counts (log CFU/mL)		
Strain	0 h	1.5 h	Delta log CFU/mL
A60	8.86	5.29	3.57
D02	9.40	7.58	1.82
4-36	9.08	5.50	3.58
4-43	8.98	5.62	3.36
4-44	9.03	5.24	3.79
GG	9.26	8.89	0.37

Results are presented as the mean of two samples. Delta log CFU/mL is expressed as the change in log CFU/mL resulting from treatment with low pH. CFUs are colony-forming units.

Table 4. Tolerance to bile of the tested strains

Strain	Absorbance at 620 nm		
	Control	Bile	Bile tolerance (%)
A60	1.70	0.84	49.4
D02	2.13	0.70	32.9
4-36	1.66	0.60	36.1
4-43	1.41	0.85	60.3
4-44	1.71	0.48	28.1
GG	2.75	1.14	41.5

All values are expressed as the mean of three samples. Bile tolerance was calculated by dividing the experimental data by control values. Bile, MRS broth containing 0.3% bile; Control, MRS broth containing no bile.

nutrients, and the production of antibacterial substances, including bacteriocins. Probiotics do not affect the intestinal environment when ingested unless the population in the intestine reaches a minimum level of 10^6 to 10^8 CFU/g in feces (Marteau & Rambaud 1993). Viable LAB can be detected at cell densities of > 10^8 CFU/g in certain fermented meats according to a study where data were described as either within the product shelf life or after ripening or maturation of sausages (Rezac et al. 2018). For example, exposure to low pH and bile reduced the survival rate of strain D02 to < 1/100 and < 1/10, respectively, in the present study. Thus, the intake of approximately 10^9 to 10^{10} CFU/g of strain D02 in fermented sausages may correspond to a cell density in the intestine of approximately 10^6 to 10^7 CFU/g. This would meet a desirable level of viability for probiotics. On the other hand, except for strain D02, a lower tolerance of low pH was observed than for strain GG. It has been reported that milk can protect cells from stress such as the conditions found in the gastrointestinal tract (Conway et al. 1987). Thus, their survival may increase by the incorporation of milk, such as milk sausage.

4. Mucin-binding activities under different growth conditions

The mucin-binding activity of the tested strains was analyzed to determine whether they could colonize the GI tract. All strains exerted comparable or significantly ($P < 0.05$) higher mucin-binding activity after 1 d of culturing at 37°C, compared with that of strain GG, which was cultured at 37°C and used as a positive control (Fig. 1-a).

The attachment of strains to GI surfaces is often a primary selection criterion when screening for potential probiotic strains (Sun et al. 2022, Yu et al. 2022). However, specific probiotic properties may be altered to a relatively greater extent by manipulating culture conditions, such as the type of food processing (Kimoto-Nira et al. 2015b). The present study also evaluated the effects of growth temperature on adhesion to mucin. After 1 d of culturing at 25°C, all strains showed adhesion to mucin. Among them, strain 4-44 showed the highest adhesion activity ($P < 0.05$, Fig. 1-b). After two weeks of culturing at 5°C, all tested strains except A60 showed mucin-binding activity, among which the activity of strain 4-44 was the highest ($P < 0.05$, Fig. 1-c). This indicates that the strains, especially strain 4-44, adhere to mucin at the usual temperature for food processing (25°C) and during storage (4°C).

The mucin-binding activities of cells cultured in MRS broth at 30°C for 1 d with and without NaCl are shown in Figure 1-d. The NaCl concentration was set to 6% (w/v), which was the experimental limit of all tested strains. Mucin-binding activity was observed in all tested strains cultured with and without NaCl; exposure to 6% (w/v) NaCl promoted significant adhesion to mucin in strains A60 and 4-36 ($P < 0.05$) and a tendency in strain D02 ($P < 0.1$). In contrast, adhesion to mucin was significantly reduced upon exposure to 6% (w/v) NaCl in strains 4-43 and 4-44 ($P < 0.05$). It has been reported that salt stress can affect the morphology and membrane composition of some LAB, reducing their adhesion to human intestinal epithelial-like Caco-2 cells (Gandhi & Nagendra 2016). Analyzing the characteristics of cells grown with and without NaCl is necessary to clarify the mechanism associated with the increased adhesion observed upon culturing with 6% (w/v) NaCl in strains A40, 4-36, and D02. High NaCl concentrations are commonly used in the food industry, as seen in the production of fermented sausage (Hu et al. 2022). When these strains are incorporated into fermented sausage, the probability of their adhesion to the intestine is increased, which is a beneficial property for probiotics. A pilot test of manufacturing fermented sausage using these strains will be necessary.

Conclusion

Reports on the *L. sakei*, *L. curvatus*, and *L. paraplantarum* strains showing both antimicrobial and mucin-binding activities or binding to intestinal epithelial cells such as Caco-2 and tolerance to GI stress (Haller et al. 2001, Martin et al. 2009) are limited. Our research studied five strains of these species to investigate the effect of growth conditions on their antimicrobial and mucin-binding activities. The study examined them for potential uses in food processing, such as the production of fermented sausage and pickles in high salt

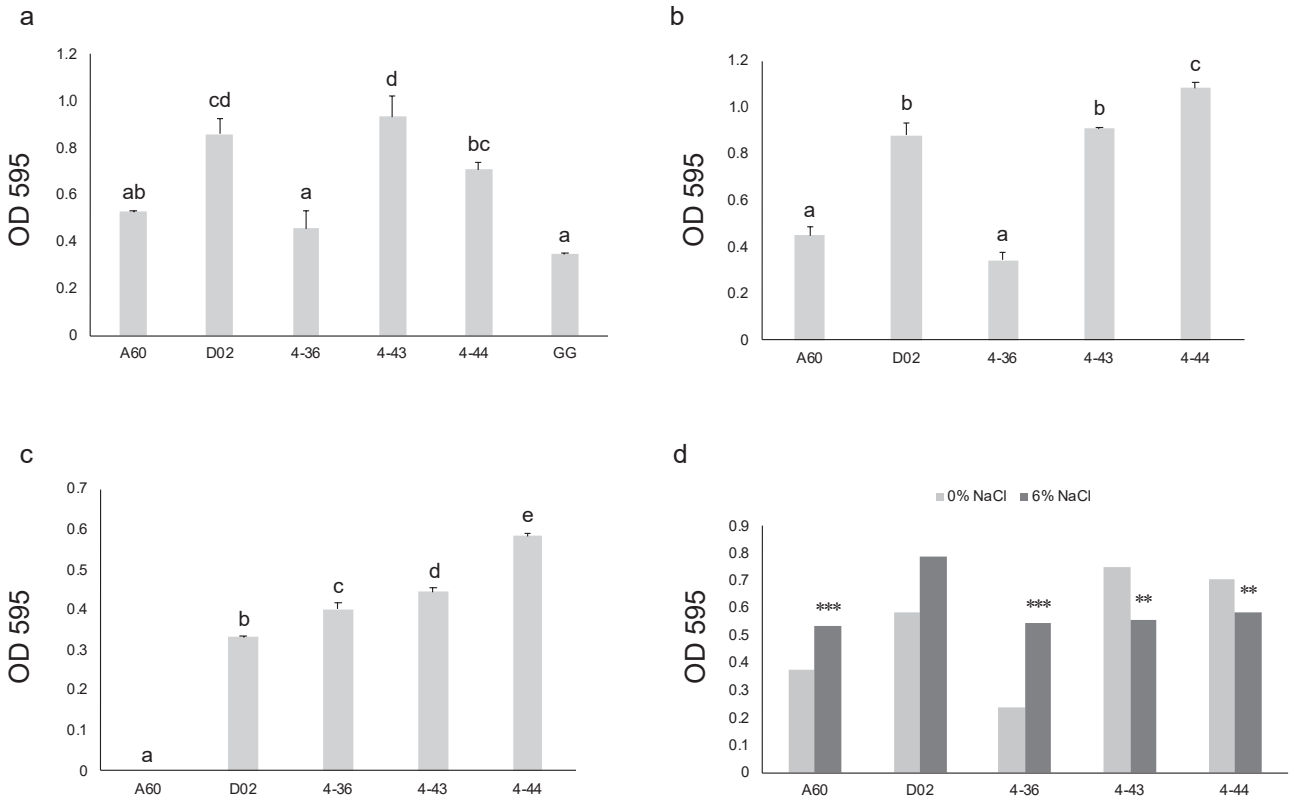

Fig. 1. Binding of the selected strains to porcine gastric mucin immobilized on a microtiter plate at different growth conditions
(a) The tested strains were cultured at 37℃ for 1 d. (b) Cultured at 25℃ for 1 d. (c) Cultured at 5℃ for 14 d. (d) Cultured at 30℃ in 0% (gray bar) or 6% NaCl (black bar) for 1 d. Data are representative of two separate experiments. Results are expressed as the mean values from 2–3 determinations, with standard deviations represented by vertical bars. Asterisks indicate values significantly different from 0% NaCl (**, $P < 0.01$; ***, $P < 0.001$). Mean values with different letters within each strain are significantly different ($P < 0.05$).

concentrations and at low temperatures, biopreservation based on antimicrobial activity, and health applications via suppression of the growth of harmful bacteria. *In vivo* evaluation of the flavor and taste of these fermented foods, as well as the effects of the selected strains on intestinal microbiota, is required before their commercial use.

Acknowledgements

This study did not receive any specific grants from funding agencies in the public, commercial, or non-profit sectors. The National Agriculture and Food Research Organization holds a patent (JP P2021-140313) for the five selected LAB strains.

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