JAST (Journal of Animal Science and Technology) TITLE PAGE
Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Antimicrobial Activity of *Pediococcus pentosaceus* Strains against Diarrheal Pathogens Isolated from Pigs and Effect on Paracellular Permeability of HT-29 cells
Running Title (within 10 words)	Antimicrobial Activity of *Pediococcus pentosaceus* and Effect on Paracellular Permeability
Author	Eunbee Cho[1,#], Yoonjeong Yoo[1,#], Yohan Yoon[1] [#]These authors have contributed equally to this work.
Affiliation	1 Department of Food and Nutrition, Sookmyung Women's University, Seoul 04310, Korea
ORCID (for more information, please visit https://orcid.org)	Eunbee Cho (https://orcid.org/0000-0002-1164-3162) Yoonjeong Yoo (https://orcid.org/0000-0003-4575-3682) Yohan Yoon (https://orcid.org/0000-0002-4561-6218)
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This research was supported by grants from the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (Z-1543081-2020-22-0103).
Acknowledgements	Not applicable.
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Yoon Y Data curation: Cho E, Yoo Y Formal analysis: Cho E, Yoo Y, Yoon Y Methodology: Cho E, Yoo Y, Yoon Y Software: Cho E, Yoo Y Validation: Cho E, Yoo Y Investigation: Cho E, Yoo Y Writing - original draft: Cho E, Yoo Y Writing - review & editing: Cho E, Yoo Y, Yoon Y
Ethics approval and consent to participate	This article does not require IRB/IACUC approval because there are no human and animal participants.

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Yohan Yoon
Email address – this is where your proofs will be sent	yyoon@sookmyung.ac.kr
Secondary Email address	hellorunjing@gmail.com
Address	Sookmyung Women's University, Seoul 04310, Korea
Cell phone number	+82-10-5007-1536
Office phone number	+82-2-2077-7585
Fax number	+82-2-710-9479

Antimicrobial Activity of *Pediococcus pentosaceus* Strains against Diarrheal Pathogens Isolated from Pigs and Effect on Paracellular Permeability of HT-29 cells

Eunbee Cho[a,†], Yoonjeong Yoo[a,†], Yohan Yoon[a]

[a]Department of Food and Nutrition, Sookmyung Women's University, Seoul 140-742, Korea

Corresponding author:

Tel: +82-2-2077-7585
Fax: +82-2-710-9479
E-mail: yyoon@sookmyung.ac.kr

[†]These authors have contributed equally to this work.

ORCID

Eunbee Cho : 0000-0002-1164-3162

Yoonjeong Yoo : 0000-0003-4575-3682

Yohan Yoon : 0000-0002-4561-6218

Abstract

This study aimed to investigate lactic acid bacteria with antimicrobial activities against infectious diarrheal pathogens in pigs and their genetic characteristics. Acid-resistant lactic acid bacteria were examined for bile resistance, pancreatic enzyme resistance, gelatinase and urease activities, and antibiotic resistance. Subsequently, selected isolates were examined for antimicrobial activities against *Campylobacter coli*, *Clostridium perfringens*, *Escherichia coli*, and *Salmonella* Typhimurium, and their effects on paracellular permeability and the expression of tight junction protein-encoding genes in HT-29 cells were assessed. Whole genome sequencing was performed to identify the genes related to safety and antibacterial activity. Of the 51 isolates examined, 12 were resistant to bile and pancreatin and did not produce gelatinase and urease. Of these 12, isolates 19, 20, 30, 36, and 67 showed tetracycline resistance and isolates 15, 19, and 38W showed antimicrobial activity against infectious diarrheal bacteria. Treatment with isolate 38W significantly reduced the paracellular permeability induced by *E. coli* in HT-29 cells and alleviated the expression of tight junction protein-encoding genes (*claudin-1*, *occludin*, and *ZO-1*) induced by *E. coli* inoculation. Isolates 15, 19, and 38W were named as *Pediococcus pentosaceus* SMFM2016-NK1, SMFM2016-YK1, and SMFM2016-WK1, respectively. Bacteriocin-related genes were *YheH*, *ytrF*, *BceA*, *BceB*, and *MccF* in SMFM2016-NK1; *YheH*, *ytrF*, *BceA*, *BceB*, *entK*, *lcnA*, *MccF*, and *skgD* in SMFM2016-YK1; and *YheH*, *ytrF*, *BceA*, *BceB*, and *MccF* in SMFM2016-WK1. SMFM2016-YK1 harbored the *tetM* gene. These results indicate that *P. pentosaceus* SMFM2016-WK1 might control diarrheal pathogens isolated from pigs. However, a further study is necessary because the results were obtained only from *in vitro* experiment.

Keywords: Antimicrobial agent, Feed additive, Probiotics, Gut health, Lactic acid bacteria

Introduction

Diarrhea frequently occurs in weaning pigs and is thus a notable issue at pig farms [1]. The major pathogens in weaning pigs are *Campylobacter* spp., *Clostridium perfringens*, *Escherichia coli*, *Salmonella* spp., group A rotaviruses, and coronaviruses [1]. Pathogenic bacteria cause intestinal infections, leading to swine morbidity and mortality, especially in weaning pigs, resulting in economic losses [2].

Antibiotics have been used in livestock feed for decades to promote health and growth [3]. However, many countries have restricted the use of antibiotics owing to antibiotic resistance. Thus, the development of alternatives to antibiotics, including probiotics, acidification agents, and functional natural extracts, has become a major research area. Among these alternatives, probiotics are mainly used because they can improve intestinal microbial balance and hence play a beneficial role in the host animal [4,5].

Probiotics are living microorganisms that provide health benefits to the host when administered appropriately [6, 7, 8]. Probiotics can enhance host health by producing short-chain fatty acids and regulating the immune system [9]. Moreover, some probiotic bacterial strains can be used as antimicrobial agents in various internal organs such as the intestine, periodontal tract, female urogenital tract, and immune organs [10]. Recently, probiotics have been introduced to feeds to protect weaning pigs from diseases and thus, increase their growth rates [11, 12, 13]. Bacteria such as *Lactobacillus*, *Pediococcus*, *Streptococcus*, *Enterococcus*, *Bifidobacterium*, and lactic acid bacteria have beneficial functional properties and are widely used as probiotics in weaning pigs [14, 15, 16]. A previous study showed that lactic acid bacteria isolated from kimchi exhibited antioxidant and anti-inflammatory effects [17]. Hence, it is worth investigating whether these isolates have antimicrobial activity against pathogenic bacteria and strengthen the gut barrier. Even though selected isolates show the antimicrobial activity, they should survive in the intestinal stress environment with no

harmful effects in the host. Thus, the resistance of isolates to acid, bile and pancreatic enzyme, and their activities of hemolysis, gelatinase, and urease need to be examined [30, 31, 35]. Therefore, this study investigated lactic acid bacteria to control diarrheal pathogens isolated from pigs.

Materials and Methods

1. Preparation of lactic acid bacteria inocula

One hundred microliters of lactic acid bacteria samples, stored at -80°C, were inoculated into 10 mL Lactobacilli de Man, Rogosa and Sharpe (MRS) broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and cultured aerobically at 37°C for 24 h. Following this, 100 μL culture medium was transferred to fresh 10 mL Lactobacilli MRS broth and incubated at 37°C for 24 h. The cultures were then centrifuged at 1,912×*g* and 4°C for 15 min. The cell pellets were washed twice with phosphate-buffered saline (PBS; pH 7.4, 0.2 g KCl, 0.2 g, KH_2PO_4, 8.0 g NaCl, and 1.5 g $Na_2HPO_4 \cdot 7H_2O$ in 1 L distilled water), resuspended in 10 mL PBS, and diluted to 7 Log CFU/mL.

2. Analysis of bile and pancreatic enzyme resistance

A modified version of the method described by Jang [17] and Casey [65] was used for bile resistance analysis. One hundred microliters of each inoculum were inoculated into 10 mL Lactobacilli MRS broth, containing 0.3% porcine bile extract (Sigma, St. Louis, MO, USA), and incubated at 37°C for 24 h. Following inoculation and incubation, 1 mL aliquots were serially diluted in 9 mL of 0.1% buffered peptone water (BPW; Becton, Dickinson, and Company). The diluents (100 μL) were spread-plated on tryptic soy agar (TSA; Becton, Dickinson, and Company). The plates were incubated at 37°C for 48 h, after which the

colonies were counted manually. Pancreatic enzyme resistance was analyzed according to the method described by Plessas et al. [19]. One hundred microliters of each inoculum were inoculated into 10 mL PBS (pH 8.0), containing 0.1% pancreatin from porcine pancreas (Sigma), and incubated at 37°C for 4 h. After inoculation and incubation, 1 mL aliquots were serially diluted in 9 mL of 0.1% BPW. The diluents (100 µL) were spread-plated on TSA. The plates were incubated at 37°C for 48 h, after which the colonies were counted manually. The bile and pancreatic enzyme resistance of the isolates was calculated using the following equations:

Bile resistance = colony counts after 24 h of culture/colony counts at 0 h × 100

Pancreatic enzyme resistance = colony counts after 4 h of culture/colony counts at 0 h × 100.

Lacticaseibacillus rhamnosus GG (LGG), which is known to be effective against diarrhea, was used as the positive control. The results of bile and pancreatic enzyme resistance of the isolates were compared with those of LGG [20].

3. Evaluation of safety

3.1. Analysis of gelatinase and urease production

Gelatinase activity was measured according to the manufacturer's instructions (MB cell, Seoul, Korea). An isolated colony of each strain on Lactobacilli MRS agar (Becton, Dickinson, and Company) was inoculated into 2 mL nutrient gelatin (MB cell). The inoculated medium was incubated at 37°C for 4 days and then stored at 4°C for 30 min. Coagulation of the medium indicated gelatinase activity. *Staphylococcus aureus* ATCC25922 inoculated into 2 mL nutrient gelatin was used as the positive control, while nutrient gelatin was used as the negative control. Urease activity was examined by modifying the method described by Brink [21]. Three microliters of each inoculum were inoculated onto urea agar (pH 6.5), which comprised 20 g yeast extract, 10 g ammonium chloride, 3 g sodium chloride,

20 g urea, 0.012 g phenol red, and 15 g agar dissolved in 1 L distilled water, and incubated at 37°C for 48 h. *Vibrio vulnificus* NCCP11887 and *Escherichia coli* NCCP14038 were used as positive controls.

3.2. Evaluation of antibiotic resistance

To determine the resistance of each isolate to antibiotics, eight antibiotics (ampicillin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, and chloramphenicol) suggested by the European Food Safety Authority [22] were used. The minimum inhibitory concentrations (MICs) of the isolates to each antibiotic were elucidated using antibiotic coated Sensititre™ CAMPY2, and CMV3AGNF MIC plates according to the manufacturer's instructions (TREK Diagnostic Systems Ltd.; Thermo Fisher Scientific Inc., Waltham, MA, USA). The MICs were determined based on the microbiological cut-off reference values suggested by the EFSA [22].

4. Analysis of antimicrobial effect of isolates against diarrheal pathogens

4.1. Preparation of isolate inoculum

One hundred microliters of each strain in 20% glycerol stock were added to 10 mL Lactobacilli MRS broth and incubated at 37°C for 24 h. After that, 100 μL aliquots of culture medium were transferred to 10 mL of a fresh Lactobacilli MRS broth and incubated at 37°C for 24 h. The cultures were then transferred to a 15 mL conical tube and centrifuged at 1,912×*g* and 4°C for 15 min. The cell pellets were washed twice with PBS, resuspended in 10 mL PBS, and diluted to 9 Log CFU/mL. For positive control (PC), 1-g amounts of three commercial probiotics (PC1, PC2, and PC3) were suspended in 9 mL distilled water. The commercial probiotic suspensions were then filtered using a filter bag (3M; St. Paul, MN, USA), and the filtrates were diluted with PBS to achieve an OD_{600} = 1.0. Each lactic acid

150 bacterial suspension and the commercial probiotic diluents (3 μL) were spot-inoculated onto
151 Lactobacilli MRS agar, and the plates were incubated at 37°C for 24 h. Cultured agar plates
152 were then used to overlay the pathogenic bacteria.
153
154 *4.2. Preparation of diarrheal pathogens*
155 Diarrheal pathogens isolated from pigs were obtained from the Korea Veterinary Culture
156 Collection (KVCC; Gimcheon-si, Gyeongsangbuk-do, Korea). A bead stock of each
157 *Campylobacter coli* strain (KVCC-BA1800493, BA1800494, and BA1800595) in 20%
158 glycerol was streaked onto Columbia blood agar (BioMerieux, Marcy-l'Etoile, Lyon, France)
159 and incubated at 42°C for 48 h under microaerobic conditions (5% O_2, 10% CO_2, and 85%
160 N_2) using a microaerobic gas pack (Oxoid Ltd., Basingstoke, UK). Colonies on the Columbia
161 agar were collected using a loop (SPL Life Sciences, Pocheon-si, Gyeonggi-do, Korea) and
162 restreaked onto fresh Columbia blood agar. The plates were incubated at 42°C for 48 h under
163 microaerobic conditions [23]. One hundred microliters of each *Clostridium perfringens* strain
164 (KVCC- BA1900009, BA1900010, BA1900011, and BA1700250) in 20% glycerol stock
165 were inoculated in 10 mL cooked meat broth and cultured at 37°C for 24 h in an anaerobic
166 chamber (Coy Laboratory Products, Grass Lake, MI, USA) containing 90% N_2, 5% CO_2, and
167 5% H_2. Next, 1 mL of the culture was transferred to 10 mL brain heart infusion broth (BHI
168 broth; Beckton Dickinson and Company) and incubated at 37°C for 24 h under anaerobic
169 conditions using an anaerobic gas pack (Oxoid). One hundred microliters of each *E. coli*
170 (KVCC-BA0001423, BA0001823, and BA1600302) and *Salmonella* Typhimurium (KVCC-
171 BA2000160 and BA2000161) strain in 20% glycerol stock were cultured in 10 mL tryptic
172 soy broth (TSB; Beckton Dickinson and Company) at 37°C for 24 h. Then, 100 μL of the
173 culture was transferred to fresh 10 mL TSB and incubated at 37°C for 24 h. Subcultures of
174 the pathogens were harvested using the procedure described in section 4.1.

175

176 *4.3. Agar diffusion assay*

177 Aliquots (100 µL) of *E. coli*, *S.* Typhimurium, and *C. perfringens* inocula were inoculated
178 into soft BHI agar (10 mL), and the inoculated BHI agar was overlaid onto the prepared
179 Lactobacilli MRS agar. The plates were then incubated aerobically (*E. coli* and *S.*
180 Typhimurium) or anaerobically (*C. perfringens*) at 37°C for 24 h. Aliquots (100 µL) of *C.*
181 *coli* inoculum were inoculated into 10 mL soft modified charcoal cefoperazone deoxycholate
182 agar (mCCDA; Oxoid Ltd.), and the inoculated mCCDA agar was then overlaid onto the
183 prepared Lactobacilli MRS agar. The plates were incubated microaerobically at 42°C for 48 h.
184 The size of the growth inhibition zone (mm) was measured using a caliper. The growth
185 inhibition zones of the isolates were compared to those of the positive control [24].

186

187 **5. Analysis of effects of lactic acid bacteria on infectious diarrhea**

188 *5.1. Cell line and culture conditions*

189 To evaluate the effects of the isolates on colonic cells, HT-29 cells,human colorectal
190 cancer cells, were obtained from the Korean Cell Line Bank (Seoul, Korea). The cells were
191 cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone, Logan, UT, USA),
192 supplemented with 10% Fetal Bovine Serum (FBS; Gibco, Thermo Fisher Scientific Inc.) and
193 1% penicillin-streptomycin solution (PS; Gibco), in a 75T flask (Corning Inc., Corning, NY,
194 USA) at 37°C under 5% CO_2 for 24 h. The cultured cells were then transferred to a fresh
195 medium, incubated for another 24 h, and washed with Dulbecco's phosphate-buffered saline
196 (DPBS; Welgene, Gyeongsan, Gyeongsangbukdo, Korea). The cultured cells were then
197 detached using 3 mL of 0.05% trypsin-0.02% EDTA (Gibco) and centrifuged at 217×*g* and
198 25°C for 5 min. The cell pellets were resuspended in 10 mL fresh DMEM supplemented with
199 10% FBS and 1% PS.

5.2. Analysis of paracellular permeability

To examine the paracellular permeability of HT-29, 500 μL HT-29 cells were seeded into the upper chamber of a 12-transwell plate (0.4 μm pore size; Corning Inc.), at a density of 2.5×10^5 cells/well, and cultured to form a monolayer at 37°C under 5% CO_2 for 24 h. The cells were then subjected to no treatment (non-treated) and treatment with *E. coli* NCCP11142 (EC), PC (positive control; LGG), isolate 15 (LAB15), isolate 19 (LAB19), isolate 38W (LAB38W), PC+EC, LAB15+EC, LAB19+EC, and LAB38W+EC. The inocula of the three selected isolates (15, 19, and 38W) and LGG were prepared using the procedure described in section 1. The isolate inocula were diluted with DMEM, containing 10% FBS, to 1×10^8 CFU/mL, and 100 μL of the diluents were inoculated on the upper layer of the transwell plate. Four hundred microliters of DMEM containing 10% FBS without isolates were added to the lower chamber of the transwell and incubated at 37°C under 5% CO_2 for 6 h. After incubation, the cells in the upper layer of the transwell plate were washed three times with DPBS. One hundred microliters of DMEM containing 10% FBS and *E. coli* (1×10^6 CFU/mL) were added to the upper layer of the transwell plate, and the plate was then placed at 37°C under 5% CO_2 for 3 h. As LGG promotes the expression of cytoprotective genes to reduce intestinal permeability and enhance intestinal defense, it was used as the positive control (PC) [25, 26]. After incubation, each upper layer of the transwell was washed three times with DPBS. One hundred microliters of DMEM supplemented with 10% FBS and 1 mg/mL FD-4 (4 kDa molecular weight; Sigma) were added in the upper chamber of the transwell. Four hundred microliters of cell-free DMEM plus 10% FBS were added in the lower layer of the transwell and incubated at 37°C under 5% CO_2 for 3 h. After incubation, the fluorescence of the medium in the lower layer of the transwell was measured to evaluate the paracellular permeability caused by bacterial treatment; this was done according to the

method described by Wang et al. [27], with some modifications. One hundred microliters of the medium in the lower chamber of the transwell plate were collected, and FD-4 concentration was quantified using SpectraMax i3 (Molecular Devices, Chicago, IL, USA) at excitation and emission wavelengths of 485 and 535 nm, respectively. The paracellular permeability caused by bacterial treatment was calculated using the following equation and was shown in "% of control".

$$\text{Paracellular permeability (\%)} = \frac{\text{fluorescence of treated sample}}{\text{fluorescence of control}} \times 100$$

5.3. Analysis of expression of tight junction (TJ) protein-encoding genes

Five hundred microliters of HT-29 cells were seeded into 6-well plates (SPL Life Sciences), at a density of 2.5×10^5 cells/well, and cultured at 37°C with 5% CO_2 for 24 h. Three selected isolates (15, 19, and 38W) were cultured using the same procedure described in section 1. The isolate suspensions were diluted with DMEM, containing 10% FBS, to 1×10^8 CFU/mL. HT-29 cells were pre-treated with the isolate diluent (150 µL) and then cultured at 37°C under 5% CO_2 for 6 h. The supernatant was discarded, and the cells were washed with DPBS. The cells were then treated with DMEM containing 10% FBS and 1×10^6 CFU/mL *E. coli* NCCP11142 and cultured at 37°C under 5% CO_2 for 3 h. After treatment, the supernatant was discarded, and the cells were washed with DPBS. The HT-29 cells were collected and lysed with TRIzol (Invitrogen, Carlsbad, CA, USA) to extract mRNA according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The expression of TJ-encoding genes (*claudin-1*, *occludin*, and *ZO-1)* was determined via quantitative reverse transcription-PCR (qRT-PCR) using the Rotor-Gene SYBR Green PCR kit and Rotor-Gene Q (Qiagen). The 25 µL reaction mixture

contained 1 µL template cDNA, 12.5 µL 2×rotor-gene SYBR® green PCR master mix, 6.5 µL RNase-free water, 2.5 µL forward primer, and 2.5 µL reverse primer. The PCR conditions were as follows: 95°C for 10 min, followed by 40 amplification cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 20 s; the primers used in this study are listed in Table 1. Relative transcription levels were normalized to those of *β-actin*. Relative gene expression was calculated using the $2^{-\triangle\triangle Ct}$ method [28].

6. Whole genome analysis

6.1. DNA library preparation and sequencing

Whole-genome *de novo* sequencing was performed to analyze the genomic characteristics of the selected isolates 15, 19, and 38W. The DNA of each isolate was extracted with the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions. Briefly, 5 µg of each DNA sample was used to construct a library. The library was constructed with SMRTbell™ Template Prep Kit 1.0 (PN 100-259-100) according to the manufacturer's instructions (PacBio, MenloPark, CA, USA). The prepared libraries were sequenced with the PacBio RS II platform (PacBio), which produced continuous long reads. The 20 kb libraries consisting of DNA fragments were then assembled into longer sequences called "contigs". The genomic characteristics of the contigs were analyzed.

6.2. Gene annotation and prediction

The contigs were used for gene annotation and prediction. The Glimmer ver. 3.02 [29] system was used to identify putative gene coding sequences (CDSs) from the contigs and open reading frames (ORFs). Functional gene ontology was predicted and annotated with BLAST2GO (BioBam BioInformatics SL, Valencia, Spain), and the genes were classified into biological processes, cell components, and molecular functions.

6.3. Genomic comparison

Gene sequence and phylogenetic analysis of the selected isolates 15, 19, and 38W were performed with CLC Genomics Workbench ver. 12.0 (Qiagen) and the NCBI database. Whole-genome alignment was used to construct a phylogenetic tree, and an Average Nucleotide Identity (ANI) analysis was performed to confirm the degree of agreement with each genetic sequence.

6.4. Analysis of antibiotic resistance and bacteriocin-related genes

The genetic characteristics of the selected isolates (15, 19, and 38W) were analyzed for antibiotic resistance factors with the CLC Genomics Workbench ver. 12.0 (Qiagen). The sequences of these factors were obtained from the NCBI GenBank database. The presence of any genetic factors related to antibiotic resistance and bacteriocins in the isolates was determined with the Basic Local Alignment Search Tool (BLAST). Antibiotic resistance was assessed by comparing the sequences of all genes.

7. Statistical analysis

Data on bile and pancreatic enzyme resistance, antimicrobial activities, and paracellular permeability were analyzed with PROC MIXED procedure of SAS® version OnDemand for Academics (SAS Institute Inc., Cary, NC, USA). The random effect of replication on treatment group (isolate) was tested, and significant differences in LS means among the treatment groups were determined with Tukey at $\alpha = 0.05$. Data on gene expression level of tight junction proteins were analyzed with PROC GLM procedure of SAS® version OnDemand for Academics (SAS Institute Inc.). Significant differences in LS means among the treatment groups were determined with Tukey at $\alpha = 0.05$.

Results and Discussion

1. Probiotic characteristics of the isolates

1.1. Bile and pancreatic enzyme resistance

For probiotics to function in the intestines, the isolates must resist any digestive enzymes secreted into the duodenum through the stomach at low pH [30]. In this study, 51 acid-resistant isolates identified by Jang [17] were evaluated for bile and pancreatic enzyme resistance (Table 2). Of the 51 isolates, 45.5%–137.1% and 77.5%–104.0% showed resistance against bile and pancreatic enzymes, respectively. Furthermore, 12 bile- and pancreatic enzyme-resistant isolates (2, 9, 11, 15, 19, 20, 30, 36, 38W, 66, 67, and 70) showed significantly higher ($p < 0.05$) efficacy than or similar efficacy as that of the PC (Table 3). Pancreatic enzyme resistance of isolate 50 was the lowest among the significant isolates. Thus, it was excluded for a further analysis. These findings indicate that the isolates 2, 9, 11, 15, 19, 20, 30, 36, 38W, 66, 67, and 70 might survive under conditions similar to those found in the pig intestine.

1.2. Gelatinase and urease activities

None of the 12 isolates hydrolyzed gelatin and were considered gelatinase-negative (data not shown). Gelatinase is considered a pathogenic factor in probiotics when it is secreted extracellularly and hydrolyzes or digests gelatin and collagen [31, 32, 33, 34]. The 12 isolates did not exhibit urease activity (data not shown). Urease activity is an important factor in bacterial pathogenesis. Urease catalyzes the hydrolysis of urea to yield ammonia and carbamate, thereby increasing the pH [35]. Urease is a virulence factor in human and animal infections in the urinary tract or gastrointestinal region [35]. Ammonia production by this

enzyme can lead to renal failure, hepatic failure, and nephrotic syndrome [36]. The results of this study indicated that none of the 12 isolates produced gelatinase or urease.

1.3. Antibiotic resistance

Among the 12 isolates, five (19, 20, 30, 36, and 67) showed tetracycline resistance (Table 4). Antibiotic resistance is an emerging issue, as antibiotic resistance genes can be transferred to commensals or pathogens in the gut [37]. Therefore, it is necessary to confirm the antibiotic resistance ability of probiotic bacteria [38, 61].

2. Effect of isolates on infectious diarrhea

2.1. Antimicrobial effect against diarrheal pathogens

Twelve lactic acid bacteria isolates were selected based on the results of bile and pancreatic enzyme resistance, gelatinase and urease activity analysis, and antibiotic resistance. To select probiotic strains for pigs, the antimicrobial activities of the isolate were examined to diarrheal pathogens such as *C. coli, C. perfringens, E. coli,* and *Salmonella* isolated from pigs [62, 63, 64]. The antimicrobial activities of the 12 isolates against pathogens are presented in Table 5. The diameters of the inhibition zones of the isolates against *C. coli, C. perfringens, E. coli,* and *Salmonella* strains were 16.9–22.2 mm, 13.1–24.7 mm, 14.5–23.3 mm, and 14.4–23.7 mm, respectively. The diameters of the inhibition zones for the positive controls for *C. coli, C. perfringens, E. coli,* and *Salmonella* were 10.3-12.2 mm, 8.7-13.8 mm, 10.3-11.7 mm, and 8.7-14.0 mm, respectively. These results show that the aforementioned 12 isolates exhibit a high antimicrobial activity against diarrheal pathogens. Isolates 15, 19, and 38W showed significantly higher ($p < 0.05$) antimicrobial activities than the other isolates, with isolate 38W exhibiting the highest antimicrobial activity. *C. coli, C. perfringens, E. coli,* and *Salmonella* infections are common causes of severe diarrhea in weaning pigs [39]; these

results suggest that isolates 15, 19, and 38W could be candidate probiotics for further analysis.

2.2. Paracellular permeability

Paracellular permeability was measured FD-4 transport in order to evaluate the protective effects of the three isolates (15, 19, and 38W) on epithelial integrity (Fig. 1). The paracellular permeability was significantly increased ($p < 0.05$) in the EC group compared to that in the non-*E. coli* infected groups (non-treated, PC, LAB15, LAB19, and LAB38W); however, the groups LAB15+EC, LAB19+EC, and LAB38W+EC, which were infected with *E. coli* and treated with isolates 15, 19, and 38W, had lower permeability than the EC group (Fig. 1). The permeability of the LAB38W+EC group was similar to that of the LAB38W group. These results indicate that isolate 38W can protect the gut barrier from increased permeability caused by *E. coli* infection. An imbalance between the abundance of beneficial and pathogenic bacteria in the gut increases the mucosal epithelial permeability, leading to chronic inflammatory diseases [40]. Several external factors, including bacteria, affect intestinal permeability. Furthermore, the primary pathogen in piglets is *E. coli*, which causes an increase in the gut permeability [41]. Acute and persistent diarrhea are associated with increased intestinal permeability, and repeated diarrhea results in malnutrition [42]. Thus, epithelial permeability must be lowered to maintain and enhance intestinal barrier function [43]. Some lactic acid bacteria reduce pathogen-induced permeability of the small intestine [44, 45, 46, 47]. Our results indicate that isolate 38W might alleviate the epithelial damage caused by diarrheal pathogens.

2.3. Expression of genes encoding TJ proteins

The relative expression of genes encoding TJ proteins in HT-29 cells significantly reduced after *E. coli* infection. However, the PC+EC, LAB15+EC, LAB19+EC, and

LAB38W+EC groups did not show this reduction (Fig. 2). TJ proteins play crucial roles in maintaining the integrity and function of the gut barrier. They include transmembrane proteins, such as *claudin* and *occludin*, and cytoplasmic scaffolding proteins, such as *ZO-1*, which have linking and sealing effects [48]. TJ protein expression decreases during weaning, thereby reducing the barrier integrity [49]. Reduced barrier integrity facilitates pathogen penetration and allows toxins to enter the body [50]. Thus, it is important to increase TJ protein expression. Particularly, the LAB38W+EC group showed expression levels of genes encoding TJ proteins (*claudin-1*, *ZO-1*, and *occludin*) similar to those in the *E. coli* untreated group (Fig. 2). This result indicates that isolate 38W may protect the gut barrier from *E. coli* infection. Similarly, various other probiotic strains have been shown to protect and maintain these barriers *in vivo* and *in vitro* [50, 51, 52]. These findings indicate that isolate 38W might be an appropriate probiotic that enhances intestinal epithelial resistance to pathogens by increasing the expression of tight junction proteins.

3. Genomic characteristics of probiotics

3.1. De novo sequencing

The whole genome was obtained by sequencing the DNA of isolates 15, 19, and 38W using *de novo* assembly. The *de novo* assembly yielded six contigs for isolate 15; the sizes were 1,797,082 (contig 1), 56,451 (contig 2), 53,170 (contig 3), 23,413 (contig 4), 18,038 (contig 5), and 15,252 bp (contig 6). The GC contents of contigs 1, 2, 3, 4, 5, and 6 were 37.28%, 39.74%, 38.91%, 36.43%, 37.61%, and 39.14% respectively. Contig 1 of isolate 15 was identified as the chromosome of *P. pentosaceus* using BLAST 2.9.0+ and the NCBI database. Contigs 2, 3, 4, 5, and 6 from isolate 15 were identified as plasmids. Isolate 19 had three contigs; the sizes were 1,795,482 (contig 1), 65,469 (contig 2), and 36,563 bp (contig 3). The GC contents of contigs 1, 2, and 3 were 37.31%, 39.67%, and 35.97%, respectively.

Contig 1 of isolate 19 was identified as *P. pentosaceus* chromosome. Contigs 2 and 3 of isolate 19 were identified as plasmids. Isolate 38W had two contigs, with sizes of 1,809,731 (contig 1) and 12,226 bp (contig 2). The GC contents of contigs 1 and 2 of isolate 38W were 37.32% and 36.19%, respectively. Contig 1 was identified as *P. pentosaceus* chromosome, and contig 2 was identified as a plasmid. Accordingly, isolates 15, 19, and 38W were named as *Pediococcus pentosaceus* SMFM2016-NK1, *Pediococcus pentosaceus* SMFM2016-YK1, and *Pediococcus pentosaceus* SMFM2016-WK1, respectively; their whole-genome sequences were registered at the NCBI under the accession numbers NZ_CP127866.1, NZ_CP127868.1, and NZ_CP127867.1, respectively.

3.2. Gene annotation and prediction

Among the whole-genome sequences of the three isolates, only contig 1 for each isolate had more than 1,000,000 bp (Figs. 3–5). Thus, contig 1 (chromosome) was identified as the complete genome, and contig 1 of each isolate was analyzed. Contig 1 of *P. pentosaceus* SMFM2016-NK1 comprised 1,761 coding sequences (CDS), 15 rRNAs, and 55 tRNAs. Contig 1 of *P. pentosaceus* SMFM2016-YK1 comprised 1,749 CDSs, 15 rRNAs, and 57 tRNAs. Contig 1 of *P. pentosaceus* SMFM2016-WK1 comprised 1,811 CDSs, 15 rRNAs, and 55 tRNAs (Figs. 3–5). The predicted functional genes were divided into three gene ontology categories (biological processes, cellular components, and molecular functions) (Figs. 3B, 4B, and 5B). The transcripts of *P. pentosaceus* SMFM2016-NK1 were found to contain 3,406 biological processes, 1,837 cellular components, and 1,880 molecular functions based on multiple gene ontologies. The transcripts of *P. pentosaceus* SMFM2016-YK1 were found to contain 3,350 biological processes, 2,156 cellular components, and 1,848 molecular functions. The transcripts of *P. pentosaceus* SMFM2016-WK1 contained 3,237 biological processes, 1,828 cellular components, and 1,794 molecular function transcription factors.

These results indicate that the three *P. pentosaceus* isolates possess different genes and thus, exhibit distinct biological functions.

3.3. Genomic comparison with other probiotic bacteria

The genetic characteristics of *P. pentosaceus* strains SMFM2016-NK1, SMFM2016-YK1, and SMFM2016-WK1 were compared with those of 15 reference strains in the NCBI database. The ANI values obtained indicated that the *P. pentosaceus* strains SMFM2016-NK1, SMFM2016-YK1, and SMFM2016-WK1 were the closest to *P. pentosaceus* SS1-3 (99.93%), *P. pentosaceus* SRCM102734 (99.69%), and *P. pentosaceus* SL4 (99.43%), respectively (Fig. 6). According to the phylogenetic tree derived from ANI, the *P. pentosaceus* strains SMFM2016-NK1, SMFM2016-YK1, and SMFM2016-WK1 were genetically distinct from the other *P. pentosaceus* strains. Furthermore, the three selected isolates were genetically distinct from the other *P. pentosaceus* strains (Table 6, Figs. 6 and 7).

3.4. Antibiotic resistance and antimicrobial genes

Through mapping and predicted gene analysis, *P. pentosaceus* SMFM2016-YK1, which was found to be resistant to tetracycline in the MIC analysis, was identified as a carrier of the *tetM* gene (tetracycline resistance ribosomal protection protein) (data not shown). The SMFM2016-NK1 and SMFM2016-WK1 strains, which showed no tetracycline resistance in the MIC analysis, were found to harbor the *tetA* gene (tetracycline efflux gene). The difference in the results of MIC and predicted gene analysis could be due to the low expression levels of genes encoding tetracycline resistance. Lim et al. [53] observed differences in the MICs of isolates with the same resistance gene and found that the expression of resistance-related genes was significantly different among the isolates, resulting

in different MICs. Antimicrobial substances produced by lactic acid bacteria include lactic acid, organic acids, ammonia, and bacteriocins [54, 55]. Bacteriocins are antibacterial extracellularly secreted peptides or proteins, and bacteriocin-producing bacteria are capable of antimicrobial activity [56, 57]. Pediocin, sakacin, nisin, and leucocin are some well-known bacteriocins; the *BceA, BceB,* and *MccF* genes are involved in pediocin synthesis [55, 58]. *P. pentosaceus* SMFM2016-NK1 harbors bacteriocin-related genes (*YheH, ytrF, BceA, BceB,* and *MccF*) and organic acid-related genes (*rackA, ALS, ccl, larA,* and *ldh*). *P. pentosaceus* SMFM2016-YK1 harbors bacteriocin-related genes (*YheH, ytrF, BceA, BceB, entK, lcnA, MccF,* and *skgD*) and organic acid-related genes (*ackA, CcpA, ALS, ALS1, aldC, ccl, ldhA, lldP, larA, larR,* and *ldh). P. pentosaceus* SMFM2016-WK1 harbors bacteriocin-related genes (*YheH, ytrF, BceA, BceB, and MccF*) and organic acid-related genes (*ackA, CcpA, ALS, aldC, ccl, ldhA, larA, larR,* and *ldh)*. Overall, our results indicate that these antimicrobial factors may inhibit the growth of diarrheal pathogens, as shown in Table 5.

Conclusion

Among 51 lactic acid bacteria strains, *P. pentosaceus* SMFM2016-NK1, SMFM2016-YK1, and SMFM2016-WK1 exhibited higher antimicrobial activity against diarrhea-causing pathogens. Of the three isolates, *P. pentosaceus* SMFM2016-WK1 was the most effective on protecting the gut barrier from increased permeability caused by *E. coli* with the increased gene expression associated with tight junction proteins. These results suggest that among the examined isolates, *P. pentosaceus* SMFM2016-WK1 might be a suitable strain to control diarrheal pathogens isolated from pigs. However, since these results were obtained only from *in vitro* experiments, the implication of the results from this study should be limited. Thus, a further study is necessary.

Funding

This research was supported by grants from the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food, and Rural Affairs, Republic of Korea (Z-1543081-2020-22-0103).

Conflict of interest

The authors declare no conflict of interest.

References

1. Ruiz VL, Bersano JG, Carvalho AF, Catroxo MH, Chiebao DP, Gregori F, Miyashiro S, Nassar AFC, Oliveira TMFS, Ogata RA, Scarcelli EP, Tonietti PO. Case–control study of pathogens involved in piglet diarrhea. BMC Res Notes. 2016; 9: 22. https://doi.org/10.1186/s13104-015-1751-2

2. Thomson JR, Friendship RM. Digestive system. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Zhang J, editors. Diseases of swine. Hoboken: Wiley-Blackwell; 2019. p. 234–63.

3. Kirchhelle C. Pharming animals: a global history of antibiotics in food production (1935–2017). Palgrave Commun. 2018; 4: 1–13. https://doi.org/10.2174/1381612053382322

4. Fuller R. Probiotics in man and animals. J Appl Bacteriol. 1989; 66: 365–378.

5. Medellin-Peña MJ, Wang H, Johnson R, Anand S, Griffiths MW. Probiotics affect virulence-related gene expression in Escherichia coli O157: H7. Appl Environ Microbiol 2007;73:4259–67. https://doi.org/10.1128/aem.00159-07

6. De Melo Pereira GV, de Oliveira Coelho B, Magalhães Júnior AIM, Thomaz-Soccol V, Soccol CR. How to select a probiotic? A review and update of methods and criteria. Biotechnol Adv. 2018; 36: 2060–2076. https://doi.org/10.1016/j.biotechadv.2018.09.003

7. Wulandari E, Yurmiati H, Subroto T, Suradi K. Quality and probiotic lactic acid bacteria diversity of rabbit meat Bekasam-fermented meat. Food Sci Anim Resour. 2020; 40: 362–376. https://doi.org/10.5851/kosfa.2020.e16

8. Zhang Y, Yao D, Huang H, Zhang M, Sun L, Su L, Zhao L, Guo Y, Jin Y. Probiotics increase intramuscular fat and improve the composition of fatty acids in Sunit sheep through the adenosine 5′-monophosphate-activated protein kinase (AMPK) signaling pathway. Food Sci Anim Resour. 2023; 43: 805–825. https://doi.org/10.5851/kosfa.2023.e37

9. Lee S, Eom S, Lee J, Pyeon M, Kim K, Choi KY, Lee JH, Shin DJ, Lee KH, Oh S, Lee JH. Probiotics that ameliorate cognitive impairment through anti-inflammation and anti-oxidation in mice. Food Sci Anim Resour. 2023; 43: 612–624. https://doi.org/10.5851/kosfa.2023.e22

10. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Reid G. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017; 14: 491–502. https://doi.org/10.1038/nrgastro.2017.75

11. Barba-Vidal E, Martín-Orúe SM, Castillejos L. Practical aspects of the use of probiotics in pig production: a review. Livest Sci. 2019; 223: 84–96. https://doi.org/10.1016/j.livsci.2019.02.017

12. Xiong X, Tan B, Song M, Ji P, Kim K, Yin Y, Liu Y. Nutritional intervention for the intestinal development and health of weaned pigs. Front Vet Sci. 2019; 6: 46. https://doi.org/10.3389/fvets.2019.00046

13. Zimmermann JA, Fusari ML, Rossler E, Blajman JE, Romero-Scharpen A, Astesana DM, Olivero CR, Berisvil AP, Signorini ML, Zbrun MV, Frizzo LS, Soto LP. Effects of probiotics in swines growth performance: a meta-analysis of randomised controlled trials. Anim Feed Sci Technol.2016; 219: 280–293. https://doi.org/10.1016/j.anifeedsci.2016.06.021

14. Anadón A, Martínez-Larrañaga MR, Aranzazu Martínez MA. Probiotics for animal nutrition in the European Union. Regulation and safety assessment. Regul Toxicol Pharmacol. 2016; 45: 91–95. https://doi.org/10.1016/j.yrtph.2006.02.004

15. Guo H, Fan L, Ding L, Yang W, Zang C, Guan H. Separation and purification of antioxidant peptide from fermented whey protein by Lactobacillus rhamnosus B2-1. Food Sci Anim Resour. 2023; 43: 10–24. https://doi.org/10.5851/kosfa.2022.e52

16. Widodo W, Kusumaningrum HRP, Wihadmadyatami H, Wicaksana AL. Milk fermented with Pediococcus acidilactici strain BE improves high blood glucose levels and pancreatic Beta-cell function in diabetic rats. Food Sci Anim Resour. 2023; 43: 170–183. https://doi.org/10.5851/kosfa.2022.e69

17. Jang HJ. Potential use of lactic acid bacteria isolated from kimchi as probiotics [Master's thesis]. Seoul: Sookmyung Women's University; 2018.

18. Watson D, Sleator RD, Hill C, Gahan CG. Enhancing bile tolerance improves survival and persistence of Bifidobacterium and Lactococcus in the murine gastrointestinal tract. BMC Microbiol. 2008; 8: 176.

19. Plessas S, Nouska C, Karapetsas A, Kazakos S, Alexopoulos A, Mantzourani I,

Chondrou P, Fournomiti M, Galanis A, Bezirtzoglou E. Isolation, characterization and evaluation of the probiotic potential of a novel Lactobacillus strain isolated from Feta-type cheese. Food Chem. 2017; 226: 102–108. https://doi.org/10.1016/j.foodchem.2017.01.052

20. Ann S, Choi Y, Yoon Y. Comparative genomic analysis and physiological properties of Limosilactobacillus fermentum SMFM2017-NK2 with ability to inflammatory bowel disease. Microorganisms. 2023; 11: 547. https://doi.org/10.3390/microorganisms11030547

21. Brink B. Urease test protocol. Amer Soc Microbiology. 2010; 1-7.

22. EFSA panel on additives and products or substances used in animal feed (FEEDAP), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos MDL, Bories G, Chesson A, Cocconcelli P, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, Puente SL, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R, Galobart J. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal. 2018; 16(3): e05206. https://doi.org/10.2903/j.efsa.2018.5206

23. Ha JM, Seo YE, Cho EB, Choi SH, Kim HJ, Yoon YH. Improvement of the detection efficiency of 3M™ molecular detection system for Campylobacter in poultry using nitrogen-doped carbon nanodots. J Microbiol Methods. 2021; 184: 106211. https://doi.org/10.1016/j.mimet.2021.106211

24. Choi YK, Park EY, Kim SJ, Ha JM, Oh HM, Kim YJ, Lee YW, Seo YE, Kang JH, Lee SM, Lee HY, Yoon YH, Choi KH. Alleviation of periodontal disease using Lactobacillus curvatus SMFM2016-NK. J Funct Foods. 2021; 83: 104531. DOI: 10.3168/jds.2020-19625

25. Lin PW, Nasr TR, Berardinelli AJ, Kumar A, Neish AS. The probiotic Lactobacillus GG may augment intestinal host defense by regulating apoptosis and promoting cytoprotective responses in the developing murine gut. Pediatr Res. 2008; 64: 511–516. https://doi.org/10.1203/PDR.0b013e3181827c0f

26. Patel RM, Myers LS, Kurundkar AR, Maheshwari A, Nusrat A, Lin PW. Probiotic bacteria induce maturation of intestinal claudin 3 expression and barrier function. Am J Pathol. 2012; 180: 626–635. https://doi.org/10.1016/j.ajpath.2011.10.025

27. Wang Z, Wang L, Chen Z, Ma X, Yang X, Zhang J, Jiang Z. In vitro evaluation of swine-derived Lactobacillus reuteri: probiotic properties and effects on intestinal porcine epithelial cells challenged with enterotoxigenic Escherichia coli K88. J Microbiol Biotechnol. 2016; 26: 1018–1025. https://doi.org/10.4014/jmb.1510.10089

28. Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 2002; 30: e36. https://doi.org/10.1093/nar/30.9.e36

29. Delcher AL, Bratke KA, Powers EC, Salzberg SL. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics. 2007; 23: 673–679. https://doi.org/10.1093/bioinformatics/btm009

30. Yasmin I, Saeed M, Khan WA, Khaliq A, Chughtai MFJ, Iqbal R, Tehseen S, Naz S, Liaqat A, Mehmood T, Ahsan S, Tanweer S. In vitro probiotic potential and safety evaluation (hemolytic, cytotoxic activity) of Bifidobacterium strains isolated from raw camel milk. Microorganisms. 2020; 8: 354. https://doi.org/10.3390/microorganisms8030354

31. Leboffe MJ, Pierce BE. Microbiology: Laboratory Theory and Application, third ed. Morton Publishing Company, Englewood. Morton Publishing Company. Colorado. 2015.

32. Gupta A, Sharma N. Characterization of potential probiotic lactic acid bacteria-Pediococcus acidilactici Ch-2 isolated from Chuli-A traditional apricot product of Himalayan region for the production of novel bioactive compounds with special therapeutic properties. J Food Microbiol Saf Hyg. 2017; 2:119.

33. Oruc O, Cetin O, Onal Darilmaz DO, Yüsekdag ZN. Determination of the biosafety of potential probiotic Enterococcus faecalis and Enterococcus faecium strains isolated from traditional white cheeses. LWT. 2021; 148: 111741. https://doi.org/10.1016/j.lwt.2021.111741

34. Vergis EN, Shankar N, Chow JW, Hayden MK, Snydman DR, Zervos MJ, Linden PK, Wagener MM, Muder RR. Association between the presence of enterococcal virulence factors gelatinase, hemolysin, and enterococcal surface protein and mortality among patients with bacteremia due to Enterococcus faecalis. Clin Infect Dis. 2002; 35: 570–575. https://doi.org/10.1086/341977

35. Collins CM, D'Orazio SE. Bacterial ureases: structure, regulation of expression and role in pathogenesis. Mol Microbiol. 1993; 9: 907–913. https://doi.org/10.1111/j.1365-2958.1993.tb01220.x

36. Tiwari A, Aryal S, Pilla S, Gong S. An amperometric urea biosensor based on covalently immobilized urease on an electrode made of hyperbranched polyester functionalized gold nanoparticles. Talanta. 2009; 78: 1401–1407. https://doi.org/10.1016/j.talanta.2009.02.038

612 37. Selvin J, Maity D, Sajayan A, Kiran GS. Revealing antibiotic resistance in therapeutic
613 and dietary probiotic supplements. J Glob Antimicrob Resist. 2020; 22: 202–205.
614 https://doi.org/10.1016/j.jgar.2020.02.007

615 38. Temmerman R, Pot B, Huys G, Swings J. Identification and antibiotic susceptibility of
616 bacterial isolates from probiotic products. Int J Food Microbiol. 2003; 81: 1–10.
617 https://doi.org/10.1016/s0168-1605(02)00162-9

618 39. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL,
619 Griffin PM. Foodborne illness acquired in the United States—major pathogens. Emerg
620 Infect Dis. 2011; 17: 7–15. https://doi.org/10.3201/eid1701.p11101

621 40. Kozakova H, Schwarzer M, Tuckova L, Srutkova D, Czarnowska E, Rosiak I, Hudcovic
622 T, Schabussova I, Hermanova P, Zakostelska Z, Aleksandrzak-Piekarczyk T,
623 Koryszewska-Baginska A, Tlaskalova-Hogenova H, Cukrowska B. Colonization of
624 germ-free mice with a mixture of three Lactobacillus strains enhances the integrity of
625 gut mucosa and ameliorates allergic sensitization. Cell Mol Immunol. 2016; 13: 251–
626 262. https://doi.org/10.1038/cmi.2015.09

627 41. Haroun E, Kumar PA, Saba L, Kassab J, Ghimire K, Dutta D, Lim SH. Intestinal barrier
628 functions in hematologic and oncologic diseases. J Transl Med. 2023; 21: 233.
629 https://doi.org/10.1186/s12967-023-04091-w

630 42. Boaz RT, Joseph AJ, Kang G, Bose A. Intestinal permeability in normally nourished and
631 malnourished children with and without diarrhea. Indian Pediatr. 2013; 50: 152–153.
632 https://doi.org/10.1007/s13312-013-0030-3

633 43. Anderson RC, Cookson AL, McNabb WC, Park Z, McCann MJ, Kelly WJ, Roy NC.
634 Lactobacillus plantarum MB452 enhances the function of the intestinal barrier by
635 increasing the expression levels of genes involved in tight junction formation. BMC
636 Microbiol. 2010; 10: 316. https://doi.org/10.1186/1471-2180-10-316

637 44. Ayu IL, Ha HK, Yang DH, Lee WJ, Lee MR. Encapsulation of Lactobacillus rhamnosus
638 GG using milk protein-based delivery systems: effects of reaction temperature and
639 holding time on their physicochemical and functional properties. Food Sci Anim Resour.
640 2021; 41: 894–904. https://doi.org/10.5851/kosfa.2021.e45

641 45. Gupta P, Andrew H, Kirschner BS, Guandalini S. Is Lactobacillus GG helpful in
642 children with Crohn's disease? Results of a preliminary, open-label study. J Pediatr
643 Gastroenterol Nutr. 2000; 31: 453–457. https://doi.org/10.1097/00005176-200010000-
644 00024

46. Klingberg TD, Pedersen MH, Cencic A, Budde BB. Application of measurements of transepithelial electrical resistance of intestinal epithelial cell monolayers to evaluate probiotic activity. Appl Environ Microbiol. 2005; 71: 7528–7530. https://doi.org/10.1128/AEM.71.11.7528-7530.2005

47. Sherman PM, Johnson-Henry KC, Yeung HP, Ngo PS, Goulet J, Tompkins TA. Probiotics reduce enterohemorrhagic Escherichia coli O157:H7- and enteropathogenic E. coli O127:H6-induced changes in polarized T84 epithelial cell monolayers by reducing bacterial adhesion and cytoskeletal rearrangements. Infect Immun. 2005; 73: 5183–5188.

48. Suzuki, T. Regulation of intestinal epithelial permeability by tight junctions. Cell Mol Life Sci. 2013; 70: 631–659. https://doi.org/10.1128/IAI.73.8.5183-5188.2005

49. Zhao Y, Qin G, Sun Z, Che D, Bao N, Zhang X. Effects of soybean agglutinin on intestinal barrier permeability and tight junction protein expression in weaned piglets. Int J Mol Sci. 2011; 12: 8502–8512. https://doi.org/10.3390/ijms12128502

50. Wang J, Ji H, Wang S, Liu H, Zhang W, Zhang D, Wang Y. Probiotic Lactobacillus plantarum promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. Front Microbiol. 2018; 9: 1953. https://doi.org/10.3389/fmicb.2018.01953

51. Wu QX, Xu X, Xie Q, Tong WY, Chen Y. Evaluation of chitosan hydrochloride-alginate as enteric micro-probiotic-carrier with dual protective barriers. Int J Biol Macromol. 2016; 93: 665–671. https://doi.org/10.1016/j.ijbiomac.2016.09.034

52. Yang H, Rao JN, Wang JY. Posttranscriptional regulation of intestinal epithelial tight junction barrier by RNA-binding proteins and microRNAs. Tissue Barriers. 2014; 2: e28320. https://doi.org/10.4161/tisb.28320

53. Lim CS, Lee YS, Kahng HY, Ahn S, Jung JS. Resistance genes in high-level streptomycin resistant Escherichia coli isolated from shellfish. Korean Microbiol. 2018; 54(3): 228–236.

54. Anand SK, Srinivasan RA, Rao LK. Antibacterial activity associated with Bifidobacterium bifidum. Cult Dairy Prod J. 1985; 35: 527–529.

55. Klaenhammer TR. Bacteriocins of lactic acid bacteria. Biochimie. 1988; 70: 337–349. https://doi.org/10.1016/0300-9084(88)90206-4

675 56. Cavera VL, Arthur TD, Kashtanov D, Chikindas ML. Bacteriocins and their position in
676 the next wave of conventional antibiotics. Int J Antimicrob Agents. 2015; 46: 494–501.
677 https://doi.org/10.1016/j.ijantimicag.2015.07.011

678 57. Simons A, Alhanout K, Duval RE. Bacteriocins, antimicrobial peptides from bacterial
679 origin: overview of their biology and their impact against multidrug-resistant bacteria.
680 Microorganisms. 2020; 8: 639.

681 58. Han SG, Kwon HC, Kim DH, Hong SJ, Han SG. In vitro synergistic antibacterial and
682 anti-inflammatory effects of nisin and lactic acid in yogurt against Helicobacter pylori
683 and human gastric cells. Food Sci Anim Resour. 2023; 43: 751–766.
684 https://doi.org/10.5851/kosfa.2023.e34

685 59. Xu Q, Li X, Wang E, He Y, Yin B, Fang D, Wang G, Zhao J, Zhang H, Chen W. A
686 cellular model for screening of Lactobacilli that can enhance tight junctions. RSC Adv.
687 2016; 6: 111812–111821.

688 60. Mora D, Arioli S. Microbial urease in health and disease. PLoS Pathog. 2014; 10(12):
689 e1004472. https://doi.org/ 10.1371/journal.ppat.1004472

690 61. Imperial I, Ibana J. Addressing the antibiotic resistance problem with probiotics :
691 reducing the risk of its double-edged sword effect. Front Microbiol. 2016; 7: 1983.
692 https://doi.org/ 10.3389/fmicb.2016.01983

693 62. Bratz K, Bücker R, Gölz G, Zakrzewski S, Janczyk P, Nöckler K, Alter T. Experimental
694 infection of weaned piglets with Campylobacter coli–excretion and translocation in a pig
695 colonisation trial. Vet Microbiol. 2013; 162(1): 136-143. https://doi.org/
696 10.1016/j.vetmic.2012.08.016

697 63. Lee D, Jang G, Min K, Lee I, Won H, Yoon I, Lee C. Coinfection with porcine epidemic
698 diarrhea virus and Clostridium perfringens type A enhances disease severity in weaned
699 pigs. Arch Virol. 2023; 168(6):166. https://doi.org/ 10.1007/s00705-023-05798-3

700 64. Pluske J, Turpin D, Sahibzada S, Pineda L, Han Y, Collins A. Impacts of feeding
701 organic acid-based feed additives on diarrhea, performance, and fecal microbiome
702 characteristics of pigs after weaning challenged with an enterotoxigenic strain of
703 Escherichia coli. Transl Anim Sci. 2021; 5(4):txab212.
704 https://doi.org/10.1093/tas/txab212

705 65. Casey P, Casey G, Gardiner G, Tangney M, Stanton C, Ross R, Fitzgerald G. Isolation
706 and characterization of anti-Salmonella lactic acid bacteria from the porcine
707 gastrointestinal tract. Lett Appl Microbiol. 2004; 39(5): 431-438.

708 https://doi.org/10.1111/j.1472-765X.2004.01603.x

709

Figure legends

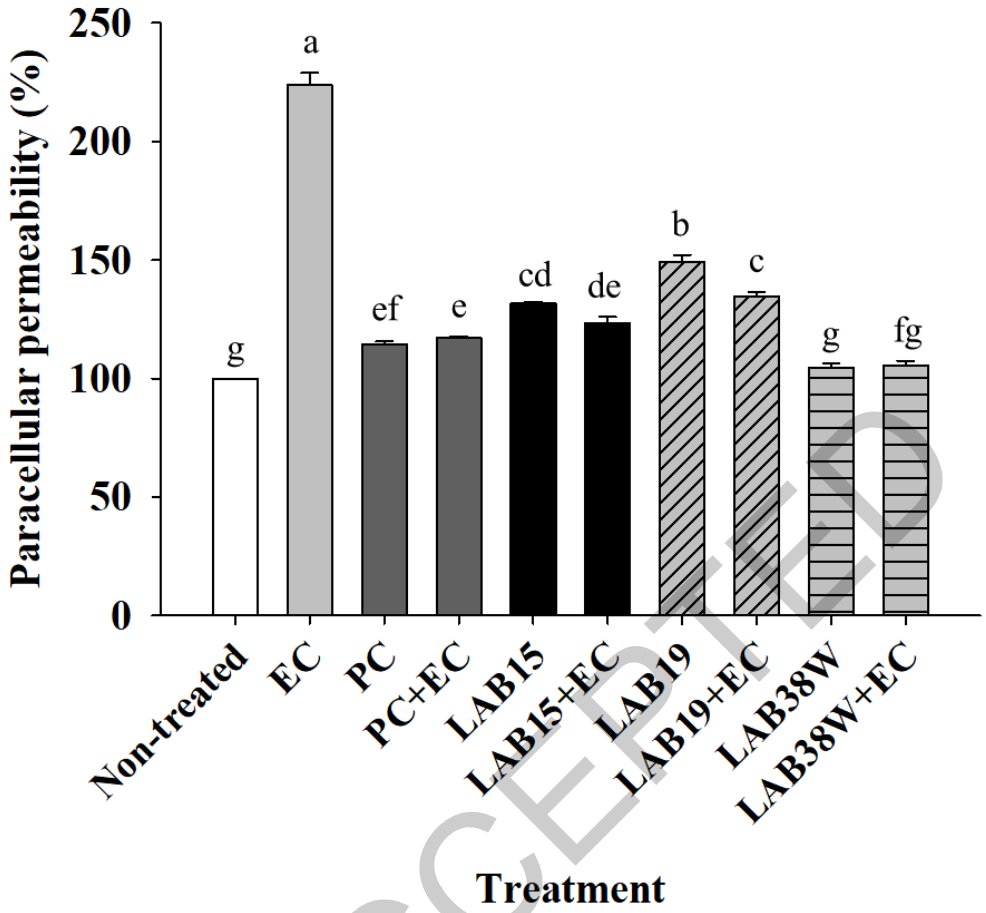
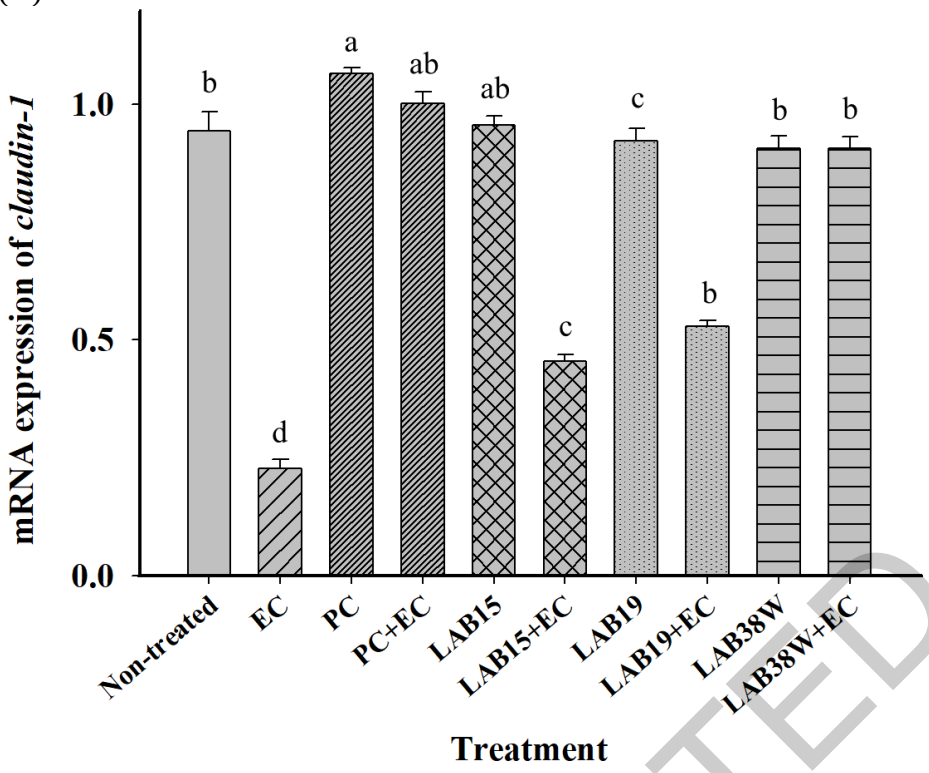
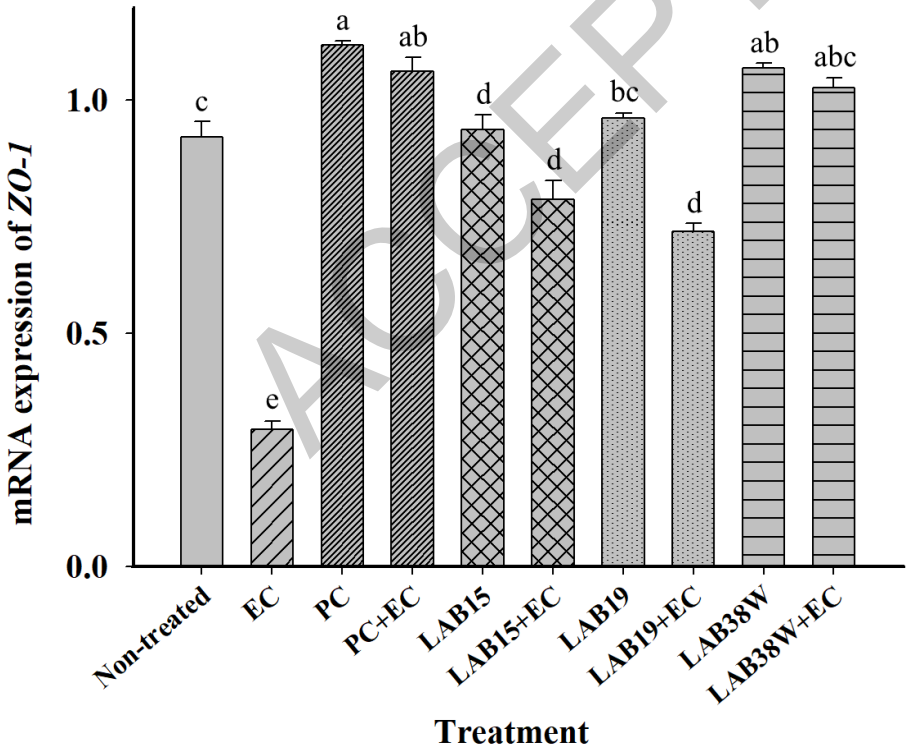


Fig. 1. Paracellular permeability of HT-29 cells treated with lactic acid bacteria isolates. Non-treated, Dulbecco's modified Eagle's medium; EC, *Escherichia coli* NCCP11142; PC, *Lacticaseibacillus rhamnosus* GG ATCC53103. [a-g]different letters indicate significant differences ($p < 0.05$).

721 (A)

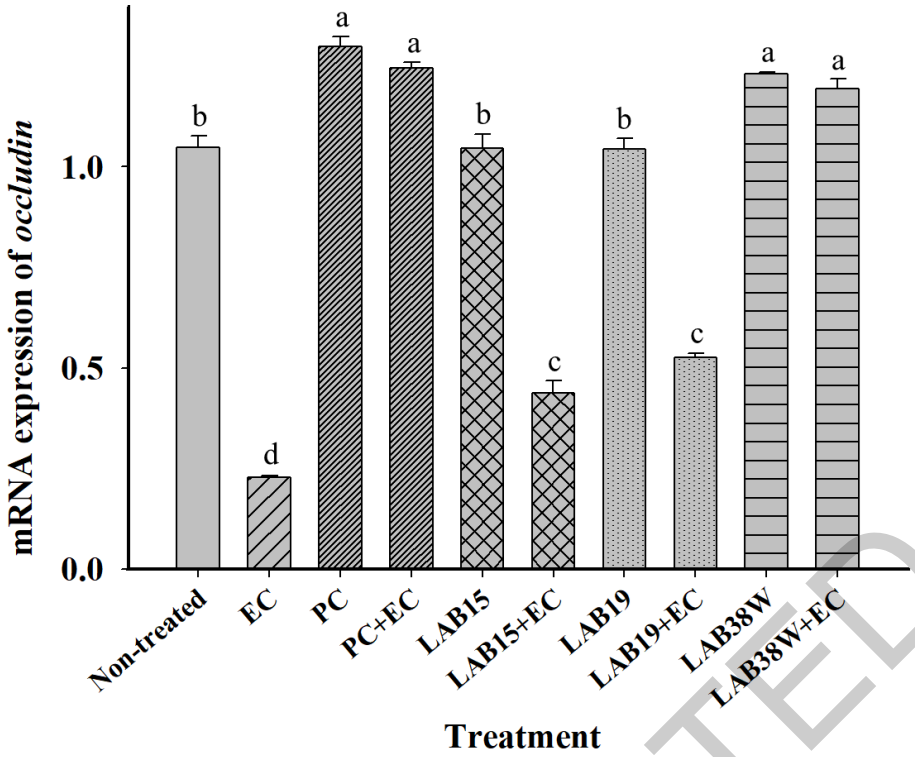


722
723 (B)



724
725
726

727 (C)



728
729

730 **Fig 2.** Relative gene expression levels of (A) *claudin-1*, (B) *ZO-1*, and (C) *occludin* in HT-29

731 cells treated with lactic acid bacteria isolates.

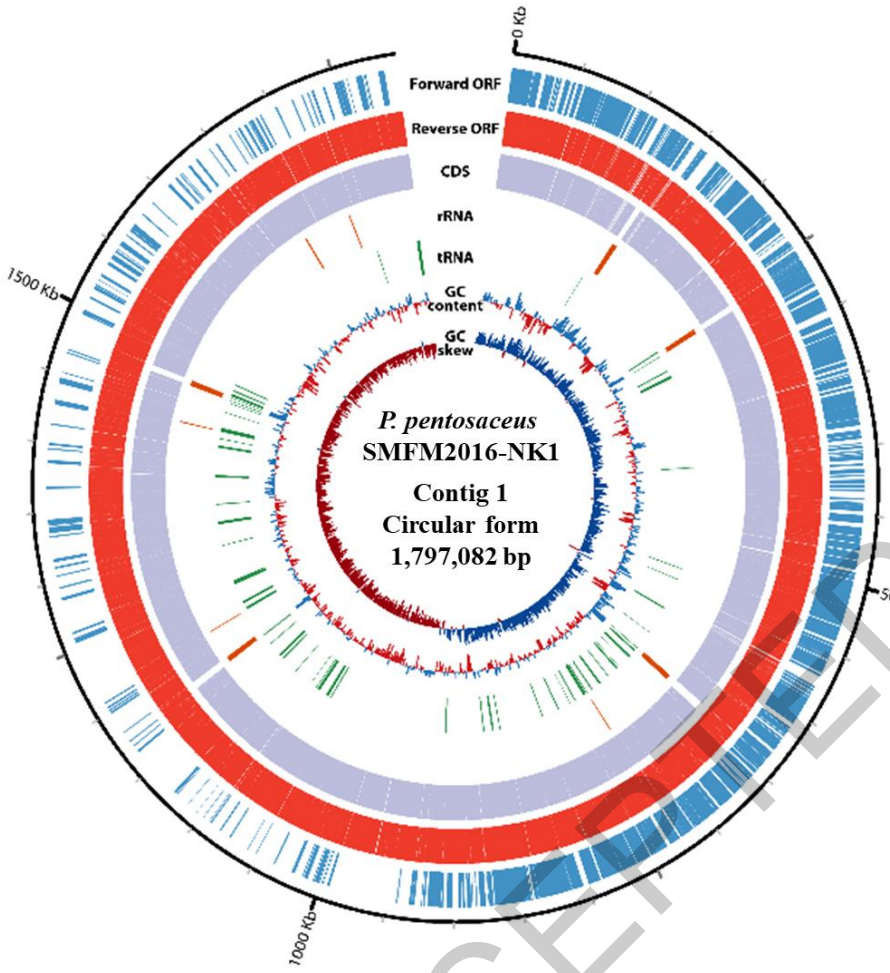
732 EC, *Escherichia coli* NCCP11142; PC, *Lacticaseibacillus rhamnosus* GG ATCC53103.

733 [a-d]different letters indicate significant differences ($p < 0.05$)

734

735

736 (A)



737
738

(B)

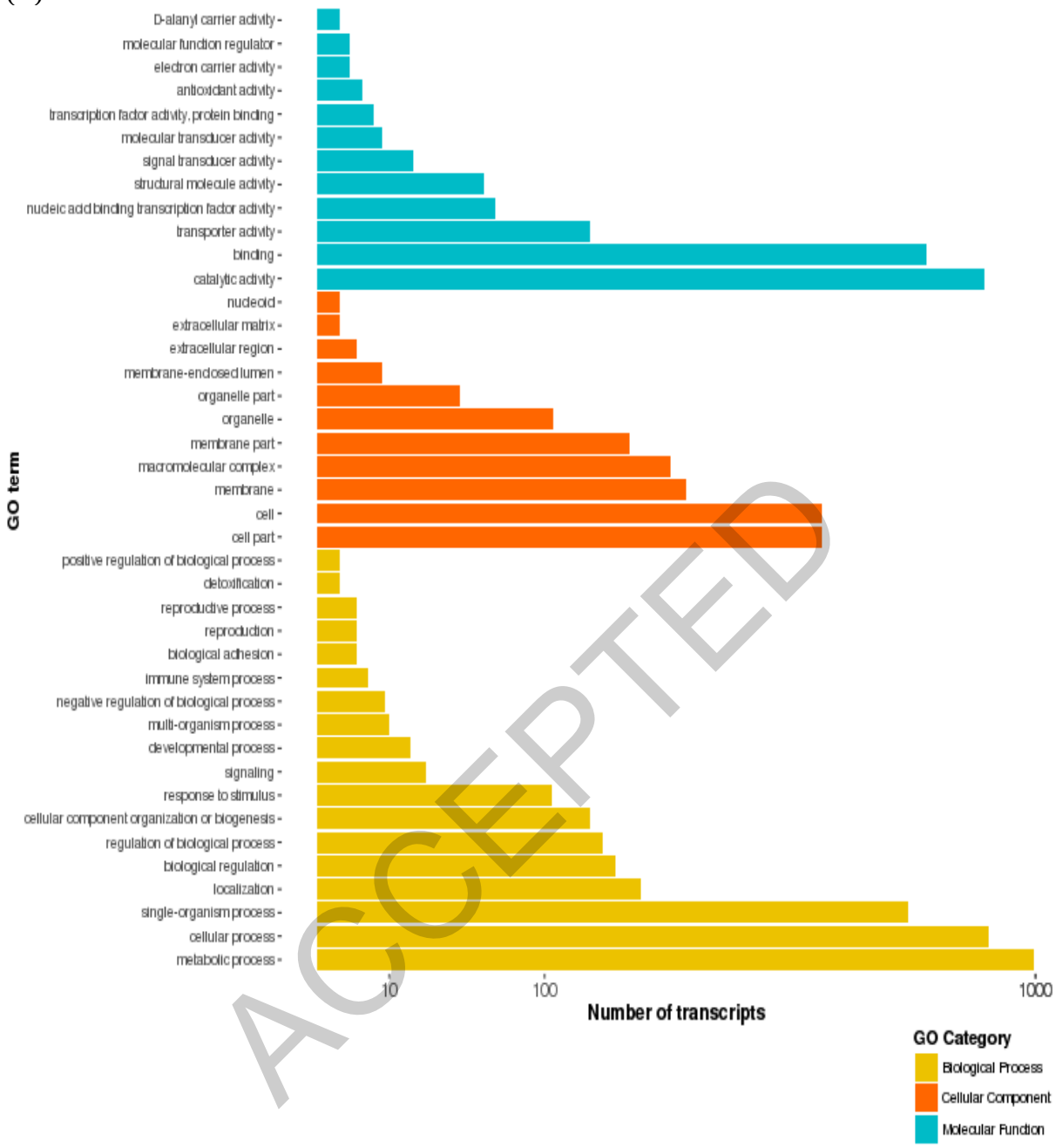
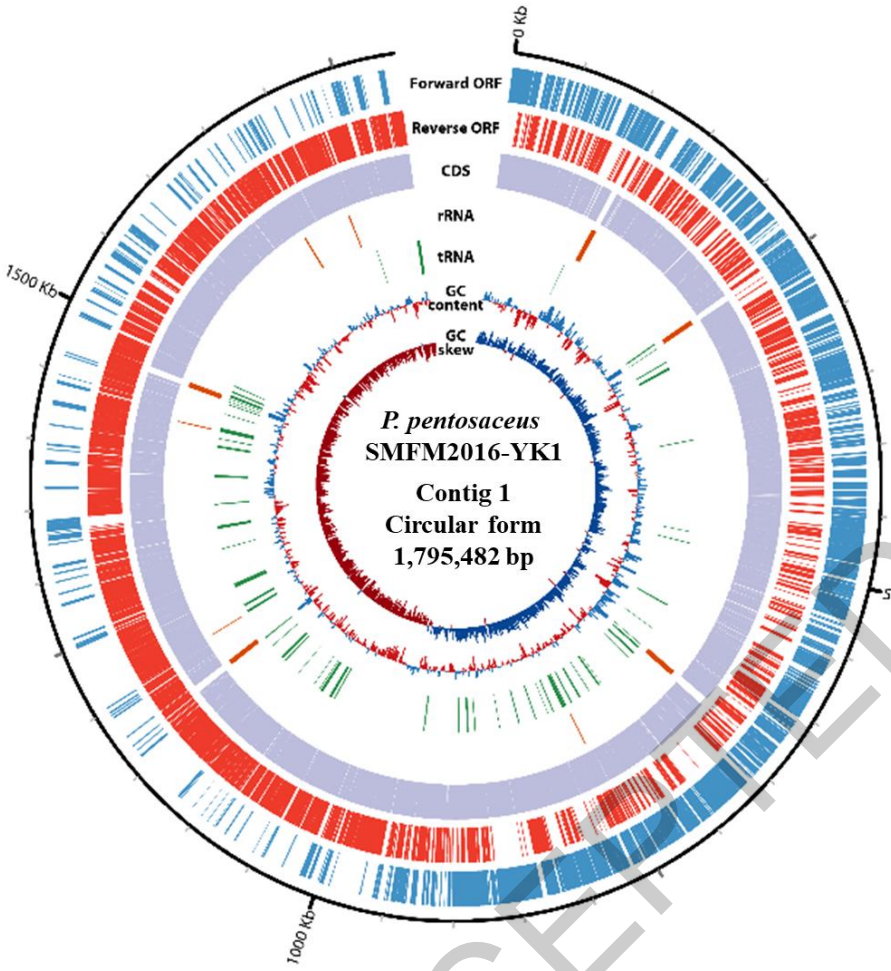


Fig. 3. Chromosomal genome properties of *Pediococcus pentosaceus* SMFM2016-NK1. (A) Overall features of the genome [outer scale; base pairs, the first (the outer-most; blue) and second pink ring; forward and reverse open reading frames (ORFs) by gene annotation, the third ring; coding sequences, the fourth ring; rRNA values, the fifth ring; tRNA values, the sixth ring; GC contents, the inner most; GC skew] and (B) gene ontology classification (biological process, cellular component, and molecular function) via gene prediction and annotation for *Pediococcus pentosaceus* SMFM2016-NK1.

751 (A)



752
753
754
755

(B)

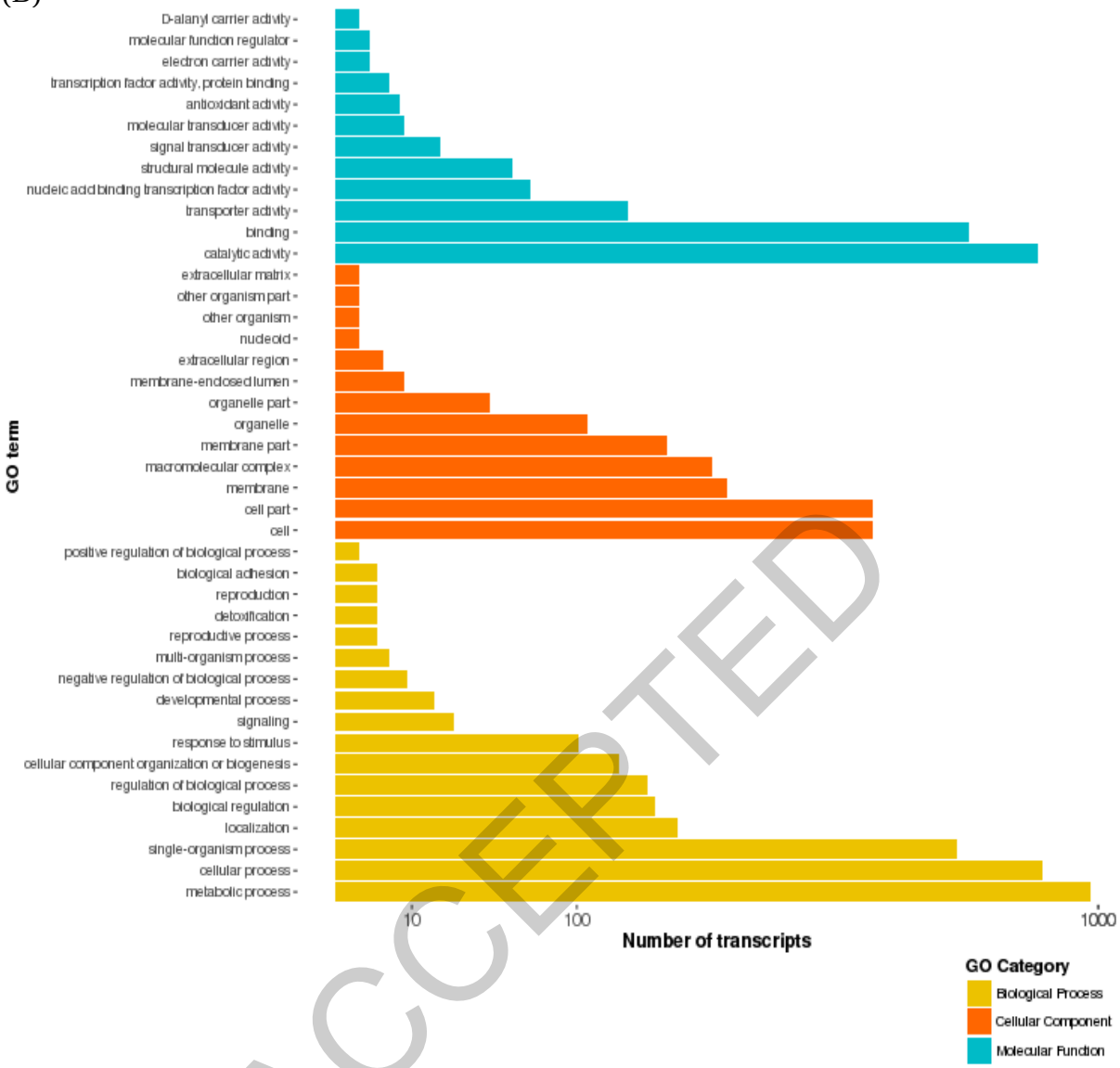
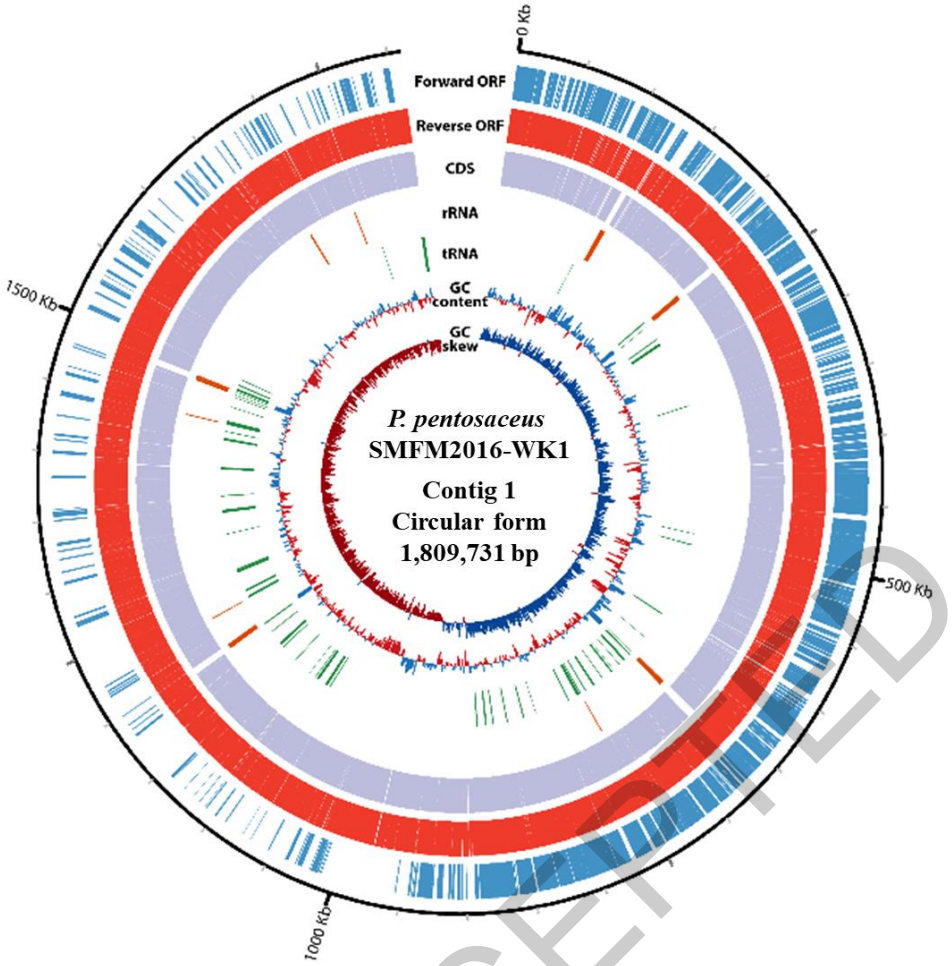


Fig. 4. Chromosomal genome properties of *Pediococcus pentosaceus* SMFM2016-YK1. (A) Overall features of the genome [outer scale; base pairs, the first (the outer-most; blue) and second pink ring; forward and reverse open reading frames (ORFs) by gene annotation, the third ring; coding sequences, the fourth ring; rRNA values, the fifth ring; tRNA values, the sixth ring; GC contents, the inner most; GC skew] and (B) gene ontology classification (biological process, cellular component, and molecular function) via gene prediction and annotation for *Pediococcus pentosaceus* SMFM2016-YK1.

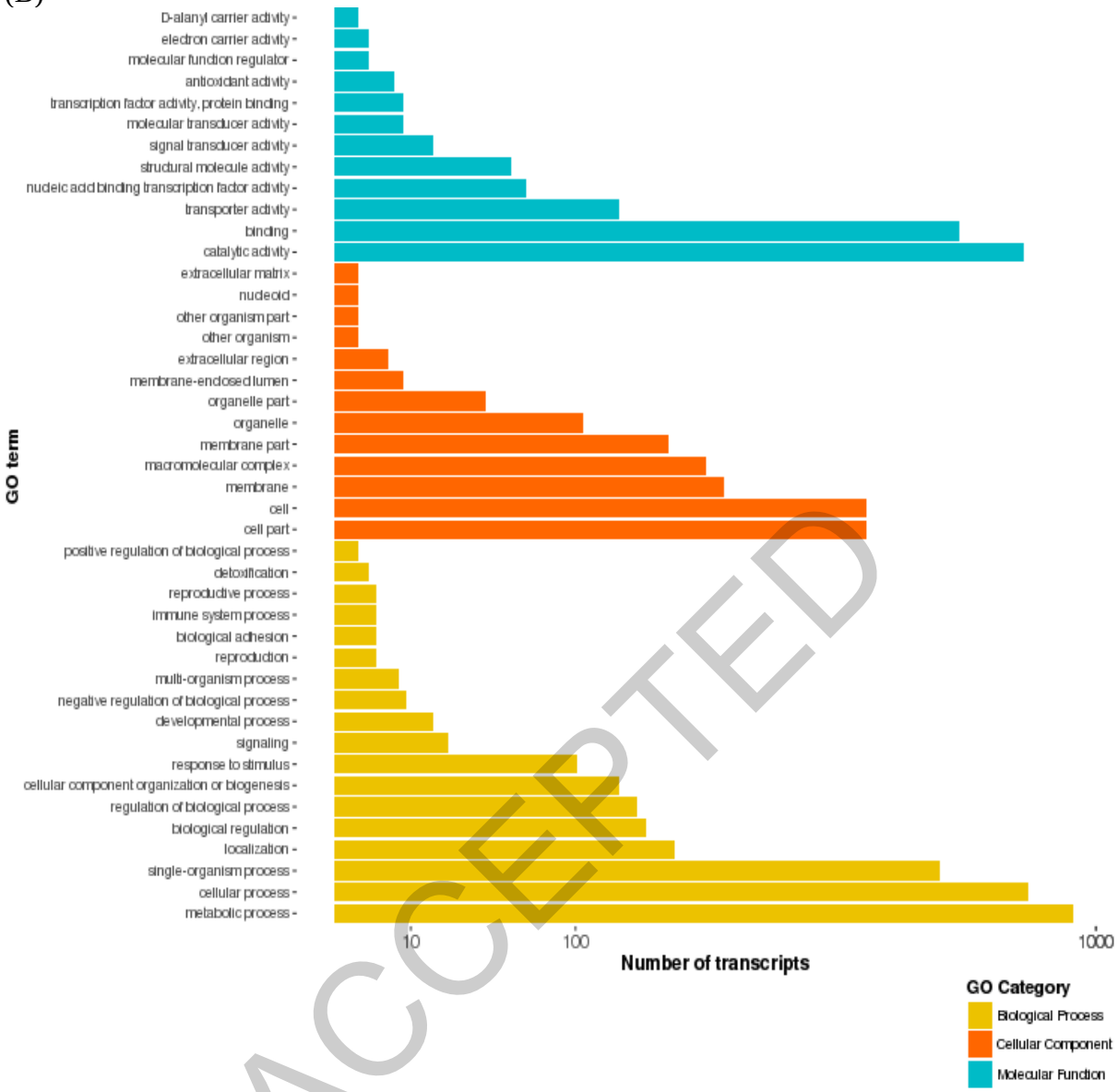
767

768 (A)



769
770

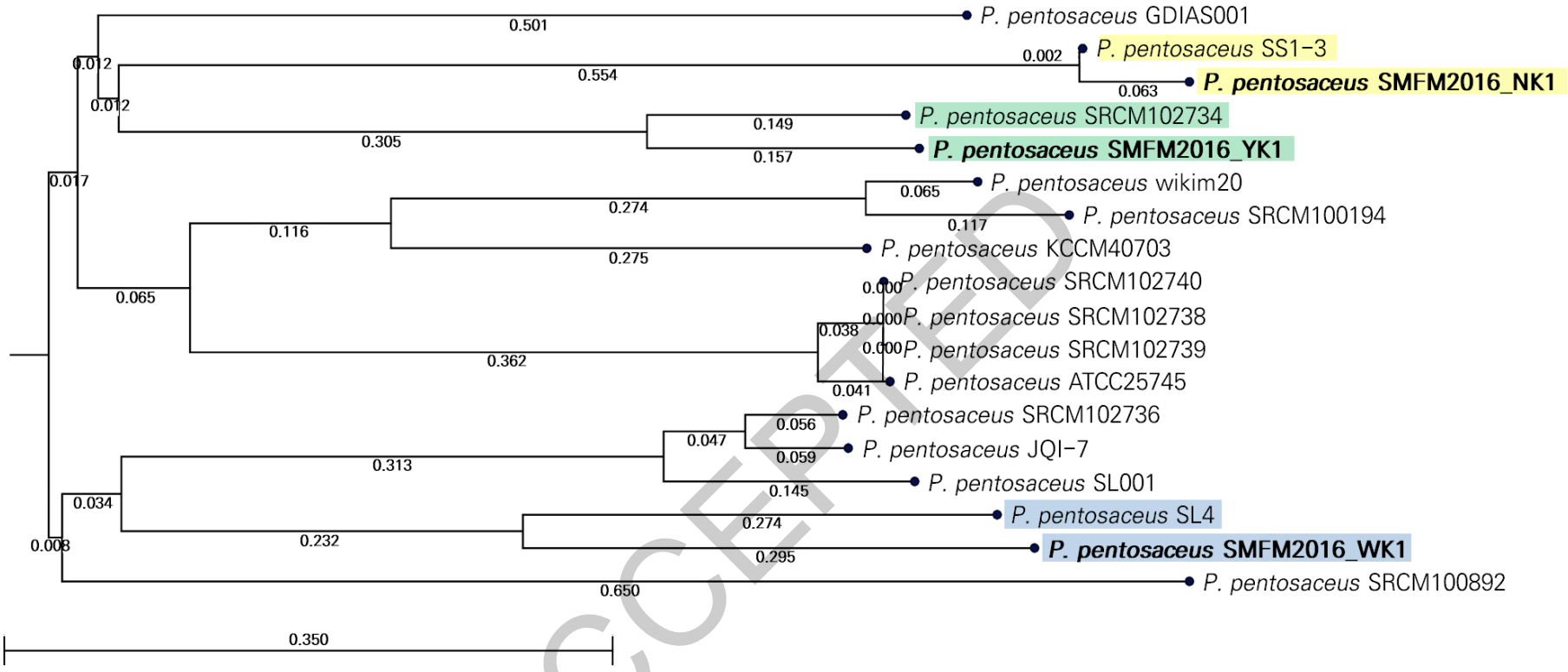
771 (B)



772
773

774 **Fig. 5.** Chromosomal genome properties of *Pediococcus pentosaceus* SMFM2016-WK1. (A)
775 Overall features of the genome [outer scale; base pairs, the first (the outer-most; blue) and
776 second pink ring; forward and reverse open reading frames (ORFs) by gene annotation, the
777 third ring; coding sequences, the fourth ring; rRNA values, the fifth ring; tRNA values, the
778 sixth ring; GC contents, the inner most; GC skew] and (B) gene ontology classification
779 (biological process, cellular component, and molecular function) via gene prediction and
780 annotation for *Pediococcus pentosaceus* SMFM2016-WK1.

781



782

783

784 **Fig. 6.** Phylogenetic tree based on the average nucleotide identity (ANI) for *Pediococcus pentosaceus* isolates.
785

786

787

788

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
P. pentosaceus SMFM2016-NK1	1		98.82	98.77	98.88	98.87	98.91	98.91	98.85	98.82	98.86	98.66	98.95	98.95	98.90	98.90	98.90	99.93	98.85
P. pentosaceus SMFM2016-YK1	2	98.82		98.91	99.13	99.01	98.94	99.01	98.89	98.98	99.05	98.74	99.69	98.97	99.12	99.12	99.12	99.02	99.07
P. pentosaceus SMFM2016-WK1	3	98.77	98.91		98.85	98.89	99.02	98.96	99.03	99.43	98.79	98.81	98.97	99.06	98.91	98.91	98.91	98.81	98.92
P. pentosaceus ATCC25745	4	98.88	99.13	98.85		99.02	99.04	99.28	99.01	98.93	99.01	98.86	99.07	99.04	99.92	99.92	99.92	98.90	99.11
P. pentosaceus GDIAS001	5	98.87	99.01	98.89	99.02		98.99	98.95	99.00	98.97	98.94	98.80	99.07	99.02	99.07	99.07	99.07	98.93	98.93
P. pentosaceus JQI-7	6	98.91	98.94	99.02	99.04	98.99		99.01	99.76	99.06	98.94	98.94	99.02	99.88	99.08	99.08	99.08	99.01	98.96
P. pentosaceus KCCM40703	7	98.91	99.01	98.96	99.28	98.95	99.01		99.03	98.97	99.35	98.95	99.00	99.05	99.26	99.26	99.26	98.93	99.37
P. pentosaceus SL001	8	98.85	98.89	99.03	99.01	99.00	99.76	99.03		99.03	98.99	98.83	99.00	99.74	99.00	99.00	99.00	98.97	98.92
P. pentosaceus SL4	9	98.82	98.98	99.43	98.93	98.97	99.06	98.97	99.03		98.76	98.78	99.01	99.10	98.91	98.91	98.91	98.86	98.90
P. pentosaceus SRCM100194	10	98.86	99.05	98.79	99.01	98.94	98.94	99.35	98.99	98.76		98.79	98.90	98.94	99.01	99.01	99.01	98.85	99.82
P. pentosaceus SRCM100892	11	98.66	98.74	98.81	98.86	98.80	98.94	98.95	98.83	98.78	98.79		98.88	98.92	98.82	98.82	98.82	98.81	98.86
P. pentosaceus SRCM102734	12	98.95	99.69	98.97	99.07	99.07	99.02	99.00	99.00	99.01	98.90	98.88		99.01	99.07	99.07	99.07	99.03	98.95
P. pentosaceus SRCM102736	13	98.95	98.97	99.06	99.04	99.02	99.88	99.05	99.74	99.10	98.94	98.92	99.01		99.05	99.05	99.05	98.98	98.98
P. pentosaceus SRCM102738	14	98.90	99.12	98.91	99.92	99.07	99.08	99.26	99.00	98.91	99.01	98.82	99.07	99.05		100.00	100.00	98.94	99.09
P. pentosaceus SRCM102739	15	98.90	99.12	98.91	99.92	99.07	99.08	99.26	99.00	98.91	99.01	98.82	99.07	99.05	100.00		100.00	98.94	99.09
P. pentosaceus SRCM102740	16	98.90	99.12	98.91	99.92	99.07	99.08	99.26	99.00	98.91	99.01	98.82	99.07	99.05	100.00	100.00		98.94	99.09
P. pentosaceus SS1-3	17	99.93	99.02	98.81	98.90	98.93	99.01	98.93	98.97	98.86	98.85	98.81	99.03	98.98	98.94	98.94	98.94		98.89
P. pentosaceus wikim20	18	98.85	99.07	98.92	99.11	98.93	98.96	99.37	98.92	98.90	99.82	98.86	98.95	98.98	99.09	99.09	99.09	98.89	

789 **Fig. 7.** Average nucleotide identity (ANI) analysis results of *Pediococcus pentosaceus* isolates.

790 **Table 1.** Primer sequences used to determine the expression of genes encoding tight junction proteins using quantitative reverse transcription-

791 PCR.

Target gene		Primer sequence (5′→3′)	Reference
claudin-1	Forward	AAGTGCTTGGAAGACGATGA	[59]
	Reverse	CTTGGTGTTGGGTAAGAGGTT	
occludin	Forward	CCAATGTCGAGGAGTGGG	
	Reverse	CGCTGCTGTAACGAGGCT	
ZO-1	Forward	ATCCCTCAAGGAGCCATTC	
	Reverse	CACTTGTTTTGCCAGGTTTTA	
β-actin	Forward	TTTTAGGATGGCAAGGGACTT	
	Reverse	GATGAGTTGGCATGGCTTTA	

794 **Table 2.** Lactic acid bacteria isolates used in this study.

Species	Strains
Limosilactobacillus fermentum	1, 3, 6, 7, 12, 22, 28, 29, 31, 32, 38Y, 44, 45, 57, 58, 59, 72, 73, 75
Levilactobacillus brevis	4W, 74
Lactiplantibacillus plantarum	8, 10, 13, 49Y, 50, 52, 53, 71, 76, 77
Lactilactobacillus sakei	14, 21, 27, 33, 34, 56, 60
Lactilactobacillus curvatus	35
Pediococcus pentosaceus	2, 9, 11, 15, 19, 20, 30, 36, 38W, 66, 67, 70

798 **Table 3**. Bile and pancreatic enzyme resistance of lactic acid bacteria isolates.
799

Bile resistance						Pancreatic enzyme resistance					
Isolate	**Tolerance (%)**	**Isolate**	**Tolerance (%)**	**Isolate**	**Tolerance (%)**	**Isolate**	**Tolerance (%)**	**Isolate**	**Tolerance (%)**	**Isolate**	**Tolerance (%)**
PC*	101.1±6.2^{JKL}	22	76.0±2.0^{PQR}	53	127.8±3.1^{ABCD}	PC	103.3±1.2^{ab}	22	103.3±1.2^{ab}	53	93.5±3.7^{cdefghijklm}
1	88.6±4.3^{MN}	27	53.6±1.3^{T}	56	65.0±3.1^{S}	1	86.5±2.3^{lmnopqrst}	27	87.8±2.4^{klmnopqrs}	56	99.6±2.5^{abcdefgh}
2	111.9±6.3^{GHI}	28	78.7±0.4^{NOPQR}	57	127.9±2.1^{ABCD}	2	99.6±0.9^{abcdefgh}	28	86.5±2.3^{lmnopqrs}	57	88.0±4.9^{klmnopqrs}
3	82.2±3.6^{NOPQ}	29	74.4±1.4^{QRS}	58	68.5±3.8^{RS}	3	100.2±1.8^{abcdefg}	29	87.8±2.4^{klmnopqrs}	58	80.1±2.1st
4W	137.1±2.2^{A}	30	118.0±4.3^{DEFGHI}	59	48.4±3.7^{T}	4W	91.4±4.0^{ghijklmnop}	30	100.5±1.1^{abcdef}	59	84.4±6.6^{nopqrst}
6	87.1±2.3^{MNO}	31	80.7±1.1^{NOPQ}	60	127.5±2.3^{ABCD}	6	82.8±1.8^{pqrst}	31	97.0±3.5^{abcdefghij}	60	92.2±4.2^{fghijklmno}
7	74.0±2.5^{QRS}	32	94.2±2.1^{LM}	66	111.3±9.8^{HIJ}	7	77.5±1.5^{t}	32	90.1±1.5^{ijklmnopqr}	66	101.2±1.4^{abcde}
8	120.5±1.4^{CDEFGH}	33	85.9±2.4^{MNOP}	67	115.8±2.6^{EFGHI}	8	85.4±1.7^{mnopqrst}	33	96.5±1.8^{abcdefghijk}	67	101.5±2.3^{abcde}
9	115.8±2.4^{EFGHI}	34	75.9±2.4^{PQR}	70	120.1±1.6^{CDEFGH}	9	102.5±4.0^{ab}	34	97.5±1.3^{abcdefghi}	70	102.2±1.0^{abc}
10	123.8±1.6^{BCDE}	35	120.4±2.4^{CDEFGH}	71	123.8±2.6^{BCDE}	10	89.3±1.8^{ijklmnopqr}	35	89.9±2.3^{ijklmnopqr}	71	92.6±2.7^{efghijklmn}
11	121.6±3.0^{CDEFG}	36	122.7±2.4^{BCDEF}	72	77.4±2.8^{OPQR}	11	103.2±2.6^{ab}	36	100.6±0.7^{abcdef}	72	81.3±10.0^{rst}
12	86.9±1.8^{MNO}	38W	119.7±2.1^{CDEFGH}	73	80.2±1.9^{NOPQ}	12	81.9±1.9^{qrst}	38W	104.0±2.0^{a}	73	83.6±4.4^{opqrst}
13	127.4±1.6^{ABCD}	38Y	115.2±3.4^{EFGHI}	74	109.3±4.2^{IJK}	13	91.1±4.0^{hijklmnop}	38Y	90.3±3.3^{ijklmnopq}	74	89.3±1.9^{ijklmnopqr}
14	45.5±2.5^{T}	44	78.5±2.9^{NOPQR}	75	78.6±5.2^{NOPQR}	14	102.8±3.0^{ab}	44	82.7±1.6^{pqrst}	75	80.0±5.0st
15	112.3±3.7^{GHI}	45	79.0±1.6^{NOPQ}	76	112.6±5.1^{FGHI}	15	103.0±1.7^{ab}	45	87.8±2.8^{klmnopqrs}	76	88.3±4.3^{jklmnopqrs}
19	99.7±1.0^{KL}	49Y	129.3±2.6^{ABC}	77	118.8±2.8^{DEFGHI}	19	102.9±1.4^{ab}	49Y	93.1±1.3^{defghijklmn}	77	90.2±2.9^{ijklmnopqr}
20	111.4±4.0^{GHI}	50	127.0±3.2^{ABCD}			20	101.6±1.6^{abcd}	50	94.6±2.4^{bcdefghijkl}		
21	54.4±5.5^{T}	52	132.2±2.3^{AB}			21	100.6±1.1^{abcdef}	52	91.7±6.2^{fghijklmnop}		

* *Lacticaseibacillus rhamnosus* GG was used as the positive control.
$^{A-T}$; different letters indicate a significant difference in bile resistance ($p < 0.05$).
$^{a-t}$; different letters indicate a significant difference in pancreatic enzyme resistance ($p < 0.05$).

800

801 **Table 4.** Antibiotic resistance of 12 lactic acid bacteria isolates.

Isolate	Minimum inhibitory concentration (mg/L)							
	Ampicillin	Gentamicin	Kanamycin	Streptomycin	Erythromycin	Clindamycin	Tetracycline	Chloramphenicol
2	2	≤0.25	16	8	≤0.25	≤0.12	4	≤2
9	≤1	≤0.25	16	16	≤0.25	≤0.12	8	≤2
11	≤1	≤0.25	16	16	≤0.25	≤0.12	8	≤2
15	≤1	≤0.25	8	8	≤0.25	≤0.12	8	≤2
19	2	0.5	16	16	≤0.25	≤0.12	**16***	≤2
20	2	0.5	16	16	≤0.25	≤0.12	**16**	≤2
30	2	≤0.25	16	16	≤0.25	≤0.12	**16**	≤2
36	≤1	≤0.25	16	16	≤0.25	≤0.12	**16**	≤2
38W	≤1	≤0.25	8	8	≤0.25	≤0.12	8	≤2
66	2	0.5	16	16	≤0.25	≤0.12	8	≤2
67	≤1	≤0.25	8	16	≤0.25	≤0.12	**16**	≤2
70	2	0.5	16	16	≤0.25	≤0.12	8	≤2
EFSA Cut-off**	4	16	64	64	1	1	8	4

*Bold number is the value more than the EFSA cut off

**Cut-off values established by EFSA (2018)

802

803

Table 5. Antimicrobial effects of 12 lactic acid bacteria isolates against the diarrheal pathogens *Escherichia coli*, *Salmonella* Typhimurium, *Campylobacter coli*, and *Clostridium perfringens*.

Isolate	*E. coli* strains			*S.* Typhimurium strains		*C. coli* strains			*C. perfringens* strains			
	KVCC-BA0001423	KVCC-BA0001823	KVCC-BA1600302	KVCC-BA2000160	KVCC-BA2000161	KVCC-BA1800493	KVCC-BA1800494	KVCC-BA1800595	KVCC-BA1900009	KVCC-BA1900010	KVCC-BA1900011	KVCC-BA1700250
PC 1	11.5±1.2^{e}	11.1±1.8^{d}	11.7±0.8^{c}	12.8±1.3^{ef}	11.8±1.2^{c}	12.2±1.8^{c}	10.9±0.7^{d}	10.5±1.0^{d}	11.0±1.4^{e}	10.3±0.8^{e}	11.7±2.1^{df}	12.1±0.8^{c}
PC 2	10.3±0.8^{e}	10.8±0.4^{d}	11.5±0.5^{c}	8.7±1.0^{f}	11.8±1.9^{c}	10.3±0.7^{c}	12.0±0.0^{cd}	11.7±0.4^{cd}	9.3±0.4^{f}	9.6±0.4^{e}	8.7±1.0^{f}	9.3±1.3^{d}
PC 3	11.3±2.0^{e}	10.8±0.8^{d}	11.7±0.8^{c}	14.0±1.2^{de}	12.0±0.6^{c}	11.8±1.2^{c}	10.8±0.8^{d}	11.2±0.8^{d}	10.0±0.9^{e}	13.3±1.5^{de}	13.8±1.7^{de}	12.3±1.5^{c}
2	16.5±1.5^{d}	17.3±2.2^{abc}	16.6±1.1^{bc}	19.0±1.7^{bc}	17.0±2.1^{abc}	17.7±2.3^{b}	18.2±1.8^{abc}	18.6±1.6^{ab}	15.5±1.4^{cd}	18.8±2.1^{bc}	17.5±1.4^{bcd}	15.9±1.6^{b}
9	18.1±1.2^{cd}	16.2±1.1^{bc}	15.3±1.1^{bc}	16.5±1.9^{cde}	14.4±1.2^{bc}	18.4±1.7^{ab}	18.0±1.6^{abc}	18.2±1.2^{ab}	15.7±2.1^{bcd}	18.3±1.7^{bcd}	17.3±1.7^{bcd}	16.9±1.0^{b}
11	20.0±1.4^{abc}	16.8±1.6^{bc}	17.2±2.9^{ab}	18.2±2.7^{bcd}	17.3±3.6^{abc}	18.9±1.7^{ab}	19.5±2.4^{ab}	19.2±1.1^{ab}	15.2±1.7^{cd}	20.9±2.0^{abc}	19.2±1.7^{abc}	21.6±1.6^{a}
15	20.9±1.6^{abc}	18.3±1.9^{abc}	17.8±2.4^{ab}	20.0±3.7^{abc}	18.9±1.3^{ab}	19.5±1.0^{ab}	20.0±0.6^{ab}	20.4±1.7^{ab}	18.8±2.9^{ab}	21.5±2.8^{abc}	19.7±1.5^{ab}	23.3±1.3^{a}
19	20.7±3.0^{abc}	18.7±2.1^{abc}	19.0±1.8^{ab}	21.7±2.3^{ab}	19.8±1.3^{ab}	20.8±1.0^{ab}	19.9±1.2^{ab}	20.6±1.2^{ab}	17.8±2.6^{abc}	22.7±2.5^{ab}	20.4±2.7^{ab}	22.5±1.9^{a}
20	20.9±1.6^{abc}	19.1±2.8^{abc}	19.3±2.2^{ab}	18.9±1.3^{bc}	17.3±0.9^{abc}	19.3±1.1^{ab}	19.5±1.8^{ab}	20.2±1.1^{ab}	16.2±1.2^{abcd}	21.0±3.0^{abc}	19.1±2.3^{bc}	22.0±1.2^{a}
30	21.8±1.7^{ab}	20.4±2.8^{de}	19.3±2.2^{ab}	20.0±2.8^{abc}	18.8±3.8^{ab}	19.3±1.2^{ab}	18.8±0.9^{ab}	20.2±1.6^{ab}	17.1±1.7^{abc}	22.7±3.8^{ab}	19.7±2.5^{ab}	22.2±1.5^{a}
36	18.2±1.5^{cd}	18.5±1.0^{abc}	19.4±2.2^{ab}	19.6±2.1^{abc}	18.7±1.2^{ab}	17.9±1.4^{ab}	18.6±2.3^{ab}	17.6±1.7^{b}	16.2±1.6^{abcd}	20.3±2.3^{abc}	18.0±2.0^{bc}	17.3±0.9^{b}
38W	23.3±2.2^{a}	21.7±1.6^{a}	21.9±3.2^{a}	23.7±1.0^{a}	23.2±3.8^{a}	22.0±1.4^{a}	22.0±2.1^{a}	22.2±1.5^{a}	19.3±1.9^{a}	24.7±3.4^{a}	22.8±1.5^{a}	24.3±1.4^{a}
66	18.2±3.1^{cd}	15.4±1.4^{c}	14.5±1.9^{bc}	17.1±2.6^{cde}	13.9±3.1^{bc}	17.5±1.8^{b}	16.9±2.2^{bc}	17.3±3.0^{bc}	13.1±1.1^{de}	18.7±1.2^{bcd}	15.5±1.8^{cd}	17.1±1.5^{b}
67	18.3±2.1^{bcd}	17.1±0.9^{bc}	16.3±1.7^{bc}	17.1±2.7^{cde}	17.1±0.9^{abc}	18.4±1.9^{ab}	17.8±2.1^{abc}	18.3±1.8^{ab}	14.8±1.3^{cd}	18.2±1.5^{bcd}	17.0±2.4^{bcd}	16.1±1.2^{b}
70	17.9±2.1^{cd}	16.3±1.0^{bc}	17.1±1.8^{ab}	18.1±2.5^{bcd}	17.3±3.2^{abc}	18.6±1.3^{ab}	17.8±1.7^{abc}	18.9±2.0^{ab}	15.8±1.2^{bcd}	16.8±2.5^{cd}	17.5±1.0^{bcd}	17.8±1.6^{b}

Values are expressed as inhibition zone (mm); mean ± standard deviation
PC; commercial probiotics for feeding.
[a-f] different letters in a column indicate a significant difference ($p < 0.05$).

Table 6. Comparison of the chromosomal properties of *Pediococcus pentosaceus* strains registered in the NCBI database.

Chromosomal properties		Strain																
		SMFM2016_NK1	SMFM2016_YK1	SMFM2016_WK1	ATCC 25745	GDIAS001	JQI-7	KCCM 40703	SL001	SL4	SRCM 100194	SRCM 100892	SRCM 102734	SRCM 102736	SRCM 102738	SRCM 102739	SRCM 102740	SS1-3	wikim20
Genome size (Mb)		1.85	1.79	1.72	1.83	1.83	1.73	1.76	1.92	1.79	1.87	2.00	1.71	1.81	1.88	1.90	1.88	1.84	1.83
GC content (%)		38.26	37.31	36.76	37.40	37.10	37.20	37.20	37.44	37.30	37.38	37.27	37.40	37.39	37.41	37.37	37.41	37.28	37.26
tRNA		55	57	55	55	56	56	55	56	51	55	56	56	56	56	56	56	55	55
rRNA		15	15	15	15	15	5	15	15	15	15	15	15	15	15	15	15	15	15
ANI (%)	NK1	-	98.82	98.77	98.88	98.87	98.91	98.91	98.85	98.82	98.86	98.66	98.95	98.95	98.90	98.90	98.90	99.93	98.85
	YK1	98.82	-	98.91	99.13	99.01	98.94	99.01	98.89	98.98	99.05	98.74	99.69	98.97	99.12	99.12	99.12	99.02	99.07
	WK1	98.77	98.91	-	98.85	98.89	99.02	98.96	99.03	99.43	98.79	98.81	98.97	99.06	98.91	98.91	98.91	98.81	98.92
Source		Kimchi	Kimchi	Kimchi	-	Plant feed material	Fermented dairy	Sake mash	Soil	Sausages	Food	Food	Doenjang	Chong-gugjang	Chong-gugjang	Chong-gugjang	Chong-gugjang	Adult feces	Kimchi
Location		Korea	Korea	Korea	-	China	China	Japan	China	Denmark	Korea	Korea	Korea	Korea	Korea	Korea	Korea	Korea	Korea