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Investigation of Lactic Acid Bacteria Isolated from Giant Panda Feces for Potential Probiotics In Vitro

Qian Liu[1] · Xueqin Ni[1] · Qiang Wang[2] · Zhirong Peng[1] · Lili Niu[2] · Meiling Xie[1] · Yicen Lin[1] · Yi Zhou[1] · Hao Sun[3] · Kangcheng Pan[3] · Bo Jing[3] · Dong Zeng[1,3]

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Abstract
The present study aimed to isolate an optimal lactic acid bacterial strain from the feces of healthy giant pandas. The strain exhibited good stability at low pH and high bile salt concentrations, activity against pathogens relevant to pandas, and antibiotic susceptibility. In the current study, 25 isolates were obtained from de Man, Rogosa, and Sharpe agar. Two (E21 and G83) and eight (E1, E2, E16, E18, E21, E69, E70, and G83) isolates demonstrated good performance at pH 2.0 and bile 2% (*w*/*v*), respectively. Three isolates (G83, G88, and G90) possessed better antimicrobial effect on enterotoxigenic *Escherichia coli* CVCC196 (ETEC) than the rest. One isolate (G83) strongly affected *Salmonella,* whereas three (G83, G87, and G88) exhibited inhibitory activity against *Staphylococcus aureus*. All isolates were multi-drug resistant. These isolates were identified as *Lactobacillus* (5 isolates) and *Enterococcus* (20 isolates) by 16S rRNA sequencing. Virulence genes were detected in *Enterococcus* isolates. Isolate G83 was identified as *Lactobacillus plantarum* and was considered as the best probiotic candidate among all of the experimental isolates. This study provided necessary and important theoretical guidance for further experiments on G83 in vivo.

Keywords Giant panda · Probiotic · Lactic acid bacteria · Isolation

Introduction

The giant panda, *Ailuropoda melanoleuca*, is famous as a living fossil. It is a vulnerable, endemic species that is extremely popular worldwide. Conservation strategies have resulted in the survival of about 1800 giant pandas worldwide. The giant panda is a herbivore that has retained a typical carnivorous digestive system. It is easily afflicted by various intestinal diseases when the feed structure changes. Under conditions of captivity, the prevention and treatment of diseases rely on antibiotics. However, scholars sought alternative treatments because of the disadvantages of antibiotics. One of the best choices is probiotics, which maintain or restore normal gut microbiota, inhibit pathogen invasion, and prevent inflammation [1–3].

Studies on the effects of intestinal probiotics on giant pandas focused mostly on cellulolytic bacterium and bacillus [4–6]. The present work is the first to investigate lactic acid bacteria (LAB) isolated from the giant panda feces, thereby providing crucial data to guide further study. We analyzed the intestinal microflora structure of captive giant pandas of different ages and focused on the survival in extreme acid and bile condition, antagonistic activity, and antibiotic susceptibility of the strains [7]. The pathogenic strains used in the antagonistic test were hyperendemic enteric pathogens of giant pandas [8, 9]. The present study is the first step in the search for probiotics that prevent and treat gut diseases in giant pandas. The next steps must involve in vivo safety test and the extensive assessment of LAB. We aim to develop new probiotics for giant pandas in the future.

Xueqin Ni, Qiang Wang and Zhirong Peng are joint first authors.

✉ Dong Zeng
zend@sicau.edu.cn

[1] Animal Microecology Institute, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China

[2] Chengdu Wildlife Institute, Chengdu Zoo, Chengdu 610081, China

[3] Key Laboratory of Animal Disease and Human Health of Sichuan Province, Chengdu 611130, China

Materials and Methods

Strains and Feces Samples

The feces samples were collected from seven healthy giant pandas (Chengdu Research Base of Giant Panda Breeding); ETEC (O8:H19:F4ac$^+$, LT$^+$, STa$^-$, STb$^+$), *Escherichia coli* ATCC25922, and *Lactobacillus rhamnosus* GG ATCC53103 (LGG) were purchased from Chinese Veterinary Drug Control; *Salmonella* and *Staphylococcus aureus* were received from Laboratory of Animal Infectious Disease and Microarray (Sichuan Agricultural University).

Isolation of LAB

Feces samples (10 g) were homogenized in sterile saline (90 mL), serially diluted and plated onto MRS agar and incubated at 37 °C for 24~48 h in constant anaerobic environment. Colonies which showed different morphologies were selected and purified by restreaking three times or more on MRS agar. The pure isolates which exhibited Gram-positive were selected and subcultured in MRS broth for further study. Biochemical reaction method (Hangzhou Microbial Reagent co., Ltd.) was the first step of screening and referenced with *Bergey's Manual Of Systematic Bacteriology* and *Isolation And Identification And Test Methods Of Lactic Acid Bacteria*.

Acid and Bile Tolerance Test

The isolates were subcultured in MRS broth for 24 h. The equal volume of suspension was added to MRS broth which was adjusted to pH 1.0, 2.0, and 3.0 with 1 M HCl and contained bile 0.3, 1.0, and 2.0% (*w*/*v*), respectively. Broth was incubated at 37 °C and then viable count was conducted at 0 and 3 h for acid tolerance test and 0 and 4 h for bile test.

Antimicrobial Activity Test

Antimicrobial activity of isolates, for ETEC, *Staphylococcus aureus*, and *Salmonella*, was assessed using the Oxford cup method [10]. The isolates were inoculated to MRS broth at 37 °C for 24 h. The cell-free supernatant was collected (15,600×*g*, 10 min). Freshly grown pathogen cultures (100 μL, 10^7 CFU/mL) were spread on LB agar plate and allowed to dry. Oxford cups were placed on plates. A 100 μL cell-free supernatant was poured into a cup on plates. The zone of inhibition was measured and recorded after inoculating at 37 °C for 24 h.

Antibiotic Susceptibility Test

Antibiotic susceptibility of the isolates was assessed using disk diffusion method [11]. Isolates and paper disk (Beijing Tiantan Biological Products co., Ltd.) were placed onto the surface of MRS agar plates. The zone of inhibition was measured and recorded after inoculating at 37 °C for 24 h. Results were compared with interpretative zone diameters described by Performance Standards for Antimicrobial Disk Susceptibility Tests [12]. The antibiotics tested were kanamycin (30 μg), gentamicin (10 μg), amikacin (30 μg), streptomycin (10 μg), sulfamethoxazole (25 μg), tetracycline (30 μg), doxycycline (30 μg), florfenicol (30 μg), chloramphenicol (30 μg), cefotaxime (30 μg), cephradine (30 μg), ceftriaxone (30 μg), cefoperazone (75 μg), and ciprofloxacin (5 μg).

Molecular Identification

The isolates were inoculated at 10 ml MRS broth at 37 °C overnight and the culture was centrifuged (4000 rev/min) to harvest the cells and wash 2–3 times by sterile saline. The genomic DNA was extracted by using E.Z.N.A.® Stool DNA kit (Omega Biotechnology, USA). The primers are 27F and 1492R. The amplified DNA fragment was separated on a 2% agarose gel. The fragment was used directly for DNA sequencing (Beijing BGI Sequencing). The resulting

Table 1 Differential phenotypic characteristic of 25 isolates from fecal samples of pandas

Characteristic	E	G83	G87,G88,G89,G90
Morphology	Ellipsoidal	Rod	Bend
Gram reaction	+	+	+
Catalase	–	–	–
Gelatin	10	–	–
Nitrate reduction	–	–	–
H_2S production	–	–	–
Sucrose	6	+	+
Xylose	–	–	–
Glucose	18	+	+
Lactose	7	+	+
Cellobiose	17	+	–
Esculin	18	+	+
Maltose	+	+	+
Sorbitol	9	+	–
Mannitol	11	+	–
Motile	–	–	–
15 °C growth test	+	+	+
45 °C growth test	+	+	+
pH 4.5 growth test	18	+	+
6.5% NaCl	+	–	–

E: E1, E2, E3, E4, E5, E7, E9, E16, E18, E19, E20, E21, E23, E27, E33, E57, E67, E69, E70, E75, 20 isolates

+: positive or weakly positive reaction

−: negative reaction

Number: the number of positive reaction

Table 2 Viable cell counts of isolates after 3 h at pH 3.0 to 1.0 (log CFU/mL)

Strains	Initial concentration	pH 3.0	pH 2.0
E1	8.75 ± 0.05	8.09 ± 0.08	7.59 ± 0.26
E2	8.95 ± 0.05	8.68 ± 0.17	6.26 ± 0.24
E3	8.77 ± 0.14	4.26 ± 0.24	–
E4	9.17 ± 0.10	7.65 ± 0.16	–
E5	9.00 ± 0.15	8.36 ± 0.10	7.36 ± 0.10
E7	8.98 ± 0.07	8.63 ± 0.06	6.83 ± 0.13
E9	8.73 ± 0.05	8.10 ± 0.17	5.10 ± 0.17
E16	8.75 ± 0.05	7.36 ± 0.32	7.11 ± 0.10
E18	8.75 ± 0.09	8.32 ± 0.28	7.46 ± 0.15
E19	9.07 ± 0.02	8.20 ± 0.17	7.46 ± 0.15
E20	8.92 ± 0.03	8.42 ± 0.10	7.23 ± 0.05
E21	8.42 ± 0.10	8.10 ± 0.10	8.15 ± 0.10
E23	8.80 ± 0.04	8.20 ± 0.17	6.36 ± 0.32
E27	8.78 ± 0.08	8.75 ± 0.05	7.68 ± 0.17
E33	8.67 ± 0.08	6.81 ± 0.20	6.63 ± 0.06
E57	8.59 ± 0.11	7.10 ± 0.06	6.16 ± 0.28
E67	8.96 ± 0.12	8.49 ± 0.20	7.43 ± 0.38
E69	8.56 ± 0.07	7.52 ± 0.07	6.10 ± 0.17
E70	8.87 ± 0.15	8.20 ± 0.17	7.23 ± 0.40
E75	8.88 ± 0.03	8.10 ± 0.17	7.68 ± 0.14
G83	8.81 ± 0.13	8.46 ± 0.15	8.10 ± 0.17
G87	8.74 ± 0.13	8.36 ± 0.10	7.50 ± 0.17
G88	8.73 ± 0.15	8.26 ± 0.24	7.46 ± 0.15
G89	8.59 ± 0.11	8.16 ± 0.28	7.59 ± 0.11
G90	8.67 ± 0.06	8.42 ± 0.10	7.52 ± 0.24

The mean of six value of each sample are presented with ± SD. No growth at pH 1.0

sequences were compared with the sequences in the GenBank database using the BLAST program available on the National Center for Biotechnology Information (NCBI) website. The criterion used to identify an isolate to the species level was identity greater than 99% in the 16S rRNA gene sequence.

Statistical Analysis

All data were expressed as means and standard deviations and analyzed using SPSS version 19.0. The difference was evaluated by one-way ANOVA and statistical significance was set at $P < 0.05$.

Result

Morphological and Phenotypic Characteristics

A total of 207 isolates were obtained and initially screened. The isolates were observed for their morphological and phenotypic characteristics. Only 25 isolates were Gram-positive. G83 exhibited a rod-shaped morphology, whereas that of G87, G88, G89, and G90 was bent. The morphology of the remaining isolates was ellipsoidal (Table 1). Among the isolates, 25 were oyster white and facultative anaerobes, arranged singly, in pairs, or in short chains. Catalase, xylose, motility, nitrate reduction, and H_2S production tests showed negative results.

Acid and Bile Resistance

The majority of the isolates showed high resistance to acid after inoculation at pH 3.0 for 3 h. Isolates E21 and G83 revealed good survival ability after inoculation at pH 2.0 for 3 h. No isolates could survive at pH 1.0 (Table 2). Viable cell counts of isolates after 4 h culture at different bile concentrations are shown in Table 3. At 0.3% bile, all isolates except E3, E9, E23, E33, and E57 showed high resistance, with concentrations higher than 10^7 CFU/mL. Under 2% bile, the viable cell count of E1, E2, E16, E18, E21, E69, E70, and G83 were higher than that of 10^6 CFU/mL, whereas E3, E9, E23, E33, and E57

Table 3 Survival of isolates after 4 h at bile concentration (log CFU/mL)

Strains	Initial concentration	0.3%	1%	2%
E1	8.65 ± 0.16	7.52 ± 0.07	7.16 ± 0.28	6.52 ± 0.07
E2	8.62 ± 0.15	7.71 ± 0.24	7.36 ± 0.10	6.63 ± 0.06
E3	8.82 ± 0.07	5.46 ± 0.15	2.10 ± 0.17	–
E4	8.36 ± 0.10	7.36 ± 0.10	6.85 ± 0.13	5.01 ± 0.07
E5	8.46 ± 0.15	7.36 ± 0.10	6.70 ± 0.17	3.64 ± 0.30
E7	8.66 ± 0.10	7.67 ± 0.06	7.15 ± 0.12	5.95 ± 0.08
E9	8.80 ± 0.04	2.20 ± 0.17	–	–
E16	8.78 ± 0.08	7.53 ± 0.21	7.29 ± 0.03	6.40 ± 0.17
E18	8.59 ± 0.11	7.69 ± 0.21	7.36 ± 0.06	6.33 ± 0.35
E19	8.79 ± 0.10	7.80 ± 0.04	6.67 ± 0.06	5.49 ± 0.20
E20	8.77 ± 0.12	7.73 ± 0.15	7.20 ± 0.17	5.88 ± 0.03
E21	8.56 ± 0.24	7.46 ± 0.15	7.21 ± 0.04	6.20 ± 0.17
E23	8.55 ± 0.13	2.10 ± 0.17	–	–
E27	8.94 ± 0.15	7.32 ± 0.06	5.58 ± 0.17	5.26 ± 0.24
E33	8.65 ± 0.16	4.10 ± 0.07	–	–
E57	8.63 ± 0.06	–	–	–
E67	8.99 ± 0.08	7.68 ± 0.17	7.26 ± 0.06	5.49 ± 0.20
E69	8.72 ± 0.13	7.68 ± 0.19	7.03 ± 0.11	6.42 ± 0.10
E70	8.88 ± 0.03	7.72 ± 0.10	7.06 ± 0.08	6.36 ± 0.10
E75	8.90 ± 0.05	7.63 ± 0.06	5.64 ± 0.03	4.56 ± 0.07
G83	8.64 ± 0.19	7.59 ± 0.26	7.07 ± 0.09	6.08 ± 0.12
G87	8.68 ± 0.24	6.69 ± 0.09	4.16 ± 0.16	3.62 ± 0.28
G88	8.53 ± 0.21	6.72 ± 0.22	4.26 ± 0.24	3.58 ± 0.28
G89	8.65 ± 0.16	6.53 ± 0.21	5.66 ± 0.32	4.16 ± 0.28
G90	8.73 ± 0.15	6.72 ± 0.22	4.02 ± 0.07	3.75 ± 0.09

The mean of six value of each sample are presented with ± SD

Table 4 Antimicrobial activities of 20 isolates from fecal samples of pandas

Strains	ETEC		*Salmonella*		*Staphylococcus aureus*	
E1	++	19.93 ± 0.33^{g}	++	19.41 ± 0.80^{i}	+	14.41 ± 0.36^{cd}
E2	±	11.55 ± 1.32^{abcd}	++	17.13 ± 0.39^{g}	+	13.14 ± 1.61^{abc}
E4	+	12.31 ± 0.78^{d}	+	14.39 ± 0.33^{bc}	+	12.47 ± 0.27^{ab}
E5	±	11.80 ± 0.50^{abcd}	+	15.06 ± 0.26^{cd}	+	15.10 ± 1.09^{de}
E7	±	11.88 ± 1.24^{bcd}	+	15.24 ± 1.17^{cde}	+	13.38 ± 0.84^{abc}
E16	+	12.15 ± 0.62^{cd}	+	14.50 ± 0.57^{bcd}	+	13.72 ± 0.18^{bc}
E18	+	14.70 ± 1.12^{e}	+	15.33 ± 0.44^{cde}	+	13.73 ± 0.25^{bc}
E19	±	11.74 ± 1.53^{abcd}	+	13.62 ± 0.90^{b}	+	12.21 ± 0.22^{a}
E20	±	11.06 ± 0.40^{abc}	+	13.75 ± 0.86^{b}	+	13.61 ± 0.34^{bc}
E21	±	11.61 ± 0.27^{abcd}	+	13.84 ± 0.52^{cde}	+	13.28 ± 1.54^{abc}
E27	±	11.03 ± 0.46^{abc}	+	15.36 ± 0.46^{de}	+	12.10 ± 0.55^{a}
E67	±	11.04 ± 0.35^{abc}	+	15.33 ± 0.52^{cde}	+	13.28 ± 1.54^{abc}
E69	±	10.88 ± 0.23^{ab}	±	11.67 ± 0.53^{a}	+	13.26 ± 0.28^{abc}
E70	±	10.64 ± 0.33^{a}	++	16.56 ± 0.46^{fg}	+	13.14 ± 1.61^{abc}
E75	±	10.98 ± 0.68^{abc}	+	14.76 ± 0.57^{cd}	+	13.01 ± 0.30^{ab}
G83	+++	22.48 ± 0.90^{h}	+++	20.10 ± 0.71^{i}	++	17.90 ± 0.65^{g}
G87	++	19.70 ± 0.25^{g}	++	18.36 ± 0.39^{h}	++	17.49 ± 0.35^{fg}
G88	+++	20.07 ± 0.19^{g}	++	16.60 ± 0.37^{fg}	++	17.35 ± 0.26^{fg}
G89	++	17.30 ± 0.29^{f}	++	16.54 ± 0.26^{fg}	++	16.41 ± 0.35^{f}
G90	+++	22.47 ± 0.34^{h}	++	16.07 ± 0.31^{ef}	++	16.27 ± 0.34^{ef}
LGG	+++	20.34 ± 0.59^{g}	++	18.16 ± 0.58^{h}	+	15.41 ± 0.33^{de}

The different letters represent significant diverse and the same letters represent no significant diverse in one queue. The mean of 6 values of each sample are presented with ± SD

±:8 mm < zone diameters ≤ 12 mm; +: 12 mm < zone diameters ≤ 16 mm; ++: 16 mm < zone diameters ≤ 20 mm; +++: 20 mm < zone diameters

did not survive. Out of 25, only 20 isolates exhibited a good ability to resist low pH and high bile salts and were selected for further analysis.

Antimicrobial Activity

The isolates exhibited significant antimicrobial effects on enterotoxigenic *Escherichia coli* CVCC196 (ETEC), *Staphylococcus aureus*, and *Salmonella* (Table 4). Isolates G83, G88, and G90 demonstrated better antimicrobial effect on ETEC (> 20 mm) than the other isolates. Isolate G83 showed a stronger effect on *Salmonella* (> 20 mm) than the others, and isolates G83, G87, and G88 possessed inhibitory activity against *S. aureus* (> 17 mm). The inhibition zones of G83 were longer with respect to LGG on each pathogen. G83 showed excellent antimicrobial ability. Antimicrobial activity was not detected after excluding the interference of acid materials by adjusting the supernatant to pH 6.5 using NaOH.

Antibiotic Susceptibility

Selected isolates exhibited multi-drug resistance. However, the antibiotic resistance ratio of isolates was not high, except that of E1 (Table 5). All isolates were sensitive to florfenicol, chloramphenicol, and

Table 5 Antibiotic susceptibility test of part of isolates from fecal samples of pandas

Antibiotics	%R	ATCC25922	E1	E69	E70	E75	G83
Kanamycin	40	S	R	MS	S	MS	MS
Gentamicin	50	S	R	MS	S	R	MS
Amikacin	80	S	R	R	MS	R	R
Streptomycin	100	S	R	R	R	R	R
Cotrimoxazole	25	S	R	S	S	S	R
Tetracycline	5	S	R	S	S	S	S
Doxycycline	0	S	MS	S	S	S	S
Florfenicol	0	S	S	S	S	S	S
Chloramphenicol	0	S	S	S	S	S	S
Cefotaxime	10	S	R	S	S	S	S
Cephradine	5	S	R	S	S	S	S
Ciprofloxacin	30	S	R	MS	MS	MS	R
Ceftriaxone	5	S	R	S	S	S	S
Cefoperazone	5	S	R	S	S	S	MS

ATCC25922 is standard indicator strains

R: resistance; *MS*: moderately sensitive; *S*: sensitive

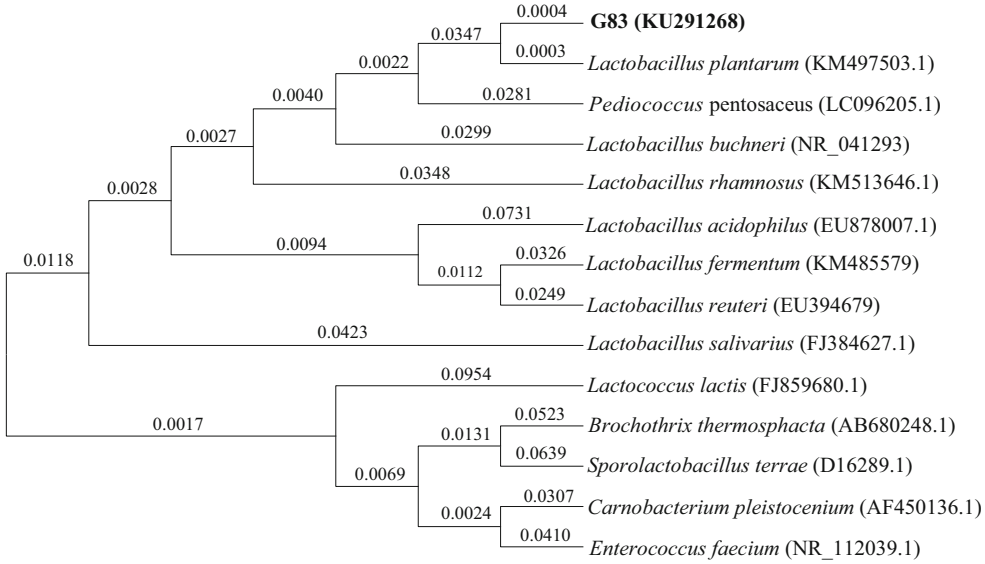

Fig. 1 Phylogenetic relationships of strain G83 to related species based on 16S rRNA gene sequences

doxycycline. Isolate G83 was only resistant to amikacin, streptomycin, cotrimoxazole, and ciprofloxacin.

Phylogenetic Analysis

All isolates were further identified by 16S rRNA sequencing and phylogenetic analysis. The five isolates were species of *Lactobacillus,* whereas the rest of the isolates were species of *Enterococcus*. G83 showed the highest sequence similarity (99%) to *L. plantarum* based on BLASTn. Its sequence was uploaded to NCBI (GenBank accession number KU291268). A phylogenetic tree was built using DNAMAN5.2 and MEGA6.0 (Fig. 1).

Table 6 The detection of enterotoxin genes in 15 isolates of *Enterococcus*

Strains	*esp* [15]	*ccf* [13]	*gelE* [16]	*cylA* [17]	*agg* [13]	*ace* [18]	*efaAfs* [13]	*efaAfm* [13]	%
E1	–	+	+	–	–	+	+	–	50
E2	–	+	+	–	–	+	+	–	50
E4	–	+	+	–	–	+	+	–	50
E5	–	+	–	–	–	–	+	+	37.5
E7	–	+	+	–	–	+	+	+	62.5
E16	–	+	–	–	–	+	+	+	50
E18	–	+	–	–	–	–	+	+	37.5
E19	–	+	+	–	–	+	+	+	62.5
E20	–	+	+	–	–	+	+	–	50
E21	–	+	–	–	–	+	+	–	37.5
E27	–	+	+	–	–	+	+	–	50
E67	–	+	+	–	–	+	+	–	50
E69	–	+	+	–	–	+	+	–	50
E70	–	+	–	–	–	+	+	–	37.5
E75	–	+	+	–	–	+	+	–	50
%	0	100	66.7	0	0	86.7	100	33.3	/

+: negation reaction; −: positive reaction; %: positive rate

Enterococcal surface protein gene (*esp*), sex pheromone (*ccf*), gelatinase gene from *Enterococcus* (*gelE*), cytolysin A (*cylA*), aggregation substance gene (*agg*), accessory colonization factor (*ace*), *Enterococcus faecalis* endocarditis antigen (efaA)

Detection of Virulence Genes

Enterococcus is one of the dominant bacteria in the gut of giant pandas [7]. *Enterococcus* can be used as probiotics without virulence genes [13, 14]. We performed virulence gene detection on the isolates for security. Some common virulence genes were detected via PCR (Table 6). A total of 15 isolates of *Enterococcus* exhibited different virulence genes, whereas 5 isolates of *Lactobacillus* did not. Eight kinds of virulence genes were detected, namely sex pheromone (*ccf*), gelatinase gene from *Enterococcus* (*gelE*), accessory colonization factor (*ace*), cytolysin A (*cylA*), aggregation substance gene (*agg*), enterococcal surface protein gene (*esp*), endocarditis antigen in *E. faecalis* (*efaAfs*), and *E. faecium* (*efaAfm*), with ratios fluctuating from 37.5 to 62.5%. The detection rates of *ccf* and *efaAfs* reached 100%, whereas those of *gelE*, *ace,* and *efaAfm* were 66.7, 86.7, and 33.3%, respectively. Overall, G83 was selected for further analysis.

Discussion

Probiotics, such as *Lactobacillus*, *Bifidobacterium*, and *Bacillus*, can prevent gut bacterial disease, maintain or restore the normal microbiota, and maintain intestinal integrity [19–22]. The current work aimed to screen potential probiotics from giant panda feces that exhibit outstanding abilities. We aimed to use the probiotics as an alternative for

antibiotics, as demonstrated in giant panda. Mimicking gastrointestinal tract conditions, the acid and bile tolerance of isolates were initially screened [23]. Acid and bile tolerance are important selection criteria for probiotic strains. Gastric acidity and bile conditions are relevant for digestion and metabolic activity [24]. The isolates must withstand acidic fluid with a pH of 1.5 to 3.0 and bile concentrations that fluctuate between 0.5 and 2.0% [25]. Isolate G83 grew well at pH 2.0 and under 2.0% bile conditions, thereby showing its ability to survive the harsh conditions in the stomach and small intestines. The LAB isolated from canine feces could live in broth with a pH level of 2.0 for 4 h [26]. In addition, nitrate and H_2S reactions were negative, thereby indicating that isolates can enhance the safety of the host. Carbohydrates were also produced, and these provide energy for host and gut microflora.

Captive giant pandas are easily afflicted by gut bacterial diseases. These gut diseases are always caused by *E. coli*, *Salmonella*, *S. aureus*, *Shigella*, *Klebsiella*, and *Proteus* [8, 9]. In the current study, selected isolates were assessed for antimicrobial activity against hyperendemic enteric pathogens of giant pandas. Isolate G83 showed maximum inhibition zones against ETEC, *Salmonella*, and *S. aureus*. The antagonistic activity of the acknowledged probiotic LGG was inferior to that of G83. Other studies reported the antimicrobial activity of probiotic strains against some common pathogens, such as *E. coli*, *Salmonella*, *Listeria*, and *S. aureus* [23, 27, 28].

Susceptibility to antibiotics is species- and strain-specific [29]. All isolates (except the indicator strain ATCC25922) showed different levels of antibiotic resistance. Consistent with previous studies, isolate G83 was resistant to amikacin, streptomycin, cotrimoxazole, and ciprofloxacin. Strains of *L. reuteri* and *L. rhamnosus* were resistant to tetracycline [30, 31]. *Corynebacterium vitaeruminis* MRU4 isolated from cow rumen was resistant to oxacillin, gentamicin, erythromycin, clindamycin, sulfa/trimethoprim, and rifampicin [32]. Antibiotic use in animals can result in the presence of antibiotic residues in their meat and milk. Antibiotic-resistant bacteria and resistance genes can transfer between animals and people [33]. However, this situation is not applicable to giant pandas. Antibiotic resistance was not detected in the strains isolated from giant panda feces.

Enterococcus is a natural flora present in traditional food. It is used for food ripening and flavor improvement [34]. Some *Enterococcus* strains have been successfully developed as probiotics to improve the health of human and animals [34, 35]. However, most *Enterococcus* strains carry various virulence factors and can cause many diseases, including urethral infections, bacteremia, endocarditis, peritonitis, and wound infections [36, 37]. We detected virulence factors in our 20 isolates to determine their safety. Five kinds of virulence genes were detected from 20 *Enterococcus* isolates. The use of the strains relies on various indexes that indicate their safety as probiotics in the Korean market [38]. These indexes not only include virulence genes but also enterotoxin genes, which are carried by some *Enterococcus* strains. Several factors have been implicated as potential virulence determinants that cause serious human diseases. Virulent strains harm the host through their adherence to host tissue, invasion and abscess formation, host inflammatory response modulation, and toxin secretion [13]. For security purposes, we selected G83 as a potential probiotic.

As previously discussed, some strains in this study showed good acid and bile resistance, activity against pathogens, and sensitivity to most antibiotics, with *Lactobacillus* G83 exhibiting the best activity. In future studies, G83 may show some interesting probiotic traits. Further sequential trials of its effects, as well as animal trials to test its in vivo effects, are required. The safety of these isolates must also be evaluated in vivo.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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