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Screening for potential probiotic bacteria from Korean fermented soybean paste: *In vitro* and *Caenorhabditis elegans* model testing

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ABSTRACT

In the present study, we screened for potential probiotics from 34 lactic acid bacteria (LAB) isolated from the Korean fermented soybean paste. The LAB were tested for their survival abilities in the simulated gastrointestinal conditions. Seven lactic acid bacteria namely *Pediococcus acidilactici* SDL 1402, *Pediococcus acidilactici* SDL 1405, *Pediococcus acidilactici* SDL 1406, *Weissella cibaria* SCCB 2306, *Streptococcus thermophilus* SCML 337, *Streptococcus thermophilus* SCML 300 and *Enterococcus faecium* SC 54 were resistant to the tested conditions. All seven LAB were sensitive to penicillin, tetracycline, kanamycin, gentamycin, erythromycin, clindamycin and chloramphenicol but resistant to streptomycin and ampicillin. Only *Streptococcus thermophilus* SCML 337 was sensitive to vancomycin. The seven lactic acid bacteria showed auto-aggregation abilities between 28 and 42.9%. They also showed good co-aggregation abilities against *Escherichia coli* 0157:H7, *Listeria monocytogenes* ATCC 15313, *Bacillus cereus* ATCC 14576, *Staphylococcus aureus* ATCC 19095 and *Salmonella enterica* ATCC14028. Additionally, all the lactic acid bacteria strongly attached to *Caenorhabditis elegans* gut indicating their strong gut colonization ability. The results from this study indicate that these seven lactic acid bacteria are promising probiotic candidates.

1. Introduction

There has been an increase in consumer interest towards functional foods in recent years. This has been attributed to increasing consumer awareness of the disease prevention and health enhancing effects of functional foods (Daliri & Lee, 2015a). Fermented foods have been associated with immense health effects such as reducing the risk of cardiovascular diseases, type 2 diabetes and obesity (Eussen et al., 2016; Tapsell, 2015). These health effects are attributed to the microbes and the products of fermentation but not directly to the starting food material. Therefore, "good microbes" in fermented foods that remain alive in the gut after ingestion may have direct health benefits on their hosts.

The Food and Agriculture Organization and the World Health Organization define probiotics as "live microorganisms (bacteria and yeast) that, when administered in adequate amounts, confer a health benefit on the host" (Morelli & Capurso, 2012). Consumption of viable, fermentation-associated microbes could therefore reduce hypercholesterolemia, improve lactose intolerance, modulate the immune system and balance the gut microbiota similar to existing probiotic strains (Daliri & Lee, 2015b; Post, 2017). Resistance to gastric acidity and intestinal fluids, adherence to intestinal surfaces, auto-aggregation and co-aggregation abilities and antimicrobial activity against pathogenic bacteria are considered as important criteria for probiotic selection (Devi, Archer, & Halami, 2015). Food-associated lactic acid bacteria including *L. plantarum* and *L. rhamnosus* (Wu et al., 2015), *P. freudenreichii* (Kwon, Lee, & Lim, 2016), *L. reuteri* (Gao et al., 2015) and *Weissella cibaria* WIKIM 28 (Lim et al., 2017) have demonstrated excellent potentials to directly affect host health. Probiotics have also been isolated from fermented beans (Jampaphaeng, Cocolin, & Maneerat, 2017) and vegetables (Kaur, Lee, Park, & Sharma, 2017).

Over the years, probiotic gut binding ability has been studied using Caco-2 cells or *Caenorhabditis elegans* (*C. elegans*) (Das, Khowala, & Biswas, 2016; Lee et al., 2015; Park, Yun, Son, Oh, & Kim, 2014). The intestinal cells of *C. elegans* have been reported to be similar to those of humans and this makes the nematode ideal for studying bacteria-host interactions in the human gut (Papadimitriou et al., 2015).

Soybean products serve as good alternatives for dairy foods for vegetarians and lactose intolerant people. However, not much study has

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Table 1
Acid resistance of lactic acid bacteria in phosphate-saline buffer (pH 2.5). Values are expressed in mean ± standard deviation (n = 3).

Strain	Initial count (cfu/ml)x 10^9	Survival after 4 h (cfu/ml)x 10^9	Percentage survival (%)
Pediococcus acidilatici SDL 1405	2.88 ± 0.31	1.61 ± 0.17	56.06[a]
Leuconostoc citreum SC 53	1.38 ± 0.11	1.06 ± 0.16	77.29[i]
Streptococcus thermophilus SCML 337	0.57 ± 0.02	0.46 ± 0.01	81.90[k]
Weissella cibaria SCCB 2306	1.45 ± 0.14	1.23 ± 0.20	85.27[m]
Weissella koreensis JBNU 2	0.15 ± 0.04	0.12 ± 0.05	83.33[l]
Pediococcus acidilatici SCL 1420	0.21 ± 0.08	0.18 ± 0.03	89.37[n]
Lactobacillus brevis SDL 1408	0.17 ± 0.04	0.11 ± 0.02	67.46[e]
Lactobacillus rhamnosus JDFM 6	0.44 ± 0.14	0.26 ± 0.01	60.94[c]
Enterococcus faecium SC 54	1.32 ± 0.12	1.22 ± 0.18	88.45[n]
Weissella confusa SCKB 2318	2.39 ± 0.11	1.70 ± 0.12	71.43[f]
Lactobacillus curvatus JBNU 38	2.45 ± 0.27	1.87 ± 0.17	76.73[i]
Pediococcus acidilatici SDL 1402	3.47 ± 0.31	2.59 ± 0.32	74.78[h]
Enterococcus lactis SCL 1421	0.13 ± 0.02	0.12 ± 0.04	91.70[o]
Pediococcus acidilatici SKL 1418	1.17 ± 0.18	1.07 ± 0.16	91.06[o]
Enterococcus faecalis MAD-13	1.74 ± 0.19	1.12 ± 0.14	64.18[d]
Pediococcus acidilatici SDL 1414	0.15 ± 0.04	0.14 ± 0.06	92.72[p]
Streptococcus thermophilus SCML 300	0.23 ± 0.06	0.18 ± 0.04	77.80[j]
Lactobacillus plantarum JDFM 44	0.32 ± 0.05	0.30 ± 0.05	95.00[q]
Lactobacillus rhamnosus JDFM 33	0.31 ± 0.05	0.26 ± 0.05	85.00[m]
Weissella confusa SCSB 2320	0.37 ± 0.07	0.28 ± 0.07	75.81[h]
Lactobacillus pentosus SC 48	1.12 ± 0.12	0.96 ± 0.11	86.22[n]
Pediococcus pentosaceus SDL 1416	2.34 ± 0.14	1.31 ± 0.22	56.38[a]
Lactobacillus arizonensis SC 25	2.29 ± 0.11	1.66 ± 0.23	72.25[g]
Pediococcus acidilactici DM-9	1.41 ± 0.17	1.06 ± 0.11	75.27[h]
Lactobacillus brevis SDL 1411	1.17 ± 0.14	0.67 ± 0.10	57.45[b]
Enterococcus faecium CK-5	0.26 ± 0.05	0.24 ± 0.04	92.78[p]
Lactobacillus plantarum SDL 1413	3.27 ± 0.15	1.66 ± 0.22	50.77[r]
Pediococcus acidilatici SDL 1406	2.75 ± 0.37	1.24 ± 0.20	45.29[s]
Pediococcus pentosaceus SDL 1415	2.45 ± 0.26	1.03 ± 0.10	42.07[t]
Pediococcus pentosaceus SDL 1401	0.25	0.08 ± 0.01	33.13[u]
Pediococcus pentosaceus MAC-11	1.55 ± 0.27	0.56 ± 0.10	36.08[v]
Pediococcus pentosaceus SDL 1409	1.91 ± 0.34	0.46 ± 0.17	24.27[w]
Leuconostoc mesenteroides JBNU 10	0.20 ± 0.01	0.08 ± 0.01	39.39[x]
Leuconostoc paramesenteroides SC 46	0.30	0.12 ± 0.01	38.38[x]

Different superscripts (^{a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u,v,w,x}) represent significantly different values ($P < 0.05$).

been done to isolate probiotic candidates from fermented soybeans (Mishra, Hati, Das, & Patel, 2017) and thus this warrants studies targeted at isolating probiotic bacteria from fermented soybean foods. In this study, we screened 34 lactic acid bacteria isolated from fermented soybeans for their resistance to pH 2.5, pepsin, bile salts (0.3% oxgall), pancreatin, ten antibiotics and their abilities on auto-aggregation and co-aggregation. We further determined the ability of the lactic acid bacteria to colonize *C. elegans* gut to ascertain their gut epithelial attachment ability.

2. Materials and methods

2.1. Bacterial strains and growth conditions

A total of 34 indigenous LAB strains, isolated from the Korean fermented soybean paste were received from Soonchang Jang Ryu Saupso Company-Korea and screened for their potential probiotic properties. The LAB were cultured at 37 °C for 24 h in de Man, Rogosa and Sharpe (MRS, MBCell- Korea) broth and/or agar. Pathogenic strains of *Escherichia coli* ATCC 0157:H7, *Listeria monocytogenes* ATCC 15313, *Bacillus cereus* ATCC 14576, *Staphylococcus aureus* ATCC 19095 and *Salmonella enterica* ATCC 14028 were obtained from the Department of Food Science and Biotechnology, Kangwon National University in South Korea. The bacteria were cultivated in trypticase soy broth (TSB, Difco- France) overnight at 37 °C and used for the experiments.

2.2. Screening for probiotic properties

2.2.1. Resistance to low pH

The resistance of lactic acid bacteria to low pH was studied according to the method described by Tokatlı, Gülgör, Bağder Elmacı, Arslankoz İşleyen, and Özçelik (2015). Briefly, LAB cultures incubated at 37 °C for 24 h were centrifuged at 6000 g for 15 min. The pellets were suspended in sterile phosphate-saline buffer (PBS, GIBCO- USA) containing 9 g/L NaCl (Sigma-Aldrich, South Korea), 9 g/L $Na_2HPO_4{\cdot}2H_2O$ and 1.5 g/L KH_2PO_4 adjusted to a pH of 2.5. The mixture was then incubated at 37 °C for 4 h. Aliquots of samples were taken at time 0 and after 4 h. The samples were serially diluted in sterile saline solution (0.85% NaCl) and the viable cells were determined by the spread plate method using MRS agar. The plates were incubated at 37 °C for 24 h and the percentage survival of the bacteria was calculated as follows:

$$\% \text{ survival} = \frac{(\text{CFU of viable cells survived})}{(\text{CFU of initial viable cells inoculated})} \text{ x } 100 \quad (1)$$

2.2.2. Resistance to pepsin

To test the viability in the presence of pepsin, simulated gastric juice was prepared by suspending 3 mg/mL pepsin (Sigma-Aldrich, South Korea) in sterile saline solution (0.85%NaCl, w/v) and adjusted to pH 2.5. The fluid was inoculated with active cultures at an inoculum size of 1%(v/v) and incubated at 37 °C for 4 h. The viable cells were determined before (T_1) and after incubation (T_2) by the spread plate method (Tokatlı et al., 2015). The percentage survival of the bacteria was calculated according to (1).

2.2.3. Resistance to bile salts and pancreatin

Resistance to intestinal juices was tested as reported by Tokatlı et al. (2015). Briefly, 0.3% (w/v) bile salt (Sigma-Aldrich, South Korea) and 1 mg/mL pancreatin (Sigma-Aldrich, South Korea) were dissolved in