Physiological Characteristics and Anti-diabetic Effect of *Pediococcus pentosaceus* KI62

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Abstract

The purpose of this study is to examine the physiological characteristics and anti-diabetic effects of *P. pentosaceus* KI62. The α-amylase and α-glucosidase inhibitory activity of *P. pentosaceus* KI62 was 94.86±3.30% and 98.59±0.52%, respectively. The amounts of short chain fatty acids (SCFA) in MRS broth containing 3% maltodextrin inoculated by *P. pentosaceus* KI62 were propionic acid 18.05 ± 1.85 mg/kg, acetic acid 1.12 ± 0.07 g/100 mL, and butyric acid 2.19 ± 0.061 g / kg. The amounts of medium chain fatty acids (MCFA) in MRS broth containing 3% maltodextrin inoculated by *P. pentosaceus* KI62 were C8 0.262±0.031 mg/kg, C10 0.279±0.021mg/kg, and C12 0.203±0.009mg/kg. Compared to sixteen antibiotics, *P. pentosaceus* KI62 had the highest sensitivity to penicillin-G and rifampicin, as well as the highest resistance to vancomycin and ampicillin. The strain also showed higher leucine arylamidase and valine arylamidase activities than other enzyme activities, but it did not produce β-glucuronidase which is carcinogenic enzymes. The survival rate of *P. pentosaceus* KI62 in 0.3% bile was 91.67%. Moreover, the strain showed a 98.63% survival rate in pH 2.0. *P. pentosaceus* KI62 exhibits resistance to *Escherichia coli*, *Salmonella* Typhimurium, *Listeria monocytogenes* and *Staphylococcus aureus* at rates of 29.41%, 38.10%, 51.72% and 50.47%, respectively. *P. pentosaceus* (23.31%) showed a similar adhesion ability to *L. rhamnosus* GG, the positive control (24.49%). These results show that *P. pentosaceus* KI62 has possibility as a probiotic with anti-diabetic effects.

Key words: *Pediococcus pentosaceus*, physiological characteristics, anti-diabetic, α-amylase inhibitory activity, α-glucosidase inhibitory activity

Introduction

Diabetes, an endocrine and metabolic disease, has become the third most non-infectious chronic disease threatening human health. Type-2 diabetes mellitus (T2DM) takes up more than 90% of people with diabetes and has become a major public health issue worldwide (Yan et al., 2019). It is characterized by increased blood glucose level, which cause damage to the body's systems, particularly blood vessels and nerves (Rittiphairoj et al., 2019).

α-glucosidase, which is a digestive enzyme present in the membrane of small intestine brush border, hydrolyzes disaccharides and/or polysaccharides into monosaccharide units for the digestion and absorption of carbohydrates. The absorption of carbohydrates by α-glucosidase generally progresses rapidly in the upper part of the small intestine, leading to a sharp rise in postprandial blood glucose levels. Therefore, it is essential to inhibit α-glucosidase and α-amylase in the postprandial glycemic management of patients with T2DM and pre-diabetes by reducing the post-prandial blood glucose lecvel increasing after carbohydrate diet (Ali et al., 2006).

Short-chain fatty acid (SCFA) produced by intestinal microbes fermenting carbohydrate has beneficial effects on humans; and a deficiency of SCFA production is associated with T2DM (Zhao et al., 2018).

Butyrate, acetate and propionate are SCFAs that are fermented by enterobacteria from dietary fiber and take an important role in energy metabolism (Cummings, 1981). In animal experiments, propionate affects the production of lipoproteins and grapes in the liver, and acetate acts as a substrate for cholesterol synthesis (Schwiertz et al., 2010).

One of the major activities of the large intestinal microbiota is to decompose substrates such as resistant starch and dietary fiber, which are not totally hydrolyzed by host enzymes in the small intestine (Bird et al., 2000; Louis et al., 2007; Topping and Clifton, 2001). Medium chain

fatty acids (MCFA) seem to offer protection from lipo-toxicity and subsequent insulin resistance without caloric restriction (Wein et al. 2009). MCFAs reduced accumulation of fat and improved glucose tolerance. So, dietary supplements including MCFAs may help prevent obesity and peripheral insulin resistance (Turner et al., 2009).

Lactic acid bacteria are industrially important microorganisms because they have been safely used in production of fermentation and functional foods for a long time (Rhee et al., 2011). *Pediococcus pentosaceus* is one of the most commonly found strain in food and dairy environments (Banwo et al., 2013).

This study was conducted to investigate the antidiabetic effect and physiological characteristics of *P. pentosaceus* KI62 to determine whether *Pediococcus pentosaceus* KI62 isolated from kimchi can be applied as a functional food or fermented milk.

Materials and Methods

Isolation of lactic acid bacteria

Using a modified MRS medium, the strain KI62 was isolated from homemade kimchi (Lim et al., 2011). The strain was incubated in *Lactobacilli* MRS broth (Difco, Detroit, MI, USA) as a growth medium at 37℃ for 18 h.

α-amylase inhibitory activity

A modified version of the method of determining α-amylase activity by Xiao et al. (2006) was used. Porcine pancreas α -amylase was purchased from Sigma (St. Louis, MO, USA). The substrate was prepared by boiling 0.5% soluble starch in distilled water for 5 min, and then leaving it to cool to room temperature. The sample (100 μL) and substrate (500 μL) were mixed in 400 μL of 0.04 M phosphate buffer (pH 5.8). After that, 0.5 mg/mL α-amylase solution (100

μL) was added, and the solution was incubated at 25°C for 10 min. The reaction was stopped by adding 100 μL 0.1M HCl, and then 100 μL of the solution was reacted with 1.5 mL iodine solution for 30 min at room temperature. Using a microplate reader (Spectramax Plus 384, Molecular Devices Corp., Sunnyvale, CA, USA), the absorbance of the reactant was determined at 660 nm.

α-glucosidase inhibitory activity

A α-glucosidase inhibition assay was carried out as previously described (Si et al., 2010), but it was modified as follows: Inhibitory activity was measured using α-glucosidase from *Saccharomyces cerevisiae* (Sigma). α-glucosidase (50 μL, 0.75 U/mL) and 0.2 M potassium phosphate buffer (pH 6.5, 50 μL) were mixed with 50 μL of the test sample. After pre-incubation at 37℃ for 15 min, 3 mM *p*-nitrophenol- αD-glucopyranoside (*p*NPG, 100 μL) was added to the mixture. The enzymatic reaction was allowed to proceed at 37℃ for 10 min and was stopped by the addition of 750 μL of 0.1 M Na_2CO_3. 4-Nitrophenol absorption was measured at 405 nm using a microplate reader.

Short chain fatty acid

The KI62 strain was inoculated 1% in MRS broth and MRS broth containing 3% indigestible polysaccharide (maltodextrin), respectively, and cultured at 37℃ for 18 hours, and the supernatant was isolated to determine the contents of propionic acid, acetic acid and butyric acid.

Acetic acid content measurement

5 mL of the sample was diluted with distilled water until the color of sample faded, then a few drops of 1% phenolphthalein solution was added to it. The total acid was titrated and calculated according to the following formula.

Total Acid (g/100 mL) = V1 × f × 0.006 × 100 / V2

V1: Amount of 0.1 N sodium hydroxide solution (mL) consumed in the titration,

f: Titer of 0.1 N sodium hydroxide solution (1.000), V2: Amount of sample liquid used for titration (mL)

Propionic acid content measurement

4 g of the sample was added to 40 mL of ACN and then extracted for 30 minutes using a sonicator. The extracted solution was centrifuged at 4000 rpm for 10 minutes to separate the supernatant. The separated supernatant was filtered with a 0.22 μM membrane filter, concentrated using a nitrogen concentrator, and analyzed by gas chromatograph / mass spectrometer (GC-MS). The GC-MS analysis conditions are shown in Table 1.

Butyric acid content measurement

Chloroform-methanol extraction was used to extract butyric acid. Samples extracted with chloroform-methanol were concentrated using an evaporator, and then esterification of fatty acids to fatty acid methyl esters was performed according to the following method. 20 mg of lipid was added to the tube, and 2 mL of 0.5N NaOH / Methanol was added to stop the stopper and hydrolyzed on a heating block (100℃.) for about 5 minutes. After cooling, 2 mL of 14% BF3 / Methanol was added and reacted for 5 minutes, followed by shaking with 2 mL of isooctane. After the reaction, 2 mL of saturated saline was added to the tube containing the sample. After stopping the plug and shaking it gently for 5 seconds, the isooctane layer was extracted and dehydrated using anhydrous sodium sulfate. A dehydrated fatty acid methyl ester test solution was received and injected into a gas chromatograph (HP-6890GC FID, Agilent Technologies, Santa Clara, Calif., USA) for analysis. The gas chromatograph analysis conditions are shown in Table 2.

Medium chain fatty acid

Another experiment was carried out using the same method of measuring the butyric acid content.

Identification of strain KI62

To analyze the DNA sequence of lactic acid bacteria, universal primers 27F 5'(AGA GTT TGA TCC TGG CTC AG) 3' and 1492R 5'(GGT TAC CTT GTT ACG ACT T) 3' were used, and PCR was performed using a Big Dye terminator cycle sequencing kit v.3.1 (Applied BioSystems, USA). The amplification process was as follows: 95℃, 5 minutes; 95℃, 30 seconds; and 55℃, 2 minutes. It was performed 30 times at 68℃and 1 minute and 30 seconds, and was finished at 68℃ and 10 minutes. After removing the dNTP and the reactant, which do not participate in the reaction with the PCR product of the Montage PCR Cleanup kit (Millipore), sequencing was performed using primers 785F 5'(GGA TTA GAT ACC .CTG GTA) 3' and 907R 5'(CCG TCA ATT CMT TTR AGT TT) 3' with an automated DNA sequencing system (model 3730XL, Applied BioSystems, USA).

Probiotics property

Antibiotic susceptibility, enzyme activity, pH and bile tolerance, antimicrobial activity, and adherence assay were conduct to measure probiotic property. The antibiotic susceptibility of *P. pentosaceus* KI62 was tested using the broth micro-dilution procedure (Phillips, et al., 1991). The LAB Susceptibility test medium with cysteine (LSM-C), which consists of a mixture of Iso-Sensitest broth (90%) and MRS broth (10%), supplemented with 0.3g/L L-cysteine (Klare et al., 2007), was used as the medium. The enzyme activity of strain was determined using an API ZYM kit (bioMérieux, Lyon, France). pH tolerance was tested as described by Clark et al. (1993). Bile tolerance was tested as method of Gilliland and Walker (1990). The *P. pentosaceus*

KI62 strain culture was inoculated into MRS broth containing 0.05% L-cysteine (Sigma) with/without 0.3% ox gall (Sigma). According to method of Gilliland and Speck (1977), antimicrobial activity of strain was measured for *Escherichia coli* ATCC 21985, *Salmonella* Typhimurium ATCC 14028, *Listeria* monocytogenes ATCC 15313, and *Staphylococcus aureus* ATCC 6538. According to method of Kim et al (2008), the intestinal adhesion ability of the strain was performed using HT-29 cells. After culturing the strain and the cells together, the number of strains adhering to the cells was counted using a BCP plate count agar

Statistical analysis

Each experiment was performed in triplicate, and the results were displayed as the mean±standard deviation (SD). Statistical analysis was performed using a XLSTAT (Addinsoft, Paris, France). All analysis was conducted on $p<0.05$ significant level.

Results and Discussion

Isolation of lactic acid bacteria

After collecting 40 kinds of kimchi in each region, 167 single colonies forming yellow colonies were isolated using a modified MRS medium.

Selection of anti-diabetic strain

To select strong inhibitory activities of α-amylase and α-glucosidase, we determined the α-amylase and α-glucosidase inhibitory activities of 167 kinds of isolated strain in kimchi. The KI62 strain exhibited α-amylase and α-glucosidase inhibitory activity of 94.86±3.30% and 98.59±0.52%, respectively (Table 3). Because the dietary habits of Korean people include far more carbohydrates than those of western countries, the mechanism of inhibiting the absorption of carbohydrates should be combined with a mechanism for inhibiting fat absorption in order

to improve obesity (Jang and Jeong, 2010).

When the KI62 strain was inoculated in MRS broth, the contents of the SCFA were propionic acid 5.95 ± 1.66 mg/kg, acetic acid 1.15 ± 0.00 g/100 mL, and butyric acid 2.38 ± 0.02g / kg. On the other hand, when the KI62 strain was inoculated in MRS broth with maltodextrin, the contents of the SCFA were propionic acid 18.05 ± 1.85 mg/kg, acetic acid 1.12 ± 0.07 g/100 mL, and butyric acid 2.19 ± 0.061 g / kg (Fig. 1).

Meanwhile, the contents of the MCFA in MRS broth were C8 0.214 ± 0.007 mg/kg, C10 0.250 ± 0.011 mg/kg, and C12 0.223 ± 0.035 mg/kg. On the other hand, the contents of the MCFA in MRS broth with maltodextrin were C8 0.262 ± 0.031 mg/kg, C10 0.279 ± 0.021 mg/kg, and C12 0.203 ± 0.009 mg/kg (Fig. 2).

Identification of strain KI62

Following sequence analysis, it was identified as *Pediococcus pentosaceus* with a similarity of 99% (Data not shown). On the basis of previous studies, it was named *Pediococcus pentosaceus* KI62.

Antibiotic tolerance

Table 4 shows the MIC values obtained for the 16 kinds of different antibiotics tested in *P. pentosaceus* KI62. The penicillin-G and rifampicin MIC value was lowest among the antibiotics. *P. pentosaceus* KI62 showed the highest vancomycin MIC. Banwo et al. (2013) reported that vancomycin resistance of pediococci is prevalent, but, fortunately, it was thought to be endogenous for a modified precursor ending in D-Ala-A-lactate. Similarly, resistance to aminoglycosides such as kanamycin, gentamicin and streptomycin is also an inherent characteristic of Pediococcus spp. (Hummel et al. 2007). According to Danielsen et al. (2007), penicillin-G, chloramphenicol and erythromycin were consistent with reports of active

antibiotics against the Pediococcus spp strain.

According to the European Food Safety Authority (EFSA, 2008) and the Scientific Committee for Animal Nutrition (SCAN, 2002), *P. pentosaceus* KI62 was susceptible to clindamycin and erythromycin. Note, however, that, according to those same sources, it was resistant to gentamycin, kanamycin, streptomycin, ampicillin, tetracycline, clindamycin, erythromycin, and chloramphenicol because the MICs were equal to or higher than the breakpoints. These results show that the *P. pentosaceus* KI62 strain generally has antibiotic tolerance.

Enzyme activity

The enzyme activities of the *P. pentosaceus* KI62 strain are shown in Table 5. The KI62 did not produce β-glucuronidase, a harmful enzyme related to the inducement of toxins, carcinogenesis, and mutagens (Dabek et al., 2008). Notably, the activity of leucine arylamidase was 5 degrees, and that of valine arylamidase was 4 degrees. β-galactosidase and β-glucosidase are useful enzymes. Especially, the KI62 displayed β-galactosidase activity that can relieve the symptoms of lactose intolerance because β-galactosidase hydrolyzes lactose to galactose and glucose in milk (De Verse et al., 2003). According to Tzanetakis and Litopoulou-Tzanetaki (1989), the average enzyme activity of leucine arylamidase and valine arylamidase among 49 strains of *P. pentosaceus* isolated from raw goat milk and Feta and Kaseri cheese were 4.98 and 4.92, respectively, and the average enzyme activity of β-galactosidase and β-glucosidase were 4.61 and 2.99, respectively. These results showed that the enzyme activity of leucine arylamidase and valine arylamidase was similar, while β-galactosidase and β-glucosidase showed slightly lower enzyme activity.

pH and bile tolerance

To be used as probiotic, bacteria should have strong resistance to acid and bile (Lee and

186 Salminen, 1995). Acid and bile tolerance is required for bacterial growth and is involved in the
187 defense mechanisms in the intestine. The bacteria should also survive passage through the
188 stomach as well as in food (Lee and Salminen, 1995; Henriksson et al., 1999; Succi et al., 2005).
189 The pH of the stomach is 2-3, and the food passes through the stomach for a period of 2-3 h
190 (Maragkoudakis et al., 2006).

191 As a result of incubation for 7 h in MRS broth, the log value of strain was reached at 9.20. But,
192 the log value of strains was 8.44 when incubation for 7 h in MRS broth adding 0.3% oxgall.
193 Consequently, the survival rate of *P. pentosaceus* KI62 in MRS broth containing 0.3% bile was
194 91.67%. *P. pentosaceus* KI62 has probiotic potential because a relatively high percentage of
195 the strain survived in MRS broth adding 0.3% bile salt.

196 Fig. 4 shows the pH tolerance of *P. pentosaceus* KI62. When incubation for 3h in pH 2.0, it
197 had a survival rate of 98.63% and the growth of the strain was not influenced by pH 3, 4, or
198 6.4. These results show that the strain was more resistant than Vidhyasagar and Jeevaratnam
199 (2013), who reported that the number of bacteria decreased by 1-2 log when inoculated into
200 MRS broth with *P. pentosaceus* at pH 2 for 2 hours.

201 In other words, *P. pentosaceus* KI62 has the best acid and bile tolerance ability because a
202 relatively high percentage of the strain survived in MRS broth adding 0.3% bile salt as well as
203 under a highly acidic condition.

204 **Antimicrobial activity**

205 Some strains of LAB produce a variety of antimicrobial substances that can prevent the growth
206 of pathogenic and spoilage bacteria. The antimicrobial metabolites of LAB include hydrogen
207 peroxide, organic acid, bacteriocins, and diacetyl (Ahmadova et al., 2013). To improve human
208 health, probiotics have to decrease the incidence of pathogenic bacteria. Therefore, the process

of choosing beneficial probiotics in the presence of pathogenic bacteria is important. the procedure for selecting probiotics, which are beneficial in the presence of pathogenic bacteria, is important in acting against these pathogens (Kesarcodi-Watson et al., 2012).

P. pentosaceus KI62 showed resistance to *E. coli, S.* Typhimurium, *L. monocytogenes,* and *S. aureus* at rates of 29.41%, 38.10%, 51.72%, and 50.47%, respectively (Table 6). The pH value of pathogens after incubation for 6 h was around 5.24-6.24, whereas the pH value of a culture with *P. pentosaceus* KI62 and pathogens was around 4.67-4.75. Although the lactic acid produced during culture was not large, it was found to have an effect on antibacterial activity. Bao et al. (2010) investigated the ability for co-aggregation with pathogens of 11 strains isolated from traditional dairy products. The 11 strains showed resistance to *E. coli, S.* Typhimurium, *L. monocytogenes,* and *S. aureus* at rates of 10.5-32.4%, 10.0-29.7%, 11.0-34.0%, and 17.7-49.9%, respectively. These results showed that the *P. pentosaceus* KI62 strain exhibited higher overall antimicrobial activity, especially *L. monocytogenes* and *S. aureus*.

Adhesion ability

Their adhesion to intestinal epithelium is one of the main screening criterion for choosing probiotics (Blum, et al, 1999). This ability takes account of precondition for showing beneficial effects, such as the bar of enteropathogenic bacteria (Bernet et al., 1993; Lee et al., 2003). HT-29 cells are generally derived from colon carcinoma, and representing the property of a differentiated absorbent enterocytes. *Lactobacillus rhamnosus* GG was demonstrated to have great ability to adhere to the epithelial cell line in many previous studies (Martin et al., 2005; Gopal et al., 2001). As shown in Fig. 5, 23.31% of *P. pentosaceus* KI62 adhered to HT-29 cell, and 24.49% of the *L. rhamnosus* GG strain adhered to the cell. These results were higher than those of Vidhyasagar and Jeevaratnam (2013), who reported that 16% of *P. pediococcus* VJ13

adhered to Caca-2 cells. Thus, one can say that *P. pentosaceus* KI62 exhibits great adherence to the epithelial surface.

Conclusion

This study was conducted to investigate the anti-diabetic effects of *P. pentosaceus* KI62 selected from among LAB isolated from kimchi, and to study its physiological characteristics to confirm the potential of health functional food or fermented milk as a starter. On the basis of the nucleotide sequence of 16s rDNA gene, it was named *P. pentosaceus* KI62. The *P. pentosaceus* KI62 strain was observed to exhibit α-amylase and α-glucosidase inhibitory activity of 94.86±3.30% and 98.59±0.52%, respectively. The contents of short chain fatty acids (SCFA) in MRS broth containing 3% maltodextrin inoculated by *P. pentosaceus* KI62 were propionic acid 8.78±1.12 mg/kg, acetic acid 1.34±0.07 g/100 mL, and butyric acid 0.876±0.003 g/kg. The contents of medium chain fatty acids (MCFA) in MRS broth containing 3% maltodextrin inoculated by *P. pentosaceus* KI62 were C8 0.262±0.031 mg/kg, C10 0.279±0.021 mg/kg, and C12 0.203±0.009 mg/kg. In a comparison of sixteen different antibiotics, *P. pentosaceus* KI62 showed higher sensitivity to penicillin-G, rifampicin, and clindamycin, as well as the highest resistance to vancomycin and ampicillin.

P. pentosaceus KI62 has the best bile and acid tolerance ability. KI62 showed resistance to *E. coli, S.* Typhimurium, *L. monocytogenes,* and *S. aureus* at rates of 29.41%, 38.10%, 51.72%, and 50.47%, respectively. KI62 exhibited 23.31% adherence to the epithelial surface. These results demonstrate that *P. pentosaceus* KI62 has potential as a probiotic with anti-diabetic effects.

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References

Ahmadova A, Todorov SD, Choiset Y, Rabesona H, Zadi TM, Kuliyev A, Franco BDGM, Chobert JM, Haertlé T. 2013. Evaluation of antimicrobial activity, probiotic properties, and safety of wild strain *Enterococcus faecium* AQ71 isolated from Azerbaijani Motal cheese. Food Control 30:631-641.

Ali H, Houghton PJ. Soumyanath A. 2006. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. J Ethnopharmacol 107:449–455.

Banwo K, Sanni A, Tan H. 2013. Functional properties of Pediococcus species isolated from traditional fermented cereal gruel and milk in Nigeria. Food Biotechnol 27:14-38.

Bao Y, Zhang Y, Zhang Y, Liu Y, Wang S, Dong X, Zhang H. 2010. Screening of potential probiotic properties of *Lactobacillus fermentum* isolated from traditional dairy products. Food Control 21:695-701.

Bernet MF, Brassart D, Neeser JR, Servin AL. 1993. Adhesion of human bifidobacterial strains to cultured human intestinal epithelial cells and inhibition of enteropathogen-cell interactions. Appl Environ Microbiol 59:4121-4128.

Blum S, Reniero R, Schiffrin EJ, Crittenden R, MattilaSandholm T, von Wright A, Saarela M, Saxelin M, Collins K, Morelli L. 1999. Adhesion studies for probiotics: need for validation and

277 refinement. Trends Food Sci Technol 10:405 – 410.

278 Clark PA, Cotton LN, Martin JH. 1993. Selection of bifidobacteria for use as dietary adjuncts
279 in cultured dairy foods: II-Tolerance to simulated pH of human stomachs. Cul Dairy Prod J
280 28:11-14.

281 Cummings JH. 1981. Short chain fatty acids in human colon. Gut 22:763-779.

282 Dabek M, McCrae SI, Stevens VJ, Duncan SH, Louis P. 2008. Distribution of β-glucosidase
283 and β-glucuronidase activity and of β-glucuronidase gene gus in human colonic bacteria.
284 FEMS Microbiol Ecol 66:487-495.

285 Danielsen M, Simpson PJ, O'Connor EB, Ross RP, Stanton C. 2007. Susceptibility of
286 *Pediococcus* spp. To antimicrobial agents. J Appl Microbiol 102:384-389.

287 De Verse M, Stegelmann A, Richter B, Fenselau S, Laue C, Schrezenmeir J. 2003. Probiotics-
288 compensation for lactose insufficiency. Am J Clin Nutr 73:421-429.

289 EFSA. 2008. Technical guidance prepared by the Panel on Additives and Products or
290 Substances in Animal Feed (FEEDAP) on the update of the criteria used in the assessment of
291 bacterial resistance to antibiotics of human and veterinary importance. The EFSA J 732:1–15.

292 Gilliand SE, Speck ML. 1977. Deconjugation of bile acids by intestinal lactobacilli. Appl
293 Environ Micobiol 33:15-18.

294 Gilliland SE, Walker DK. 1990. Factors to consider when selecting a culture of *Lactobacillus*
295 *acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. J Dairy
296 Sci 73:905-911.

297 Gopal PK, Prasad J, Smart J, Gill HS. 2001. *In vitro* adherence properties of *Lactobacillus*
298 *rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their antagonistic activity

against an enterotoxigenic *Esherichia coli*. Int J Food Microbiol 47:207-216.

Henriksson R, Bergstrom P, Franzen L, Lewin F, Wagenius G. 1999. Aspects of reducing gastrointestinal adverse effects associated with radiotherapy. Acta Oncológica 38:226–231.

Hummel AS, Hertel C, Holzapfel WH, Franz CMAP. 2007. Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. Appl Environ Microbiol 73:730-739.

Jang YS, Jeong JM. 2010. Antioxidative effect and digestive enzyme inhibition of grape seed extract (GSE). J Korean Soc Food Sci Nutr 39: 783-788.

Kesarcodi-Watson A, Miner P, Nicolas JL, Robert R. 2012. Protective effect of four potential probiotics against pathogen-challenge of the larvae of three bivalves: Pacific oyster (*Crassostrea gigas*), flat oyster (*Ostrea edulis*), and scallop (*Pecten maximus*). Aquaculture 344:29-34.

Kim SJ, Cho SY, Kim SH, Song OJ, Shin IS, Cha DS, Park HJ. 2008. Effect of microencapsulation on viability and other characteristics in *Lactobacillus acidophilus* ATCC 43121. LWT-Food Sci Technol 41:493-500.

Klare I, Konstabel C, Werner G, Huys G, Vankerckhoven V, Kahlmeter G, Goossens H. 2007. Antimicrobial susceptibilities of *Lactobacillus*, *Pediococcus* and *Lactococcus* human isolates and cultures intended for probiotic or nutritional use. J Antimicrob Chemother 59:900-912.

Lee YK, Puong KY, Ouwehand AC, Salminen S. 2003. Displacement of bacterial pathogens from mucus and Caco-2 cell surface lactobacilli. J Med Microbiol 52:925-930.

Lee YK, Salminen S. 1995. The coming age of probiotics. Trends Food Sci Technol 6:241–245.

Lim SD, Kim KS, Do JR. 2011. Physiological characteristics and production of vitamin K_2 by

Lactobacillus fermentum LC272 isolated from raw milk. Korean J Food Sci An 31:513-520.

Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulos G, Pot B, Tsakalidou E. 2006. Probiotic potential of Lactobacillus strains isolated from dairy products. Int Dairy J 16:189-199.

Martín R, Olivares M, Marín ML, Fernández L, Xaus J, Rodríguez JM. 2005. Probiotic potential of 3 lactobacilli strains isolated from breast milk. J Hum Lact 21:8-17.

Phillips I. 1991. A guide to sensitivity testing. Report of the working party on antimicrobial sensitivity testing of the british society for antimicrobial chemotherapy. J Antimicrob Chemother 27: Supplement D:1-50.

Rhee SJ, Lee JE, Lee CH. 2011. Importance of lactic acid bacteria in Asian fermented foods. Microb Cell Fact 10:1-5.

Rittiphairoj T, Pongpirul K, Mueller NT, Li T. 2019. Probiotics for glycemic control in patients with type 2 diabetes mellitus: protocol for a systematic review. Systematic Reviews 8:227.

SCAN. 2002. Opinion of the Scientific Committee on animal nutrition on the criteria for assessing the safety of micro-organisms resistant to antibiotics of human clinical and veterinary importance. European Commission, Health and Consumer Protection Directorate General; Directorate C, Scientific Opinions, 18 April 2002.

Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, Hardt PD. 2010. Microbiota and SCFA in lean and overweight healthy subjects. Obesity(Silver Spring) 18:190-195.

Si MM, Lou JS, Zhou CX, Shen JN, Wu HH, Yang B. He QJ, Wu HS. 2010. Insulin releasing and alpha-glucosidase inhibitory activity of ethyl acetate fraction of Acorus calamus in vitro and in vivo. J Ethnopharmacol 128:154-159.

342 Succi M, Tremonte P, Reale A, Sorrentino E, Grazia L, Pacifico S, Coppola R. 2005. Bile salt
343 and acid tolerance of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano
344 cheese. FEMS Microbiology Letters 244:129-137.

345 Topping DL, Clifton PM. 2001. Short-chain fatty acids and human colonic function: roles of
346 resistant starch and nonstarch polysaccharides. Physiol Rev 81:1031–1064.

347 Turner N, Hariharan K, TidAng J, Frangioudakis G, Beale SM, Wright LE, Zeng XY, Leslie
348 SJ, Li JY, Kraegen EW, Cooney GJ, Ye JM. 2009. Enhancement of muscle mitochondrial
349 oxidative capacity and alterations in insulin action are lipid species dependent : Potent tissue-
350 specific effects of Medium-Chain Fatty Acids. Diabetes 58:2547-2554.

351 Tzanetakis N, Litopoulou-Tzanetaki E. 1989. Biochemical activities of *Pediococcus*
352 *pentosaceus* isolates of dairy origin. J Dairy Sci 72:859-863.

353 Vidhyasagar V, Jeevaratnam K. 2013. Evaluation of *Pediococcus pentosaceus* strains isolated
354 from idly batter for probiotic properties in vitro. J Func Foods 5:235-243.

355 Wein S, Wolffrarm S, Schrezenmeir J, Gasperikova D, Klimes I, Sebokova E. 2009. Medium-
356 chain fatty acids ameliorate insulin resistance caused by high-fat diets in rats. Diabetes Metab
357 Res Rev 25:185-194.

358 Xiao Z, Storms R, Tsang A. 2006. A quantitative starch-iodine method for measuring alpha-
359 amylase and glucoamylase activities. Anal Biochem 351:146-148.

360 Yan F, Li N, Shi J, Li H, Yue Y, Jiao W, Wang N, Song Y, Huo G, Li B. 2019. *Lactobacillus*
361 *acidophilus* alleviates type 2 diabetes by regulating hepatic glucose, lipid metabolism and gut
362 microbiota in mice. Food Funct 10:5804-5815.

363 Zhao L, Zhang F, Ding X, Wu G, Lam YY, Wang X, Fu H, Xue X, Lu C, Ma J, Yu L, Xu C,

Ren Z, Xu Y, Xu S, Shen H, Zhu X, Shi Y, Shen Q, Dong W, Liu R, Ling Y, Zeng Y, Wang X, Zhang Q, Wang J, Wang L, Wu Y, Zeng B, Wei H, Zhang M, Peng Y, Zhang C. 2018. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. Science 359:1151-1156.

Table 1. Specification and operating condition of GC for propionic acid analysis

Device	Parameter	Condition
GC	Column	HP-FFAP (0.32 mm i.d. × 30 m, 0.25μM)
	Oven temperature program	60℃ (4 min) → 115℃(28℃/min) → 240℃(20℃/min, 5min)
	Inlet temperature	200℃
	Injector temperature	200℃
	Injection volume	1 μL
	Split ratio	Splitless
	Carrier	Helium, 1.0 mL/min
MS	Ionization mode	EI
	Electron impact mode	70 eV
	Selected ion (m/z)	74[1)], 57, 45
	MS ion source temperature	200℃

[1)] Quantitation ion

Table 2. Specification and operating condition of GC for butyric acid analysis

Instrument	GC-FDI
Column	SP-2560(Supelco, 100m x 0.2mm ID, 0.2um film)
Detector	Flame ionization detector
Oven temperature	100℃(2min) - 4℃/min - 230℃(20min)
Injection temperature	230℃
Detector temperature	250℃
Carrier gas	He
Column flow	1.5 mL/min
Injection volumn	1.0 uL
Split ratio	50:1

Table 3. Selected lactic acid bacteria having anti-diabetes

(%)

Strain	α-amylase inhibition	α-glucosidase inhibition
KI62	94.86±3.30	98.59±0.52

Values are mean ± standard deviation of three replicates.

Table 3. Antibiotics susceptibility of *Pediococcus pentosaceus* KI62

Anti-microbial agents	Minimal inhibitory concentrations (μg/mL)
Amikacin	64
Gentamycin	128
Kanamycin	128
Streptomycin	256
Ampicillin	>2048
Penicillin-G	0.5
Oxacillin	4
Bacitracin	128
Polymyxin B	>512
Ciprofloxacin	128
Tetracycline	64
Clindamycin	1
Erythromycin	2
Rifampicin	0.5
Vancomycin	>4096
Chloramphenicol	4

Table 4. Enzyme patterns of *Pediococcus pentosaceus* KI62

Enzyme	*Pediococcus pentosaceus* KI62
Alkaline phosphatase	0
Esterase (C4)	0
Esterase lipase (C8)	0
Lipase (C14)	1
Leucine arylamidase	5
Valine arylamidase	4
Cystinearylamidase	1
Trypsin	0
α-chymotrypsin	0
Acid phosphatase	2
Naphtol-AS-BI-phosphohydrolase	3
α-galactosidase	0
β-galactosidase	2
β-glucuronidase	0
α-glucosidase	0
β-glucosidase	2
N-acetyl-β-glucosaminidase	2
α-mannosidase	0
α-fucosidase	0

*: A value ranging from 0 to 2 is assigned to the standard color: zero represents a negative; 5 represents a reaction of maximum intensity. Values 1 through 4 represent intermediate reactions depending on the level of intensity. The approximate activity may be estimated from the color strength: 1 corresponds to the liberation of 5nanomoles; 2, to 10nanomoles; 3, to 20nanomoles; 4, to 30nanomoles; and 5, to 40nanomoles or more.

Table 5. Inhibition of pathogens by *Pediococcus pentosaceus* KI62 in MRS broth

Pathogens	Growth				Inhibition (%)
	Pathogens[a]		KI62+pathogens[a]		
	CFU/mL	pH	CFU/mL	pH	
Escherichia coli	$6.80\pm0.14\times10^{6}$	6.22	$4.80\pm0.28\times10^{5}$	4.72	29.41%
Salmonella Typhimurium	$3.15\pm0.64\times10^{7}$	6.17	$1.95\pm0.21\times10^{7}$	4.75	38.10%
Listeria monocytogenes	$1.45\pm0.07\times10^{5}$	6.24	$7.00\pm0.14\times10^{4}$	4.67	51.72%
Staphylococcus aureus	$7.13\pm0.75\times10^{6}$	5.24	$3.53\pm0.60\times10^{6}$	4.67	50.47%

* Initial count of *Pediococcus pentosaceus* KI62: $3.63\pm0.35\times10^{6}$ CFU/mL

[a] Determined after 6 h of incubation at 37℃

Values are mean ± standard deviation of the three replicates.

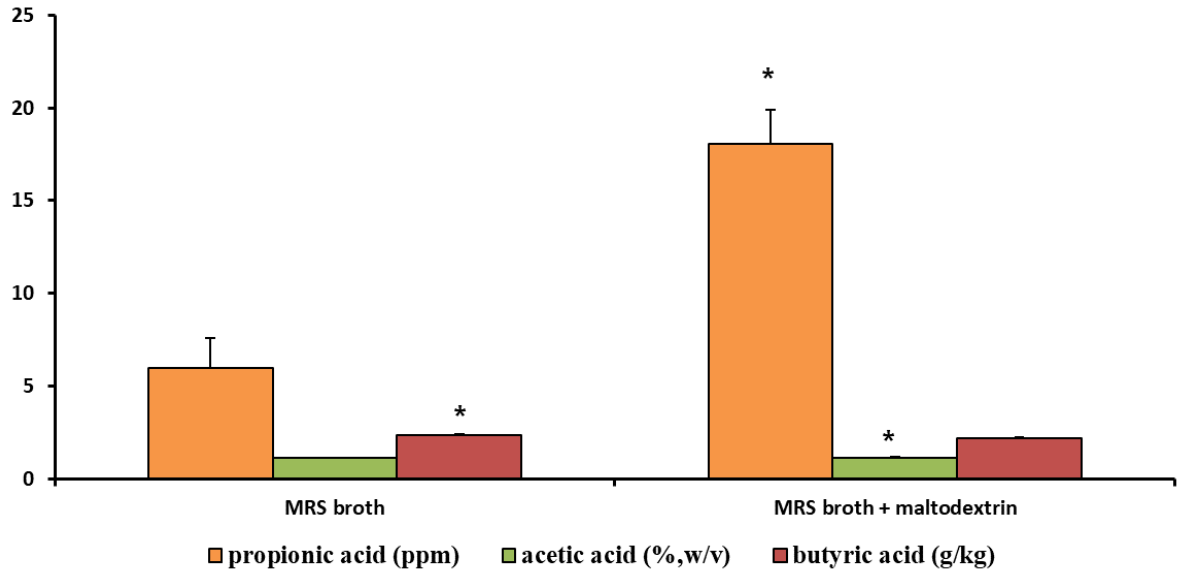

Fig. 1. Production of short chain fatty acid of *Pediococcus pentosaceus* KI62 in MRS broth and MRS broth with 3% maltodextrin. *p<0.05 between with maltodextrin and without maltodextrin (*t*-test)

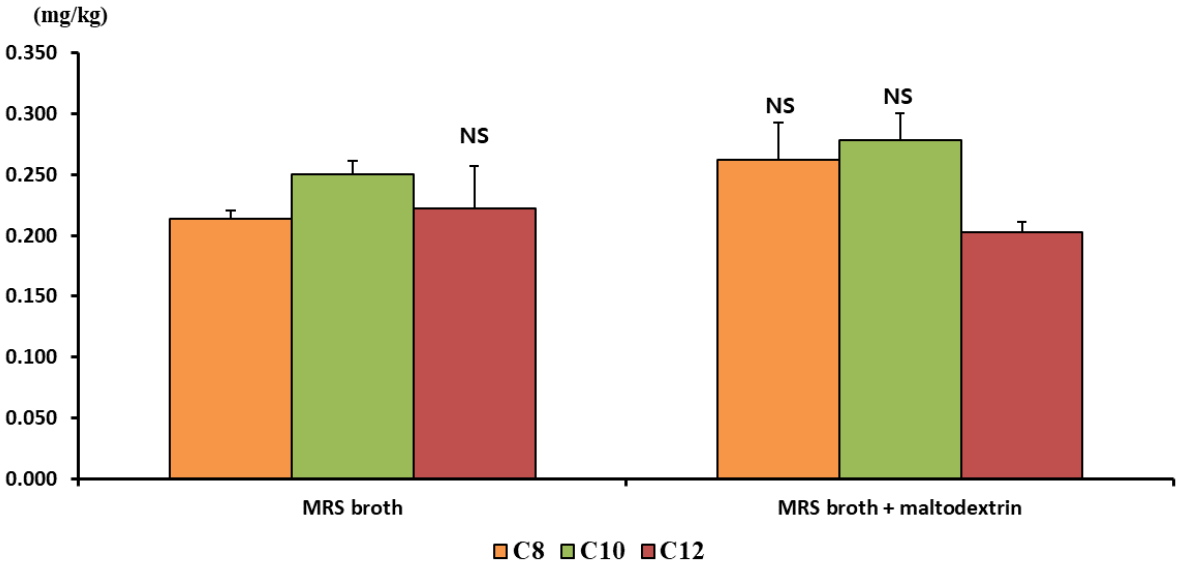

Fig. 2. Production of medium chain fatty acid of *Pediococcus pentosaceus* KI62 in MRS broth and MRS broth with 3% maltodextrin. [NS]Means that the values are not significantly different between with maltodextrin and without maltodextrin (*t*-test).

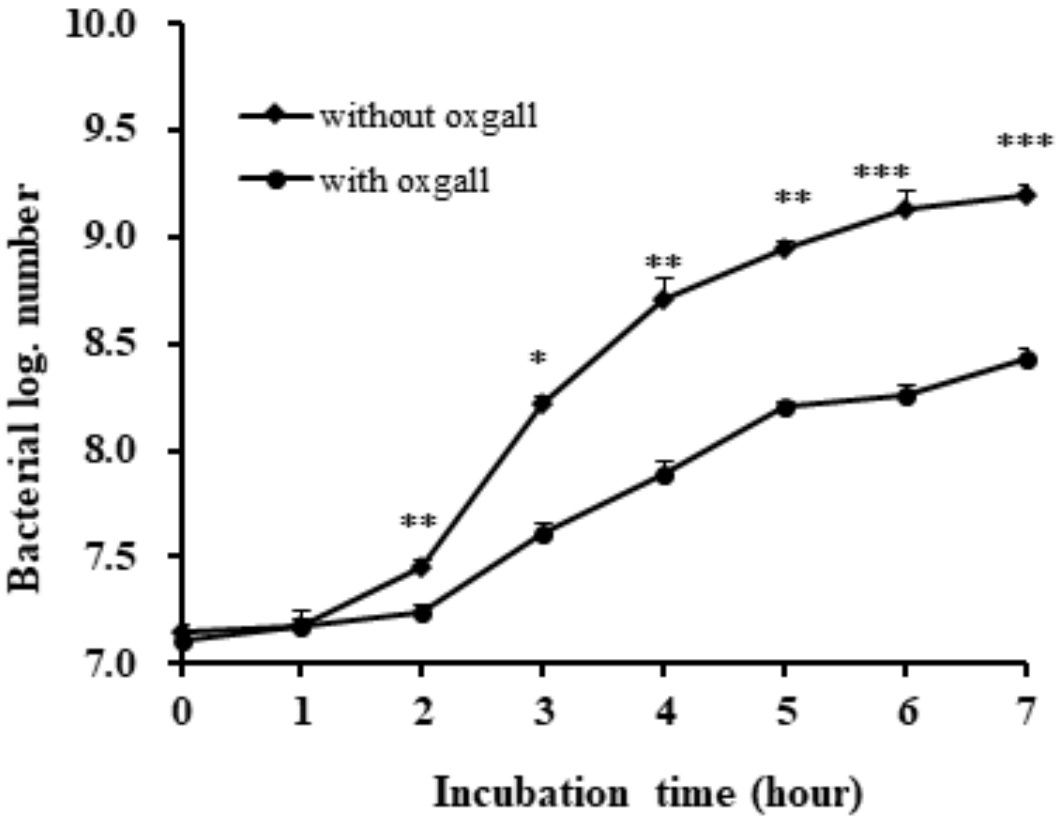

Fig. 3. Growth of *Pediococcus pentosaceus* KI62 in MRS broth containing 0.05% L-cysteine with/without 0.3% oxgall. Values are mean ± standard deviation of the three replicates; *p<0.05, **p<0.01 and ***p<0.001 between with ox gall and without oxgall (*t*-test)

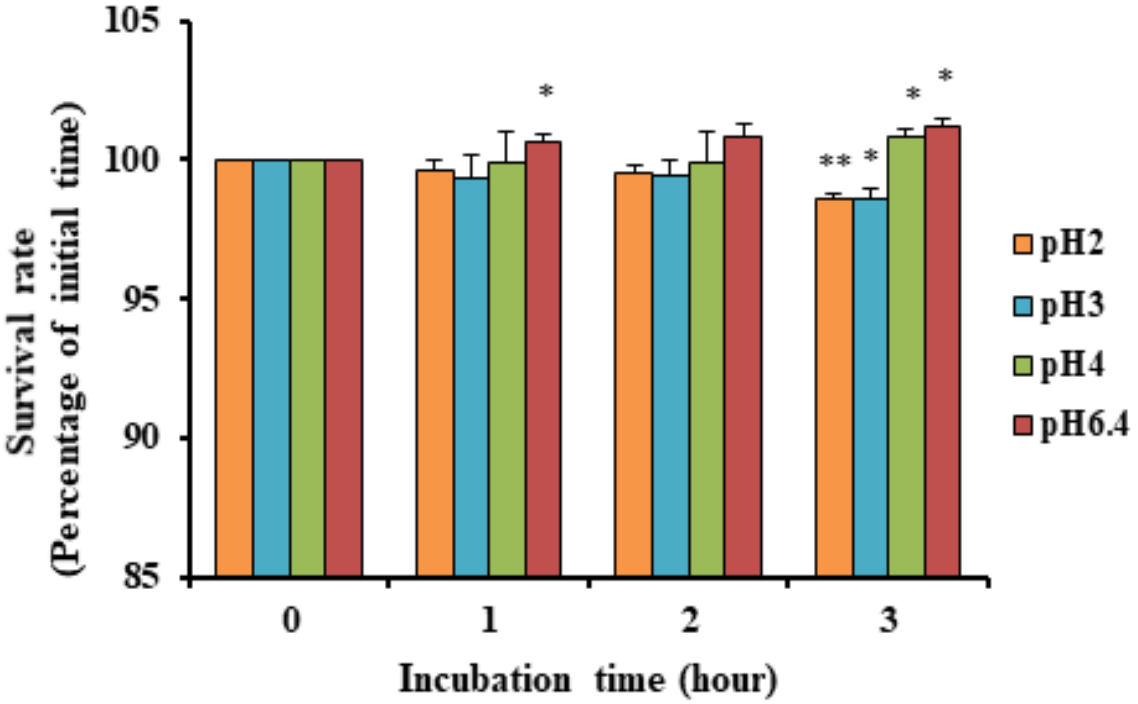

Fig. 4. Survival of *Pediococcus pentosaceus* KI62 after three hours in HCl solution. Values are mean ± standard deviation of the three replicates; *p<0.05 and **p<0.01 compared with initial time (t-test)

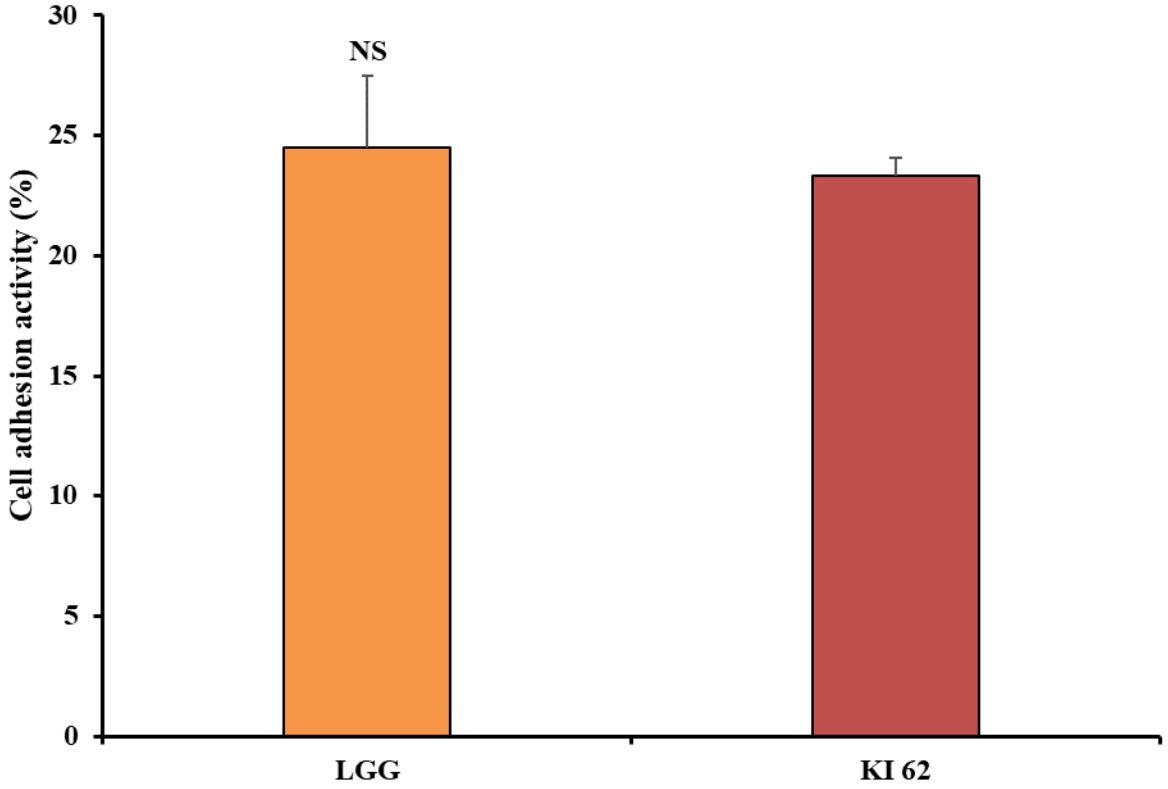

Fig. 5. Adhesion ability of *Pediococcus pentosaceus* KI62 to HT-29 cell. Values are mean ± standard deviation of the three replicates. [NS]Means that the values are not significantly different compared with *Lactobacillus rhamnosus* GG (t-test, $p<0.05$).