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ARTICLE

Physiological Characteristics and Anti-obesity Effect of *Lactobacillus plantarum* Q180 Isolated from Feces

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Abstract

Obesity is strongly associated with several metabolic and chronic diseases and has become a major public health problem of worldwide concern. This study aimed to investigate the physiological characteristics and anti-obesity effects of *Lactobacillus plantarum* Q180. *Lactobacillus plantarum* Q180 was isolated from the faces of healthy adults and found to have a lipase inhibitory activity of 83.61±2.32% and inhibited adipocyte differentiation of 3T3-L1 cells (14.63±1.37%) at a concentration of 100 μg/mL. The strain was investigated for its physiological characteristics. The optimum growth temperature of *L. plantarum* Q180 was 37°C. *Lactobacillus plantarum* Q180 showed higher sensitivity to novobiocin in a comparison of fifteen different antibiotics and showed the highest resistance to rifampicin, polymyxin B and vancomycin. The strain showed higher β-galactosidase and N-acetyl-β-glucosaminidase activities. It also did not produce carcinogenic enzymes such as β-glucuronidase. The survival rate of *L. plantarum* Q180 in MRS broth containing 0.3% bile was 97.8%. Moreover, the strain showed a 97.2% survival rate after incubation for 3 h in pH 2.0. *Lactobacillus plantarum* Q180 was displayed resistance to *Escherichia coli*, *Salmonella* Typhimurium and *Staphylococcus aureus* with rates of 55.6%, 38.0% and 47.6%, respectively. These results demonstrate that *L. plantarum* Q180 has potential as a probiotic with anti-obesity effects.

Keywords: *Lactobacillus plantarum*, physiological characteristics, anti-lipase activity, anti-adipogenic activity

Introduction

Obesity, a condition in which an abnormally large amount of fat is stored in adipose tissue, causing an increase in body weight, has become a major public health concern worldwide. It is now generally acknowledged that obesity results from an imbalance between energy intake and consumption (Wood *et al*., 1998), and that this condition is strongly associated with several metabolic and chronic diseases including heart disease, cancer, obstructive sleep apnea, arthritis, hypertension, hyperlipidemia, and type 2 diabetes associated with insulin resistance (Wickelgren, 1998). To date, pharmacological treatments have not appeared to be effective in promoting continuous long-term weight loss (Glenny *et al*., 1997). Therefore, further research is needed to discover new drug therapies that can be used to reduce the prevalence of obesity.

Probiotics are defined as viable microbial dietary supplements that have various beneficial effects on a host's health. These effects include, among others, the amelioration of hypercholesterolemia (Park *et al*., 2007) and hypertension (Aihara *et al*., 2005); the prevention of cancer (Rafter *et al*., 2004); and modulation of the immune system (Baken *et al*., 2006). In addition, recent experimental studies have demonstrated the preventive effects of some bacterial strains on obesity. *L. curvatus* HY7601 and *L. plantarum* KY1032 have been reported to reduce adipogenesis in 3T3-L1 cells (Park *et al*., 2011). According to Moon *et al*. (2012), when the lactic acid bacterial (LAB) strain *Weissella koreensis* OK1-6 isolated from kimchi is used to treat differentiating pre-adipocyte 3T3-L1 cells, the cells accumulate significantly less intracellular lipid.

A potentially probiotic strain is expected to have several desirable properties in order to exert its beneficial effects. It has been reported that the basic requirements for the use of LAB strains as probiotics include the following: (1) they should be generally recognized as safe (GRAS); (2) they should be tolerant to acid and bile; (3) they should be able to adhere to the intestinal epithelium of the hosts; (4) they should be able to demonstrate antagonistic activity against pathogenic bacteria; and (5) they

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should be able to retain their viability during processing and storage (Lin *et al*., 2006; Lonkar *et al*., 2005; Rial, 2000).

The aim of this study was to isolate and screen a specific LAB with potentially significant anti-obesity activity with a view to applying it to functional food products.

Materials and Methods

Isolation of lactic acid bacteria

The strain Q180 was isolated from the feces of healthy adults in a modified MRS medium (Lim *et al*., 2011). The strain was incubated in *Lactobacilli* MRS broth (Difco, USA) as the growth medium at 37°C for 18 h.

Identification of strain Q180

The properties of the strain Q180 were investigated by testing the Gram staining and microscopic observation after cultivation on MRS broth for 18 h at 37°C. Bergey's Manual of Systematic Bacteriology by Buchanan and Gibbons (1974) was used to examine the morphological and physiological properties of the isolated strains. The Q180 strain was identified by using the 16S rDNA sequencing method. The chromosomal DNA of the isolated strain was separated by using a SolGent Genomic DNA prep kit (SolGent, Korea). The DNA extracts were used for the polymerase chain reaction (PCR) with universal primers [27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 14 92R (5'-GGT TAC CTT GTT ACG ACT T-3')]. PCR was carried out in a programmable therma cycler (SolGent EF-Taq, Korea), according to the following steps: one cycle of denaturation at 95°C for 15 min, followed by 30 cycles of 95°C for 20 s, 50°C for 40 s, and 72°C for 90 s. The final extension was carried out at 72°C for 5 min. The purified PCR product obtained by using a SolGent PCR purification kit (SolGent, Korea) was used for sequencing with an ABI 3730XL DNA analyzer (Applied Biosystems, USA).

Lipase activity

The method of Lee *et al.* (1993) for lipase activity determination was modified. The pancreatic lipase activity was measured using porcine pancreatic lipase (Sigma, USA). 0.1 mg/mL of a sample solution dissolved in water, and 0.167 mM *p*-Nitrophenylpalmitate (PNP; Sigma, USA) solution and 0.061 M (pH 8.5) Tris-HCl buffer were mixed in the well of a plate, and 0.3 mg/mL of the lipase solution was then added to start the enzyme reaction. After incubation at 25°C for 10 min, its absorbance was measured at 405 nm.

Cell line and cell culture

3T3-L1 cells were obtained from the American Type Culture Collection (ATCC, USA) and were cultured at 37°C in humidified 5% CO_2 atmosphere. 3T3-L1 cells were cultured as described by Hemati *et al.* (1997). Briefly, the cells were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO, USA) containing high glucose supplemented with 10% bovine calf serum (BCS, GIBCO, USA) and 1% penicillin/streptomycin in six well culture plates. Two days later, confluence cells were cultured in an adipocyte differentiation cocktail medium containing 0.5 mM 3-isobutyl-1-methylxanthine (IBMX, Sigma, USA), 1 M dexamethasone (Dex, Sigma, USA), and 5 g/mL insulin (Sigma, USA) in DMEM supplemented with 10% fetal bovine serum (FBS, GIBCO, USA) for 2 d. The differentiation was complete after 6 d.

Sample preparation and Treatment of *L. plantarum* Q180

L. plantarum Q180 was incubated at 37°C for 18 h in MRS broth. All of the purified strains were kept at 70°C until use. After culturing *L. plantarum* Q180, all of the strains were harvested in a refrigerated centrifuge (1,500 *g* for 15 min at 4°C) and washed three times with distilled water to remove any remaining MRS broth. The washed *L. plantarum* Q180 was freeze-dried and re-suspended in distilled water at a concentration of 10 mg/mL and homogenized for 50 sec followed by 1 min of rest (repeated 3 times) using a sonicator. The 3T3-L1 cells were treated with three different concentrations of supernatant, i.e., 0 g/mL (control), 10 g/mL, and 100 g/mL.

Oil Red O staining of 3T3-L1 adipocytes

Intracellular lipid accumulation was measured using oil red O (Sigma, USA). Oil red O straining of 3T3-L1 cells was done using a modified version of the method described by Ramirez-Zacarias *et al.* (1992). 3T3-L1 cells were washed with PBS twice, fixed with 10% formaldehyde/PBS at 4°C for 1 h and stained with filtered oil red O solution (stock solution: 3.5 mg/mL in isopropanol; working solution: 60% oil red O stock solution and 40% distilled water) at room temperature for 30 min. The quantification of lipid accumulation was achieved by oil red O obtained from stained cells with isopropyl alcohol and measured spectro-photometrically at 520 nm. The material stained with oil red O was expressed on a per cell basis using the cell number determined from similar plates. The

percentage of the material stained with oil red O relative to the control wells containing the cell culture medium without compounds was calculated as 520 nm (Q180)/ 520 nm (control) × 100.

Growth of strain

The number of viable *L. plantarum* Q180 was determined by serial ten-fold dilution in 0.1% peptone water. 50 L (9.6×10^5/mL) of *L. plantarum* Q180 was inoculated into 150 mL of 10% reconstituted skimmed milk, and then the culture was incubated at 3 h intervals for 24 h at 34°C, 37°C and 40°C. All pour plates were incubated aerobically at 37°C for 48 h using a BCP plate count agar (Eiken, Japan).

Antibiotic tolerance

L. plantarum Q180 was grown at 37°C for 18 h in MRS broth and inoculated (1%, v/v) into a MRS broth supplemented with antibiotics (amikacin, gentamicin, kanamycin, neomycin, streptomycin, penicillin-G, methicillin, oxacillin, ampicillin, bacitracin, rifampicin, novobiocin, lincomycin, polymyxin B and chloramphenico, Sigma, USA) at various concentrations in a two-fold dilution step. The minimal inhibitory concentration (MIC) was determined by checking the moment at which the strain stopped growing after incubation at 37°C for 48 h.

Enzyme activity

An API ZYM kit (bioMerieux, Lyon, France) was used to study enzyme activity. *L. plantarum* Q180 was grown at 37°C for 18 h in MRS broth. Sediment from the centrifuged broth culture was used to prepare the suspension at 10^5-10^6 CFU/mL. After inoculation, the cultures were incubated for 5 h at 37°C. The addition of a surface active agent (ZYM A reagent) in the cupules facilitated the solubilization of the ZYM B reagent in the medium. Color was allowed to develop for at least 5 min, and values ranging from 0-5 (corresponding to the colors developed) were assigned. The approximate number for the free nmol hydrolyzed substrate was determined based on the color strength: 0, negative reaction; 1, 5 nmol; 2, 10 nmol; 3, 20 nmol; 4, 30 nmol; 5, 40 nmol or higher.

Bile tolerance

Bile tolerance was tested as described by Gilliland and Walker (1990). *L. plantarum* Q180 was grown at 37°C for 18 h in MRS broth. Each 1% of the *L. plantarum* Q180 strain culture was inoculated into sterilized MRS broth containing 0.05% L-cysteine (Sigma, USA) with or without 0.3% oxgall (Sigma, USA), and then the growth potential was compared in the presence of the bile. Then, the cultures were incubated anaerobically at 1 h intervals for 7 h at 37°C. All pour plates were incubated anaerobically at 37°C for 48 h using the BCP plate count agar.

Acid tolerance

Acid tolerance was tested as described by Clark *et al*. (1993). Solutions of 37% HCl in double-distilled water were adjusted to pH levels of 2.0, 3.0, and 4.0. Sterile double-distilled water (pH 6.4) served as the control. 10 mL of each pH solution was transferred into sterile test tubes. 1 mL of stock culture containing approximately 10^9 CFU/mL of *L. plantarum* Q180 using MRS agar containing 0.05% cysteine was then transferred into each of the four pH solutions. The pH solutions containing *L. plantarum* Q180 were then incubated anaerobically at 37°C, followed by intermittent plating after 1, 2, and 3 h to stimulate the survival of *L. plantarum* Q180 under pH conditions common to the human stomach. Samples from the pH solution were re-suspended and subjected to serial dilutions. About 100 μL of the abovementioned sample solution was spread onto the surface of the BCP plate count agar plates and incubated anaerobically at 37°C for 48 h.

Antimicrobial activity

Antimicrobial activity was tested as described by Gilliland and Speck (1977). *Escherichia coli* KFRI 174, *Salmonella* Typhimurium KFRI 250, and *Staphylococcus aureus* KFRI 219 were obtained from the culture collection of the Korea Food Research Institute (Sungnam, Korea). *Escherichia coli* was enumerated on EMB agar (Difco, USA), *Salmonella* Typhimurium on Bismuth sulfite agar (Difco, USA), and *Staphylococcus aureus* on Baird Parker agar (Difco, USA). All the plates were incubated for 48 h at 37°C. Both the control culture and the associative culture were incubated for 6 h at 37°C. At the end of the incubation period, the samples were removed and placed in an ice bath until analysis. The number of CFU of pathogens per mL was determined using the appropriate selective medium. Percentages of inhibition were determined using the following formula:

$$\text{Inhibition (\%)} = \frac{(\text{CFU/mL in control}) - (\text{CFU/mL in associative culture})}{(\text{CFU/mL in control})} \times 100$$

Statistical analysis

The results are expressed as the mean±standard deviation (SD). The statistical analysis was performed with a statistical analysis system (SAS, SAS Institute Inc., USA). The significance of the differences was analyzed by conducting a one-way analysis of variance (ANOVA) with Duncan's multiple range tests. Values of $p<0.05$ were considered statistically significant.

Results and Discussion

Isolation of lactic acid bacteria

The feces samples were collected from healthy adult staff members of the Korea Food Research Institute (Sungnam, Korea), and 188 strains were isolated as lactic acid bacteria in a modified MRS medium. The strain was incubated in lactobacilli MRS broth as the growth medium at 37°C for 18 h.

Selection of strain of anti-obesity activity

Several approaches to the prevention and treatment of obesity have been reported (Birari *et al.*, 2007). Among these, both natural and synthetic pancreatic lipase inhibitors are effective in preventing obesity, which is likely to be due to their inhibition of intestinal lipid absorption (Hirose *et al.*, 2013). After being incubated in MRS broth at 37°C for 18 h, three kinds of strains having a lipase inhibitory activity of over 60% were selected from among the 188 strains by measuring their lipase inhibitory activity. Table 1 showed that then Q180 strain exhibited the highest lipase inhibitory activity among the three selected strains. The Q180 strain exhibited a lipase inhibitory activity of 83.61±2.31% compared with the control. Fig. 1 showed that an 8-day treatment at various concentrations [0-100 μg/mL] of the Q180 strain during the differentiation period significantly and dose-dependently inhibited 3T3-L1 adipogenesis in terms of lipid accumulation compared with the control cells. Among the tested concentrations of Q180, 100 μg/mL was the most effective at reducing the lipid content in differentiated cells (by 14.63±1.37% compared with the control cells). These results suggest that Q180 inhibits pancreatic lipase (PL) and the differentiation of 3T3-L1 preadipocytes by suppressing lipid accumulation.

Identification and DNA sequencing of the selected Q180 strain

Physiological and biochemical tests were conducted to determine the genus and species of the selected Q180 strain. The Q180 strain consisted of non-spore, rod-type, gram-positive bacteria and exhibited negative properties on catalase and motility. In addition, it cannot grow at 15°C and 45°C. As it does not produce gas and ammonia from glucose and arginine, it has been identified as a genus *Lactobacillus* (Table 2). After PCR amplification using universal primers targeting 16S rDNA and the following sequence analysis, it was identified as *Lactobacillus plantarum* with a similarity of 99% (data not shown). Based on the results of previous studies, it was named *L. plantarum* Q180.

Table 1. Anti-lipase activity of selected lactic acid bacteria

Strains	Anti-lipase activity (%)	Source
F22	68.01±0.86	Feces of adults
Q180	83.61±2.32	Feces of adults
Q185	70.90±0.55	Feces of adults

All values are mean±standard deviation of three replicates.

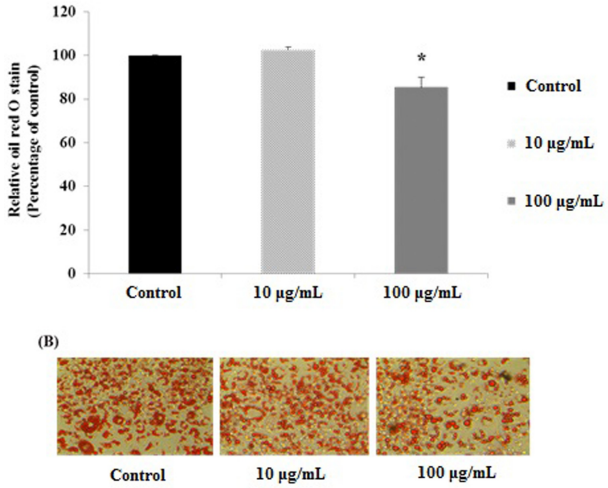

Fig. 1. The effects of *L.plantarum* Q180 on oil red O stained in 3T3-L1 adipocyte: (A) quantification of oil red O staining * significant difference from control at $p<0.05$; (B) photograph of oil red O staining. Cells were stained with oil red O observed by using a microscope (original magnification ×200).

Growth of strain

The number of viable *L. plantarum* Q180 was determined by serial ten-fold dilution in 0.1% peptone water. 10 μL (9.6×10^5/mL) of *L. plantarum* Q180 was inoculated into 150 mL of 10% reconstituted skimmed milk, and then the culture was incubated at 34°C, 37°C and 40°C for 24 h, and checked at intervals of 3 h, with the highest growth rate identified at 37°C. The optimum growth temperature of *L. plantarum* Q180 was found to

Table 2. Physiological characteristics of *Lactobacillus plantarum* Q180

Gram reaction		+	
Cell type		rod	
Spore forming		−	
Motility		−	
Aerobic growth		+	
Anaerobic growth		+	
Catalase reaction		−	
Growth at 15°C		−	
Growth at 45°C		−	
Gas forming from glucose		−	
Ammonia production from alginin		−	
Acid production from			
Glycerol	−	Salicin	+
Erythritol	−	D-Celiobiose	+
D-Arabinose	−	D-Maltose	+
L-Arabinose	+	D-Lactose	+
D-Ribose	+	D-Melibiose	+
D-Xylose	−	D-Saccharose	+
L-Xylose	−	D-Trehalose	+
D-Adonitol	−	Inulin	−
Methyl-βD-Xylopyranoside	−	D-Melezitose	+
D-Galactose	+	D-Raffinose	+
D-Glucose	+	Amidon (starch)	−
D-Fructose	+	Glycogen	−
D-Mannose	+	Xylitol	−
L-Sorbose	−	Gentiobiose	+
L-Rhamnose	+	D-Turanose	+
Dulcitol	−	D-Lyxose	−
Inositol	−	D-Tagatose	−
D-Mannitol	+	D-Fucose	−
D-Sorbitol	−	L-Fucose	−
Methyl-aD-Mannopyranoside	+	D-Arabitol	+
Methyl-aD-Glucopyranoside	−	L-Arabitol	−
N-AcetylGlucosamine	+	Potassium Gluconate	+
Amygdalin	+	Potassium 2-KetoGluconate	−
Arbutin	+	Potassium 5-KetoGluconate	−
Esculin	+		

be 37°C (Fig. 2).

Antibiotic tolerance

When using lactic acid bacteria as probiotics, the ability to survive in antibiotic circumstances is an essential factor (Havinaar *et al*., 1992). Table 3 shows the tolerance of the *L. plantarum* Q180 strain to sixteen kinds of antibiotics. The results showed that the *L. plantarum* Q180 strains were susceptible to Gram-positive spectrum antibiotics (bacitracin, novobiocin, lincomycin). In fact, *L. plantarum* Q180 showed itself to be more sensitive to novobiocin in a comparison of fifteen different antibiotics, and exhibited the greatst resistance to rifampicin, polymyxin B and vancomycin. Among antibiotic resistances, vancomycin resistance is of major concern because vancomycin is one of the last antibiotics to remain broadly efficacious against clinical infections caused by multidrug-resistant pathogens (Zhou *et al*., 2005). Some LABs including strains of *L. rhamnosus, L. casei, L. plantarum, Leuconostoc* spp. and *pediococci* are resistant to vancomycin. Such resistance is usually intrinsic, it is chromosomally encoded and non-transmissible (Handwerger *et al*., 1994; Klein *et al*., 1998; Ruoff *et al*., 1988; Swenson *et al*., 1990). These results show that the *L. plantarum* Q180 strain generally showed slightly higher susceptibility against antibiotics.

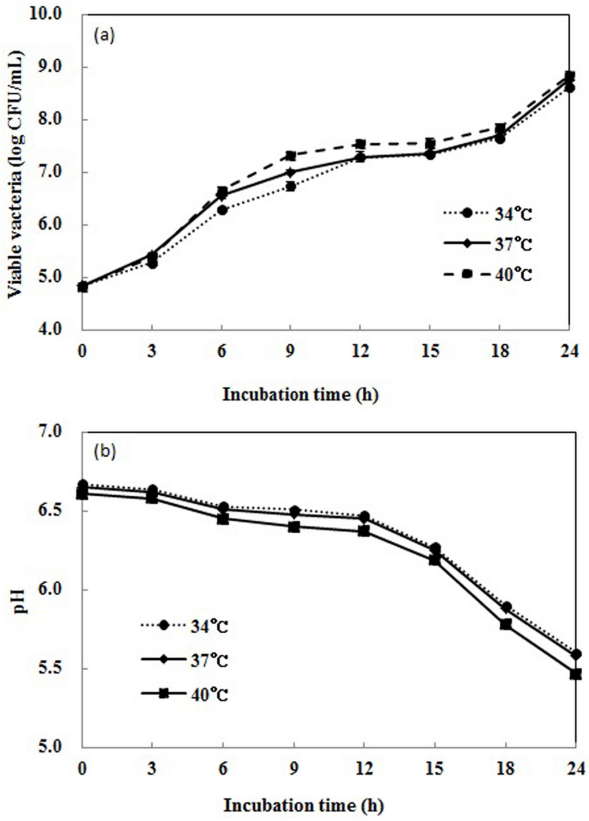

Fig. 2. Growth curve (a), and pH changes (b) of *Lactobacillus plantarum* Q180 in MRS broth at various temperatures. (a) All values are within the mean±standard deviation of the three replicates.

Enzyme activity

Enzyme activity is also important when using lactic acid bacteria as probiotics. Probiotics should not produce β-glucuronidase, a toxic enzyme which has been implicated in the formation of carcinogens (Borriello *et al*., 2003). When carcinogenic substances such as benzo(a)pyrene enter the human body, their poisonous effects are counter-

Table 3. Antibiotics susceptibility of *Lactobacillus plantarum* Q180

Antimicrobal agents	minimal inhibitory concentrations (μg/mL)
Aminoglycosides	
Amikacin	40±0
Gentamycin	10±0
Kanamycin	100±0
Neomycin*	25±0
Streptomycin	200±0
β-lactams	
Penicillin-G*	10±0
Methicillin	40±0
Oxacillin	30±0
Ampicillin	120±0
Gram-positive spectrum	
Bacitracin*	15±0
Rifampicin	960±0
Novobiocin	7.5±0
Lincomycin*	12.5±0
Gram-negative spectrum	
Polymyxin B*	1200±0
Broad spectrum	
Chloramphenicol	40±0
Vancomycin	1600±0

*units/mL
All values are the mean±standard deviation of three replicates.

Table 4. Enzyme patterns of *Lactobacillus plantarum* Q180

Enzyme	*L. plantarum* Q180
Alkaline phosphatase	1
Esterase (C4)	2
Esterase Lipase (C8)	2
Lipase (C14)	1
Leucine arylamidase	3
Valine arylamidase	2
Cystinearylamidase	2
Trypsin	1
α-chymotrypsin	1
Acid phosphatase	1
Naphtol-AS-BI-phosphohydrolase	3
α-galactosidase	2
β-galactosidase	4
β-glucuronidase	1
α-glucosidase	3
β-glucosidase	1
N-acetyl-β-glucosaminidase	4
α-mannosidase	3
α-fucosidase	2

*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 5 nanomoles, 2 to 10 nanomoles, 3 to 20 nanomoles, 4 to 30 nanomoles, and 5 to 40 nanomoles or more.

acted due to conjugation with glucuronic acid in the liver. If this conjugated product is excreted with bile acid in the intestine, cleavage β-glucuronidase can liberate these substances to become toxic once again. On the other hand, enzymes such as lipase, protease, and β-galactosidase have advantages for digestion and the treatment of lactose intolerance (Kumar *et al*., 2012). *L. plantarum* Q180 did not produce β-glucuronidase; rather, it produced such enzymes as leucine arylamidase, naphtol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, N-acetyl-β-glucosaminidase, and α-mannosidase. Notably, the activities of β-galactosidase and N-acetyl-β-glucosaminidase were 4 degree (Table 4). According to Lee *et al*. (2006), *L. plantarum* NK181 isolated from *jeotgal* produced such enzymes as leucine arylamidase, valine acrylamides, acid phosphatase, naphtol-AS-BI-phosphohydrolase, β-galactosidase, α, β-glucosidase, and N-acetyl-β-glucosaminidase. This result shows that the enzyme profiles of the *L. plantarum* Q180 strain were similar to those of *L. plantarum* NK181.

Bile tolerance

Resistance to human gastric transit constitutes an essential evaluation criterion for probiotic bacteria (Goldin *et al*., 1989; Klaenhammer *et al*., 1982). Moreover, bile salt tolerance is considered one of the necessary properties required for lactic acid bacteria to survive in the small intestine (Park *et al*., 1998; Saarela *et al*., 2000). Bile secreted in the small intestine reduces the survival of bacteria by destroying their cell membranes, whose major components are lipids and fatty acids, and these modifications may affect not only cell permeability and viability, but also the interactions between the membrane and the environment (Gilliland *et al*., 1984). Fig. 3 shows the growth curves in MRS broth or MRS broth containing 0.3% bile. A 0.3% concentration of bile salt is considered the critical screen for probiotics in the human gastrointestinal tract (Gilliland *et al*., 1984). The log value of the population after incubation for 7 h without 0.3% oxgall was 8.9, but it was 8.7 with the addition of 0.3% bile. Therefore, the survival rate of *L. plantarum* Q180 in MRS broth containing 0.3% bile was 97.8%. *L. plantarum* Q180 has probiotic potential because a comparatively high percentage of the strain survived in MRS broth containing 0.3% bile salt.

Acid tolerance

Therefore, before a probiotic can benefit human health,

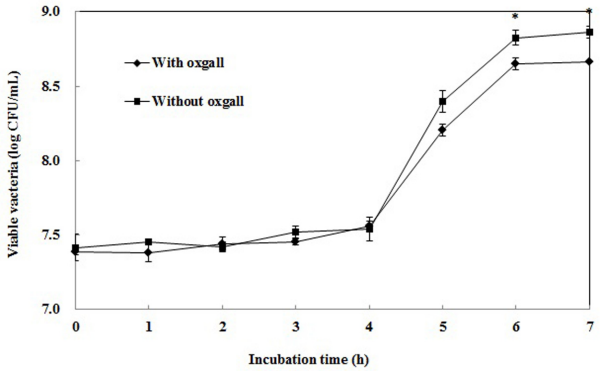

Fig. 3. Growth of *Lactobacillus plantarum* Q180 in MRS broth containing 0.05% L-cysteine with/without 0.3% oxgall. *$p<0.05$ between oxgall and without oxgall (*t*-test).

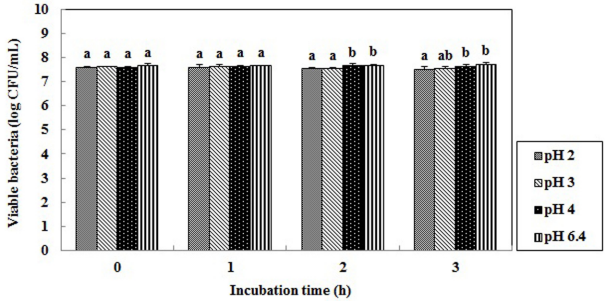

Fig. 4. Survival of *Lactobacillus plantarum* Q180 after 3 h in HCl solution (pH 2.0, 3.0, 4.0 and 6.4). [a-b]Means values with different superscript within same time are significantly different ($p<0.05$).

it must satisfy several criteria such as the ability to tolerate acid and to grow in the lower intestinal tract (Hirayama *et al*., 2000; Ouwehand *et al*., 2002; Pereira *et al*., 2002; Zhu *et al*., 2000). To be a good probiotic, it is necessary to survive in a pH lower than 3 so that it can reach the small intestine via the stomach (Booth, 1985; McDonald *et al*., 1990). Fig. 4 shows the pH tolerance of *L. plantarum* Q180. It showed a 97.2% survival rate after incubation for 3 h in highly acidic conditions (pH 2.0). Lim and Im (2009) reported that *L. plantarum* isolated from kimchi was resistant in acidic condition, showing a 98.8% survival rate after incubation for 2 h at pH 2.5. Pennacchia *et al*. (2004) reported a 60-80% survival rate for LAB incubated in a PBS buffer at pH 2.5 for 3 h at 37°C.

Antimicrobial activity

Antagonism against pathogens is one of the main criteria for selecting probiotics (Ouwehand *et al*., 2002). The antagonistic activities demonstrated by lactic acid bacteria may be due to the production of substances with antibacterial properties in particular: hydrogen peroxide, organic acid and bacteriocins (Tejero-Sarimena *et al*., 2012). According to previous studies, *Lactobacillus* strains show a variable ability to inhibit pathogens even within a same species (Jacobsen *et al*., 1999; Larsen *et al*., 1993; Strahinic *et al*., 2007).

Table 5 shows the antimicrobial activity of *L. plantarum* Q180 against various pathogenic strains. *L. plantarum* Q 180 showed resistance against *E. coli, S.* Typhimurium and *S. aureus* at rates of 55.6%, 38.0%, and 47.6%, respectively. The pH value of pathogens after incubation for 7 h was 6.4, but the pH value of a mixed culture with *L. plantarum* Q180 and pathogens was around 5.5-5.6. It means that even lactic acid produced during incubation affected on antimicrobial activity, it is not large effect.

Conclusion

We investigated whether lactic acid bacteria isolated from the feces of healthy adults exhibit any anti-obesity activity such as a pancreatic lipase (PL) inhibitory activity, inhibition of adipocyte differentiation of 3T3-L1 cells. The selected Q180 strain was identified as *L. plantarum* as a result of the API carbohydrate fermentation test and 16S rDNA sequence. The optimum growth temperature of Q180 was 37°C. *L. plantarum* Q180 could survive in antibiotic conditions at a low concentration and did not produce carcinogenic enzymes such as β-glucuronidase. Moreover, it was found to be comparatively tolerant to bile juice and acid, and displayed resistance to pathogenic strains. These results demonstrate that *L. plantarum* Q180

Table 5. Inhibition of pathogens by *Lactobacillus plantarum* Q180 in MRS broth

Pathogens	Pathogens[a]		*L. plantarum* Q180[a] + Pathogens		Inhibition (%)
	CFU/mL	pH	CFU/mL	pH	
Escherichia coli	$2.0\pm0.4\times10^8$	6.4	$8.7\pm0.3\times10^7$	5.6	56.5
Salmonella Typhimurium	$7.2\pm0.3\times10^6$	6.4	$4.7\pm0.2\times10^6$	5.5	34.7
Staphylococcus aureus	$3.3\pm0.5\times10^8$	6.4	$1.7\pm0.3\times10^8$	5.5	48.5

*Initial count of *L. plantrum* Q180: $3.6\pm0.5\times10^6$ CFU/mL
[a]Determined after 6 h of incubation at 37°C
All values are the mean±standard deviation of three replicates.

could be an excellent strain for anti-obesity activity.

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