



Probiotic potential of *Lactobacillus* strains with anti-allergic effects from kimchi for yogurt starters

Na-Kyoung Lee, So-Yeon Kim, Kyoung Jun Han, Su Jin Eom, Hyun-Dong Paik*

Department of Food Science and Biotechnology of Animal Resources, Konkuk University, #1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea

ARTICLE INFO

Article history:

Received 3 September 2013

Received in revised form

22 January 2014

Accepted 17 February 2014

Keywords:

Kimchi

Lactobacillus plantarum

Anti-allergic effects

Probiotic

ABSTRACT

Lactobacillus strains were isolated from kimchi and investigated for their potential use for probiotic yogurt starters. Two of the isolated strains have over 90% survival rate to artificial gastric acid (pH 2.5, 1% pepsin) and artificial bile acid (0.3% oxgall). These strains were identified as *Lactobacillus plantarum* by 16S rRNA sequencing, and named as *L. plantarum* SY11 and *L. plantarum* SY12. The known carcinogenic enzyme, β -glucuronidase was not produced by *L. plantarum* SY11 and *L. plantarum* SY12. These 2 strains were found to be resistant to several antibiotics and strongly adhered to intestinal cells. Antiallergic effect of *L. plantarum* SY11 and *L. plantarum* SY12 was demonstrated using nitric oxide (NO) and cytokine production. *L. plantarum* SY11 and *L. plantarum* SY12 were capable of significantly decreasing NO production, and reduced T helper 2-associated cytokines, cyclooxygenase-2, tumor necrosis factor- α , and inducible nitric oxide synthase compared to control. Yogurt samples produced using *L. plantarum* SY11 and *L. plantarum* SY12 did not show significant differences in microbiological, physicochemical, and sensory properties compared with yogurt sample produced using commercial strain. Therefore, these two strains could be used as probiotic yogurt starters with antiallergic effects.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Kimchi is prepared by the fermentation of various ingredients including Korean cabbage, radish, *jeot-gal*, and spices such as garlic, ginger, green onion, and red pepper (Jung et al., 2011). Kimchi has been recognized as a functional food and mentioned as one of the world's most healthy foods (Song, 2004). Lactic acid bacteria (LAB) originated from kimchi have been evaluated for their nutritional and pharmacological aspects (Ahn, Han, Shin, Jin, & Ghim, 2003; Kim et al., 2013). These kimchi LAB exhibit strong tolerance to gastric acid and bile juice, which can cause them to persist for longer durations in the human intestine. Furthermore, acid production and acid tolerance properties of kimchi LAB indicate their potential as yogurt starters (Cho, Park, & Jeong, 2009).

Probiotics are live microorganisms and improve the health status of the host by exerting desirable effects in the gastrointestinal tract (Shanahan, 2004). These effects include antimicrobial, anticancer, antioxidant, and anti-allergic effects, reduction of blood lipids, and enhancement of immune function, and are strain-dependent. Most probiotic bacteria are LAB, such as *Lactobacillus*

sp. and *Bifidobacterium* sp., partly *Bacillus* sp., or yeasts (Hoa et al., 2000). In particular, *Lactobacillus* spp. have been detected in various environments and from the subject of considerable study because of their commercial use in the food industry (Sutula, Coulthwaite, Thomas, & Verran, 2013).

Allergic diseases have increased due to various causes such as heredity, aberrant exposure to environmental factors, and others. Allergy treatment has taken the direction of avoiding allergens or reducing inflammation; this treatment could be partly successful. Allergic diseases generally result from an inappropriate immune response characterized by a disruption of the T helper (Th)1/Th2 balance. Th1 immunoresponses are related to cytotoxicity, and generate bactericidal responses in macrophages, natural killer (NK) cells, and cytotoxic T lymphocytes, interferon (IFN)- α , IFN- γ , interleukin (IL)-12, and IL-18. On the other hand, downregulation of Th2 results in anti-allergic effects, with the generation of cytokines such as IL-4, IL-6, IL-8, and transforming growth factor (TGF)- β . Probiotics may be safe alternatives for allergy therapy; anti-allergic effects have been reported in the case of *Lactobacillus rhamnosus* GG, *Bifidobacterium lactis* Bb-12, *Lactobacillus casei*, and *Lactobacillus reuteri* DSM 122460 (Cross, 2002; Ouwehand, 2007).

The objective of this study was to isolate probiotic *Lactobacillus* spp. from kimchi, and to evaluate their probiotic characteristics

* Corresponding author. Tel.: +82 2 2049 6011; fax: +82 2 455 3082.

E-mail addresses: hdpaik@konkuk.ac.kr, hdpaik@nate.com (H.-D. Paik).

including anti-allergic effects. Additionally, this study demonstrates potential use of such strains as fermentation starters in yogurt production.

2. Materials and methods

2.1. Isolation of probiotic strain from kimchi and cell line

To isolate *Lactobacillus* sp. from kimchi, samples were diluted in 0.1% peptone water, and 100 μ L of each diluted suspension was spread onto *Lactobacillus* selective medium (LBS; BD BBL, Cockeysville, MD, USA) agar. After incubation for 24 h at 37 °C, grown colonies were isolated. The isolates were grown on lactobacilli MRS agar (BD BBL), and stored as stock solutions in 20% glycerol at –70 °C. *Lactobacillus acidophilus* CH-2 (Chr. Hansen, Hoersholm, Denmark) and *Streptococcus thermophilus* TH-4 (Chr. Hansen) were used for yogurt production.

RAW 264.7 (murine macrophage) and HT-29 (human colon adenocarcinoma) cell lines were purchased from the Korean Cell Line Bank (KCLB; Seoul, Korea). Each medium of RAW 264.7 and HT-29 cell lines were DMEM (Invitrogen, Grand Island, NY, USA) and RPMI 1640 (Invitrogen). Both medium were containing 10% FBS (Invitrogen) and 1% streptomycin-penicillin (Invitrogen), and incubated under 5% CO₂ at 37 °C.

2.2. Resistance to artificial gastric conditions

The resistance of *Lactobacillus* sp. to artificial gastric conditions was determined (Lee, Lee, & Paik, 2013). Stationary-phase-grown cells of LAB were harvested at 10,000 $\times$ g for 15 min, washed with 0.1% peptone water, and added to artificial gastric acid, and incubated at 37 °C for 2 h. The artificial gastric acid was MRS broth containing 1% pepsin (Sigma, St. Louis, MO, USA), adjusted to pH 2.5 by the addition of 0.1 M HCl. Resistance to artificial bile acid was measured by cultivating cells treated with artificial gastric juice (MRS broth containing 0.3% (w/v) oxgall (BD BBL)). Viable cell counts were measured by incubating aliquots for 24 h on MRS agar at 37 °C.

2.3. Identification of isolated strains

The selected *Lactobacillus* strains were identified by gram staining, analyzing cell morphology, measuring carbohydrate utilization using the API 50 CHL kit (BioMerieux, Lyon, France), and the 16S rRNA sequencing method by Macrogen Inc. (Seoul, Korea). Sequencing was performed using the Basic Local Alignment Search Tool (BLAST) of the GENBANK data library (<http://blast.ncbi.nlm.nih.gov>).

2.4. Enzyme activity of strains

Enzyme activity was assessed using the API ZYM kit (BioMerieux, Marcy l'Etoile, France) (Sharifuzzaman, Al-Harbi, & Austin, 2014). The isolated strains were suspended in sterile saline containing 0.85% sodium chloride at 10⁵ CFU/mL and added to each of 20 cupules. After inoculation, the cultures were incubated at 37 °C for 4 h, and then 1 drop each of the ZYM A and ZYM B reagents were consecutively added to each cupule. Color was allowed to develop for at least 5 min, and values were assigned as the colors developed. The approximate amount of substrate hydrolysis was determined.

2.5. Adherence to intestinal epithelial cells

Adherence assays were performed by adding about 1 $\times$ 10⁸ CFU/well of each isolated strain into a monolayer of HT-29 cells. This mixture was incubated at 37 °C for 2 h under 5% CO₂. Nonadherent cells were removed by washing 4 times with DPBS (Invitrogen). The

wells were treated with 1 mL of 0.1% Triton X-100, and harvested. Serial dilutions of the harvested samples were spread on MRS medium and incubated at 37 °C for 24 h. *L. acidophilus* CH-2 was used as the control. Adhesion (%) was calculated as follows:

$$\text{Adhesion}(\%) = (V_1 \times 100)/V_0$$

Where V_0 is the initial viable bacterial count tested, and V_1 is the viable bacterial count obtained from the HT-29 cells, at the end of the experiment.

2.6. Nitric oxide (NO) production and evaluation of anti-allergic effects

Viable and heat-treated (100 °C, 5 min) bacterial cells were added at concentrations of 1 $\times$ 10⁷ colony-forming units (CFUs)/well with or without lipopolysaccharide (LPS) in RAW 264.7 cells. After 3 h of incubation, the conditioned media (100 μ L) were allowed to react with an equal volume of Griess reagent (Sigma) for 15 min at room temperature. The optical density was determined at 540 nm using an ELISA plate reader (Molecular Devices, Sunnyvale, CA, U.S.A.). NO production was evaluated by comparison of the optical density with the standard curve obtained with sodium nitrite (Sigma–Aldrich).

Viable and heat-treated (100 °C, 5 min) bacterial cells were added at concentrations of 1 $\times$ 10⁷ CFUs/well with or without LPS in RAW 264.7 cells for analysis of cytokine. After 3 h of incubation, RAW 264.7 cells were harvested and washed in preparation for RNA isolation. Total RNA was isolated from the pellet using the EzWay™ Total RNA Isolation Kit (Koma Biotech, Seoul, Korea). Reverse transcription polymerase chain reaction (RT-PCR) was performed using the Reverse Transcription Master Premix (5 $\times$) (Elpis Biotech, Daejeon, Korea) on a Bioer XP Thermal Cycler (Bioer Technology, Hangzhou, China). One microliter of total RNA was reversed transcribed in a 20- μ L reaction mixture containing PCR buffer, dNTP mix, primers, Taq DNA polymerase, cDNA, and nuclease-free water. Amplification was performed in the thermal cycler programmed according to the melting point of the primers. The amplified samples were run on a 1% agarose gel at 100 V and visualized with ethidium bromide staining.

2.7. Production and analysis of probiotic yogurt

Lactobacillus plantarum SY11 and *L. plantarum* SY12 were cultivated in MRS broth at 37 °C for 24 h and harvested by centrifugation at 8000 $\times$ g for 20 min at 4 °C. The harvested cells were washed twice and freeze-dried for use as yogurt starter.

The production of probiotic yogurt was carried out using 3 different samples. Pasteurized milk (1 L; Seoul milk, Korea) was purchased at a local supermarket, repasteurized at 90 °C for 10 min and cooled down to 40 °C. The milk samples were then inoculated with a mixed culture (1:1) of *S. thermophilus* TH-4 (Chr. Hansen, Denmark) and another LAB strain (*L. plantarum* SY11, *L. plantarum* SY12, or *L. acidophilus* CH-2). These inoculated milk samples were incubated at 40 °C until a pH of 4.4–4.5 was reached.

Microbiological and physicochemical of the 3 samples were performed. The pH and titratable acidity (TA) was measured using a pH meter and titration with 0.1 N NaOH, respectively (Lee, 2001). LAB counts were estimated using bromocresol purple (BCP) plate count agar (Eiken Co., Tokyo, Japan).

2.8. Statistical analysis

The results were represented as mean $\pm$ SD. Statistical analysis was performed using the SPSS software program version 18 (SPSS Inc., Chicago, IL, USA). The results of microbial, chemical, and sensory tests were analyzed by one-way and two-way analysis of

variance (ANOVA). Comparison of means was carried out using Duncan's multiple range test ($P < 0.05$).

3. Results and discussion

3.1. Isolation and identification of probiotic strains from kimchi

Twenty-five *Lactobacillus* strains were isolated from various home-made kimchi samples. Among these, only 2 strains were found to be resistant to artificial gastric juice (pH 2.5, 1% pepsin) and artificial bile acid (0.3% oxgall). The SY11 and SY12 strains showed high survival rates of 98.5% and 99.0% in artificial gastric juice, and 93.7% and 97.9% in artificial bile acid, respectively. *L. plantarum* isolated from raw fruits and vegetables showed a high survival rate of 82% (Vitali et al., 2012). Therefore, these strains are worthy of consideration for commercial use in a base of a high survival rate. The tolerance of lactobacilli to acid was originated from presence of a constant gradient between extracellular and cytoplasmic pH by F_0F_1 -ATPase (Corcoran, Santon, Fitzgerald, & Ross, 2005). In addition, the tolerance to bile salt was originated from excretion of bile salt hydrolase (Guo et al., 2009). These bile salt hydrolase of LAB can be provided of potential to lower serum cholesterol level.

SY11 and SY12 strains were identified as *L. plantarum*, with 99.9% similarity via gram staining, carbohydrate utilization, and 16S rRNA sequencing (data not shown). Therefore, these 2 strains were named *L. plantarum* SY11 and *L. plantarum* SY12, respectively.

3.2. Production of various enzymes

Enzyme activity is an important criterion in the selection of probiotic strains, because carcinogenic enzymes like β -glucuronidase could be generated by microorganisms (Cole, Fuller, &

Table 2

Nitric oxide (NO) production by *L. plantarum* strains.

NO (μ M)	Control	SY11	Heated SY11	SY12	Heated SY12
Without LPS	17.54 $\pm$ 0.03	5.18 $\pm$ 0.02	15.23 $\pm$ 0.02	6.76 $\pm$ 0.00	22.23 $\pm$ 0.03
With LPS	29.07 $\pm$ 0.03	5.75 $\pm$ 0.00	15.88 $\pm$ 0.02	6.62 $\pm$ 0.00	18.59 $\pm$ 0.02

The values are represented as mean $\pm$ standard deviation (SD).

Carter, 1989). *L. plantarum* SY11 and *L. plantarum* SY12 produced various enzymes including alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, β -glucosidase, and N-acetyl- β -glucosaminidase (Table 1). In addition, acid phosphatase and α -glucosidase were produced by *L. plantarum* SY11, lipase was produced by *L. plantarum* SY12. However, the carcinogenic enzyme β -glucuronidase was not produced by these strains.

3.3. Adherence to human intestinal epithelial cells

The adhesion ability of bacteria may vary in a strain-dependent manner. Adhesion of *L. plantarum* SY11 and *L. plantarum* SY12 was verified using the plating method. Upon the addition of about 8 Log CFU/well of *L. plantarum* SY11 and *L. plantarum* SY12, 7.11 $\pm$ 0.02 Log CFU/well (7.2% adhesion) and 6.86 $\pm$ 0.11 Log CFU/well (5.3% adhesion), respectively, adhered to HT-29 cell lines, whereas the addition of 8.27 Log CFU/well of *L. acidophilus* CH-2 resulted in the adherence of 2.31 $\pm$ 0.10 Log CFU/well (0.0% adhesion) to HT-29 cells. The 2 isolated strains showed strong adherence compared to *L. acidophilus* CH-2. *Lactobacillus* ssp. was reported as 2–10% adherence, *Lactobacillus paracasei* 34, *L. paracasei* ATCC 27092, and *L. casei* ATCC 393 was represented between 2% and 4% (Monteagudo-Mera et al., 2012). Therefore, the adherence of *L. plantarum* SY11 and *L. plantarum* SY12 was verified a major criteria for probiotic selection.

3.4. Anti-allergic effects of *L. plantarum* SY11 and *L. plantarum* SY12

NO production and mechanism of regulation of the cytokines TNF- α , iNOS, and COX-2 in Raw 264.7 cell lines was investigated to verify their anti-allergic effects. Table 2 shows that *L. plantarum* SY11 and *L. plantarum* SY12 decreased NO production in Raw 264.7 cells, whereas NO production showed almost no change with *L. acidophilus* CH-2 (data not shown). RT-PCR analyses revealed that *L. plantarum* SY11 and *L. plantarum* SY12 inhibited the LPS-induced expression of TNF- α , iNOS, and COX-2 at the mRNA level, whereas *L. acidophilus* CH-2 did not (Fig. 1). These results suggest that *L. plantarum* SY11 and *L. plantarum* SY12 have anti-allergic effects,

Table 1
Enzyme activity of *L. plantarum* strains measured using the API ZYM kit.

Enzyme	Substrate	Enzyme activity ^a	
		SY11 ^b	SY12 ^b
Control	—	0	0
Alkaline phosphatase	2-naphthyl phosphate	3	2
Esterase	2-naphthyl butyrate	0	0
Esterase lipase	2-naphthyl caprylate	0	0
Lipase	2-naphthyl myristate	0	1
Leucine arylamidase	L-leucyl-2-naphthylamide	2	3
Valine arylamidase	L-valyl-2-naphthylamide	1	3
Cystine arylamidase	L-cystyl-2-naphthylamide	1	2
Trypsin	N-benzoyl-DL-arginine-2-naphthylamide	0	0
α -Chymotrypsin	N-glutaryl-phenylalanine-2-naphthylamide	0	0
Acid phosphatase	2-naphthyl phosphate	2	0
Naphthol-AS-BI-phosphohydrolase	Naphthol-AS-BI-phosphate	3	1
α -Galactosidase	6-Br-2-naphthyl-aD-galactopyranoside	0	0
β -Galactosidase	2-naphthyl-bD-galactopyranoside	4	2
β -Glucuronidase	Naphthol-AS-BI-bD-glucuronide	0	0
α -Glucosidase	2-naphthyl-aD-glucopyranoside	1	0
β -Glucosidase	6-Br-2-naphthyl-bD-glucopyranoside	1	1
N-Acetyl- β -glucosaminidase	1-naphthyl-N-acetyl-bD-glucosaminide	2	2
α -Mannosidase	6-Br-2-naphthyl-aD-mannopyranoside	0	0
α -Fucosidase	2-naphthyl-aL-fucopyranoside	0	0

^a 0, 0 nmol; 1, 5 nmol; 2, 10 nmol; 3, 20 nmol; 4, 30 nmol; 5, ≥ 40 nmol.

^b SY11, *L. plantarum* SY11; SY12, *L. plantarum* SY12.

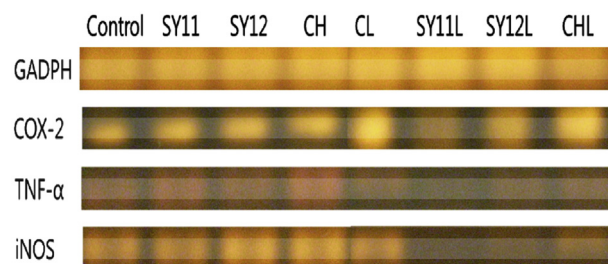


Fig. 1. Effect of probiotics on cyclooxygenase-2 (COX-2), tumor necrosis factor- α (TNF- α), and inducible nitric oxide synthase (iNOS) in RAW 264.7 cells. GAPDH were used to confirm for electrophoresis. Control, *L. plantarum* SY11 (SY11), *L. plantarum* SY12 (SY12), *L. acidophilus* CH-2 (CH), control with LPS (CL), *L. plantarum* SY11 with LPS (SY11L), *L. plantarum* SY12 with LPS (SY12L), and *L. acidophilus* CH-2 with LPS (CHL).

Table 3
pH and titratable acidity (TA) of yogurt samples during fermentation.

Treatment		Incubation time (h)					
		0	2	4	6	8	10
pH	Control	6.68 ± 0.01 ^a	6.41 ± 0.04 ^a	6.25 ± 0.02 ^a	5.53 ± 0.08 ^a	4.88 ± 0.06 ^a	4.50 ± 0.01 ^a
	SY11	6.65 ± 0.01 ^a	6.41 ± 0.02 ^a	6.23 ± 0.03 ^a	5.44 ± 0.07 ^a	4.86 ± 0.06 ^a	4.44 ± 0.01 ^a
	SY12	6.67 ± 0.01 ^a	6.47 ± 0.01 ^a	6.34 ± 0.01 ^b	5.63 ± 0.04 ^a	4.86 ± 0.07 ^a	4.47 ± 0.03 ^a
TA (%)	Control	0.16 ± 0.00 ^a	0.19 ± 0.00 ^a	0.22 ± 0.00 ^a	0.44 ± 0.02 ^a	0.64 ± 0.01 ^a	0.86 ± 0.00 ^a
	SY11	0.15 ± 0.00 ^a	0.19 ± 0.00 ^a	0.22 ± 0.00 ^a	0.45 ± 0.05 ^a	0.64 ± 0.01 ^a	0.88 ± 0.00 ^a
	SY12	0.15 ± 0.00 ^a	0.18 ± 0.00 ^a	0.21 ± 0.00 ^a	0.42 ± 0.01 ^a	0.64 ± 0.01 ^a	0.87 ± 0.01

Mean ± SD in the same column with different superscript differ significantly ($P < 0.05$).

and that such immunomodulatory effects are species-dependent. *Enterococcus faecalis* YM-73, *L. rhamnosus* GG, and *B. lactis* Bb-12 have been reported to exhibit Th2 downregulatory effects, as seen in our results (Cross, 2002; Ou, Lin, Tsai, & Lin, 2011).

3.5. Application of *L. plantarum* SY11 and *L. plantarum* SY12 as probiotic yogurt starters

Characteristics of probiotic yogurt produced using *L. acidophilus* CH-2, *L. plantarum* SY11, and *L. plantarum* SY12 mixed with *S. thermophilus* TH-4 were investigated. Changes in the pH and TA values of yogurt are shown in Table 3. The pH values of the control, SY11, and SY12 yogurt samples after 10 h incubation were 4.50, 4.44, and 4.47, respectively. The TA values of the control, SY11, and SY12 yogurt samples were 0.86, 0.88, and 0.87, respectively. The pH and TA's differences were also almost similar among the groups ($P < 0.05$).

Probiotic cultures added to fermented milk need to be tolerant to acidic conditions as the pH of fermented milk is at least 4.5 and can be much lower (Vinderola, Bailo, & Reinheimer, 2000). The LAB counts of the control, SY11, and SY12 yogurt samples before incubation were 7.53 Log CFU/g, 7.52 Log CFU/g, and 7.52 Log CFU/g, respectively (Fig. 2). After 10 h of incubation, LAB counts of the control, SY11, and SY12 yogurt samples were 8.91 Log CFU/g,

8.95 Log CFU/g, and 8.87 Log CFU/g, respectively. LAB counts were similar among the groups ($P < 0.05$). It appeared that yogurts fermented with *L. plantarum* SY11, *L. plantarum* SY12, and commercial strains did not show significant differences in microbiological, physicochemical, and sensory properties.

4. Conclusion

L. plantarum SY11 and *L. plantarum* SY12 were isolated from kimchi, and selected for probiotic yogurt production. These 2 strains showed anti-allergic effects; in particular, those of *L. plantarum* SY11 were higher. Yogurt samples produced using *L. plantarum* SY11 and *L. plantarum* SY12 did not show differences in microbiological and physicochemical properties compared to control yogurt produced using a conventional commercial starter. On the basis of these results, *L. plantarum* SY11 and *L. plantarum* SY12 could be good alternative starters to replace *L. acidophilus* CH-2. In particular, *L. plantarum* SY11 may be highly suitable for yogurt production as a starter culture, as the SY11 yogurt sample exhibited the best qualities among the 3 yogurt samples analyzed. More research is demanded on other potential of yogurt starter used these strains.

Acknowledgments

This paper was supported by Konkuk University in 2012.

References

- Ahn, D. K., Han, T. W., Shin, H. Y., Jin, I. N., & Ghim, S. Y. (2003). Diversity and antibacterial activity of lactic acid bacteria isolated from kimchi. *Korean Journal of Microbiology and Biotechnology*, 31, 191–196.
- Cho, Y. H., Park, S. N., & Jeong, S. H. (2009). A study on the physiological activity and industrial prospects of plant-origin lactic acid bacteria. *Journal of Korean Dairy Technology and Science Association*, 27, 53–57.
- Cole, C. B., Fuller, R., & Carter, S. M. (1989). Effect of probiotic supplements of *Lactobacillus acidophilus* and *Bifidobacterium adolescentis* 2204 on β -glucosidase and β -glucuronidase activity in the lower gut of rats associated with a human fecal flora. *Microbial Ecology in Health and Disease*, 2, 223–225.
- Corcoran, B. M., Santon, C., Fitzgerald, G. F., & Ross, R. P. (2005). Survival of probiotic Lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars. *Applied and Environmental Microbiology*, 71, 3060–3067.
- Cross, M. L. (2002). Immunoregulation by probiotic lactobacilli: pro-Th1 signals and their relevance to human health. *Clinical and Applied Immunology Reviews*, 3, 115–125.
- Guo, Z., Wang, J., Yan, L., Chen, W., Liu, X. M., & Zhang, H. P. (2009). In vitro comparison of probiotic properties of *Lactobacillus casei* Zhang, a potential new probiotic, with selected probiotic strains. *LWT—Food Science and Technology*, 42, 1640–1646.
- Hoa, N. T., Baccigalupi, L., Huxham, A., Smertenko, A., Van, P. H., Ammendola, S., et al. (2000). Characterization of *Bacillus* species used for oral bacteriotherapy and bacterioprophyllaxis of gastrointestinal disorders. *Applied and Environmental Microbiology*, 66, 5241–5247.
- Jung, J. Y., Lee, S. H., Kim, J. M., Park, M. S., Bae, J. W., Hahn, Y., et al. (2011). Meta-genomic analysis of kimchi, a traditional Korean fermented food. *Applied and Environmental Microbiology*, 77, 2264–2274.
- Kim, Y., Kim, H., Park, H., Lee, J., Lee, H., Shin, H., et al. (2013). Functionality and safety of lactic acid bacterial strains from Korean kimchi. *Food Control*, 31, 467–473.

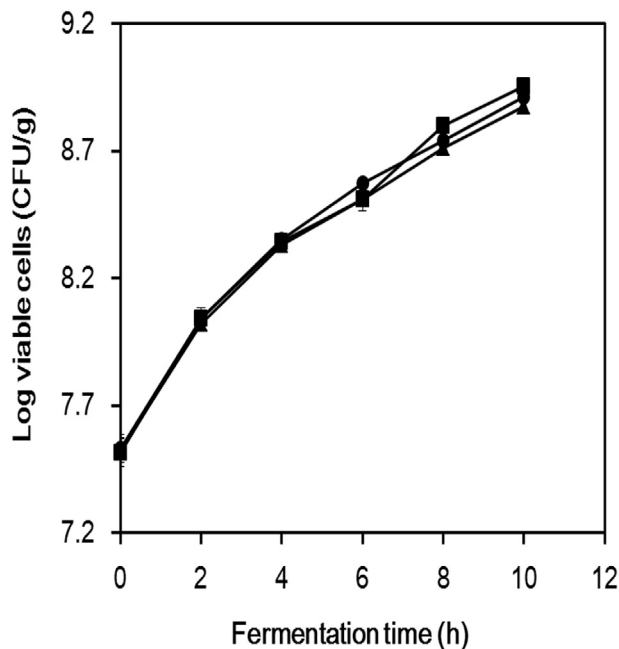


Fig. 2. Lactic acid bacteria viability of yogurt samples make with a mixed culture (1:1) of *S. thermophilus* TH-4 and another LAB strain (●, *L. acidophilus* CH-2; ■, *L. plantarum* SY11; ▲, *L. plantarum* SY12) during fermentation.

- Lee, C. (2001). Changes in n-hexanal content of peanut milk fermented with lactic acid bacteria. *Food Science and Biotechnology*, 10, 387–390.
- Lee, N. K., Lee, E. K., & Paik, H. D. (2013). Potential probiotic properties of phytase-producing *Lactobacillus salivarius* FC113. *Annals of Microbiology*, 63, 555–560.
- Monteagudo-Mera, A., Rodríguez-Aparico, L., Rúa, J., Martínez-Blanco, H., Navasa, N., García-Armesto, M. R., et al. (2012). In vitro evaluation of physiological probiotic properties of different lactic acid bacteria strains of dairy and human origin. *Journal of Functional Foods*, 4, 531–541.
- Ou, C. C., Lin, S. L., Tsai, J. J., & Lin, M. Y. (2011). Heat-killed lactic acid bacteria enhance immunomodulatory potential by skewing the immune response toward Th1 polarization. *Journal of Food Science*, 75, M260–M267.
- Ouwehand, A. C. (2007). Antiallergic effects of probiotics. *Journal of Nutrition*, 137, 794S–797S.
- Shanahan, F. (2004). Probiotics in inflammatory bowel disease – therapeutic rationale and role. *Advanced Drug Delivery Reviews*, 56, 809–818.
- Sharifuzzaman, S. M., Al-Harbi, A. H., & Austin, B. (2014). Characteristics of growth, digestive system functionality, and stress factors of rainbow trout fed probiotics *Kocuria* SM1 and *Rhodococcus* SM2. *Aquaculture*, 418–419, 55–61.
- Song, Y. O. (2004). The functional properties of kimchi for the health benefits. *Journal of Food Science and Nutrition*, 9, 27–33.
- Sutula, J., Coulthwaite, L. A., Thomas, L. V., & Verran, J. (2013). The effect of a commercial probiotic drink containing *Lactobacillus casei* strain Shirota on oral health in healthy dentate people. *Microbial Ecology in Health and Disease*, 24. <http://dx.doi.org/10.3402/mehd.v24i0.21003>.
- Vinderola, C. G., Bailo, N., & Reinheimer, J. A. (2000). Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage. *Food Research International*, 33, 97–102.
- Vitali, B., Minervini, G., Rizzello, C. G., Spisni, E., Maccaferri, S., Brigidi, P., et al. (2012). Novel probiotic candidates for humans isolated from raw fruits and vegetables. *Food Microbiology*, 31, 116–125.