Food Sci. Biotechnol. Vol. 18, No. 2, pp. 384 ~ 389 (2009)

Food Science and Biotechnology

Isolation and Identification of Lactic Acid Bacteria from Sourdough with High Exopolysaccharide Production Ability

Seung Won Jung, Wang June Kim*, Kwang Geun Lee, Cheol Woo Kim[1], and Wan Seob Noh

Department of Food Science and Technology, Dongguk University, Seoul 100-715, Korea
[1]Korea Advanced Food Research Institute, Seoul 137-850, Korea

Abstract To isolate lactic acid bacteria having high exopolysaccharides (EPS) production ability, 50 strains were initially isolated from the sourdough. Twenty-one strains formed highly mucoid colonies on the sucrose agar medium, which are indicative of active EPS synthesis. DU-07, DU-10, DU-12, DU-19, and DU-21 produced 11.51±0.167, 13.09±0.193, 12.72±0.108, 11.61±0.284, and 13.32±0.094 g/L EPS, respectively, in MRS medium. The isolated strains, DU-10, DU-12, and DU-21, were identified as *Enterococcus flavescens*, *Enterococcus faecium*, and *Lactobacillus amylovorus*, respectively, by using API 50CHL kit and determining partial sequences of their 16S rDNA. Especially, *L. amylovorus* DU-21 showed the highest production of EPS, as well as the highest inhibitory activities against pathogenic ($p<0.05$). Interestingly, the *L. amylovorus* DU-21 seem to be endemic to sourdough fermentations, as they have not been isolated from other environments.

Keywords: exopolysaccharide, sourdough, lactic acid bacteria, screening, 16S rDNA gene

Introduction

Polymers from plant, animal, and microbial origin play an important role in food formulations. For industrial practice, most are chemically modified. Their use is restricted and in the European Union (EU) all added food polymers will carry an E-number (1). Alternative biothickeners are the microbial exopolysaccharides (EPS) (2). EPS either surround the bacterial cells as a capsule or are excreted into the extracellular environment as slime, although the distinction between these 2 types of EPS is not always very clear. EPS are long-chain polysaccharides consisting of branched, repeating units of sugars or sugar derivatives. These sugar units are mainly glucose, galactose, and rhamonose, at different ratios (3). The food industry is interested in EPS producing food grade organisms such as lactic acid bacteria (LAB). EPS from LAB have found their most valuable application in the improvement of the rheology, texture and mouthfeel of fermented milk products, such as yoghurt. An additional hypothesized physiological benefit is that EPS will remain for longer in the gastrointestinal tract, thus enhancing colonization by probiotic bacteria (4). In addition, LAB EPS have been claimed to have antitumor effects, immunostimulatory activity, and to lower blood cholesterol (5-7). Therefore, EPS from LAB have potential for development and exploitation as food additives or functional food ingredients with both health and economic benefits.

Study of sourdough from a microbiological point of view barely started a hundred years ago. Sourdough is a mixture of flour (wheat, rye, rice, etc.) and water that is fermented with LAB and yeasts (8,9). It is generally considered that in sourdoughs, the ratio of LAB to yeast should be 100:1 for optimal activities (10). Many inherent properties of sourdough rely on the metabolic activities of its resident LAB: lactic fermentation, proteolysis, synthesis of volatile compounds, and anti-mould activity are among the most important activities during sourdough fermentation. During the last 3 years, several new LAB species have been isolated from traditional sourdoughs at ambient temperature. Sourdough LAB usually belong to the genus *Lactobacillus*, but occasionally *Leuconostoc* spp., *Weissella* spp., *Pediococcus* spp., and *Enterococcus* spp. have been found (11).

The main purpose of this study was to find putative starter cultures with high EPS production ability for yoghurt fermentation. Accurate species labeling is important to responsible quality control efforts to build consumer confidence in product labeling, and for safety consideration. For this reason, LAB were isolated from the sourdough and tested for antimicrobial activity and the ability to produce EPS. Further, LAB with high EPS production ability were studied using Gram-stain, catalase reaction, carbohydrate fermentation, and partial 16S rDNA sequencing methods for the characterization and identification.

Materials and Methods

Sourdough preparation Three-stage technique derived from a traditional procedure was applied to prepare the sourdough as described by Paramithiotis *et al.* (12). Dough 1 (D1) was prepared by mixing 100 g of wheat flour (strong flour, 1st grade; CJ Corp., Seoul, Korea) and 65 mL of tap water. After 24 hr incubation at 28°C, sourdough 1 (SD1) was formed. Then, 50 g of SD1 was mixed with 100 g of wheat flour and 100 mL of tap water to form dough 2 (D2). After 24 hr incubation at 30°C, sourdough 2 (SD2) was formed. Then, 50 g of SD2 was mixed with 100 g of wheat flour and 100 mL of tap water to form dough 3 (D3). After 24 hr incubation at 30°C, sourdough 3 (SD3) formed. This sourdough (SD3) was used for isolation.

*Corresponding author: Tel, Fax: +82-2-2260-3373
E-mail: wjkim@dongguk.edu
Received August 20, 2008; Revised October 7, 2008;
Accepted November 1, 2008

Isolation and identification of LAB Ten g of sourdough (SD3) were initially diluted in 90 mL of sterile 0.1% buffered peptone solution (Difco, Detroit, MI, USA), and homogenized (Nihonseiki Kaisha Ltd., Tokyo, Japan). The LAB were isolated on modified MRS (containing 0.01% brom cresol green) agar medium. Plates were incubated anaerobically (BBL Gas Pak Plus Anaerobic System; Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) at 30°C for about 48 hr. Single colonies were purified by streaking on fresh MRS (Difco) medium and incubated as above. Mucoid colonies were detected on sucrose medium (1% tryptone, 0.5% yeast extract, 0.5% dipotassium phosphate, 0.5% diammonium citrate, 5% sucrose, 1.5% agar pH 7.0). The isolates were Gram-stained and tested for catalase reaction. The carbohydrate fermentation profiles of the isolates from sourdough were investigated by using API 50CH strips and API 50CHL medium according to manufacturer's instructions (API System; Bio-Mérieux, Inc., Lyon, France). A description at species level of strains showing antibacterial activity against the indicator strains was carried out using the database of the API 50CHL.

Isolation of exopolysaccharides (EPS) Isolated LAB showing antibacterial activity were grown in MRS broth (Difco) at 38°C for 48 hr. EPS was isolated from the fermented sample, according to the modified method of Yang *et al.* (13). After incubation, cultures were heated at 100°C for 20 min to inactivate potential EPS hydrolases and improve detachment of EPS from the microbial cell walls. Trichloroacetic acid (Junsei Chemicals, Tokyo, Japan) solution was added to the fermented sample to give a final concentration of 4%(w/v), and the precipitated protein and bacteria were removed by centrifugation at 4°C using a Jouan refrigerated centrifuge (MR14.11; Jouan Inc., Winchester, VA, USA) at 16,000×g for 40 min. The supernatant was then mixed with an equal volume of ethanol, stored at 4°C for 24 hr, and centrifuged, as described above, to collect the precipitated EPS. The precipitate was re-suspended in 25 mL of distilled water. EPS solutions were dialysed (Mw cut off 12,000) at 4°C for 24 hr against distilled water. After 24 hr of freezing at −80°C, followed by 24 hr of freezed-drying by lyophilization at −18°C, the dry weight of the precipitated EPS was determined. *Streptococcus thermophilus* St-body 1 (Chr. Hansen, Hørsholm, Denmark) was used as an indicator strain for the estimation of EPS production ability.

Genetic characterization of isolated LAB DNA was extracted using a Wizard genomic DNA purification kit (Promega, Madison, WI, USA) and DNA was amplified using forward primers 27F: 5'-AGA GTT TGA TCA TGG CTC AG-3' and reverse primer 1492R: 5'-GGA TAC CTT GTT ACG ACT T-3' (14). The polymerase chain reaction (PCR) products purified by Wizard SV Gel and PCR clean-up system (Promega). The PCR-amplified fragments were sequenced by using the ABI PRISM 3700 DNA Analyzer system as specified by the manufacturer. The 16S rDNA sequence was compiled using Basic Local Alignment Search Tool (BLASTN). The 16S rDNA gene sequences of related taxa were obtained from the GenBank database. The multiple alignments were performed by the Clustal X program. The phylogenetic trees were constructed by using the neighbor-joining method and the maximum-parsimony method using the Mega 2 program with bootstrap values based on 1,000 replications (15).

Statistical analyses The statistical analysis was done using the SAS Statistical Analysis for Windows v9.1 (SAS Institute Inc., Cary, NC, USA). The means were compared with Duncan's multiple-range test at $\alpha=0.05$.

Results and Discussion

Isolation and identification of isolates Fifty strains of presumptive LAB were isolated from sourdough. All strains in pure culture were found to be Gram-positive, catalase negative, able to grow at 15°C and unable to grow at 45°C. Twenty-one strains formed highly mucoid colonies on the sucrose agar medium, which are indicative of active exopolysaccharide synthesis. Homopolysaccharides (HoPS) are synthesized by extracellular glucan and fructosyltransferases using sucrose as the glycosyl donor. The biosynthesis mechanism of heteropolysaccharides (HePS) is more complex (16). HePS application is limited to 'ropy' dairy starter cultures employed to improve the texture of yoghurt and other fermented milk products (17). Several screening methods have been applied that raised diverse problems during EPS determination and quantification, such as the presence of other polysaccharides in the media used (18). Based on those reports, a screening for EPS production was carried out using sucrose agar medium. The visual inspection of mucoid colonies on agar plates is most probably the easiest method, but unfortunately very insensitive and only indicative. The latter studies will be required to develop of rapid and reliable screening methods to be able to screen in a fast and elegant way for EPS producing LAB.

To confirm their identity, the 21 strains were tested using the API 50CHL kit. Table 1 lists 21 strains, giving details about the identification by the API 50CHL kit and probability of fit to the closest species. The isolates comprised of 2 genera, *Lactobacillus* and *Lactococcus*. The isolated LAB were identified as *Lactobacillus brevis*, *L. crispatus*, *Lactococcus lactis* subsp. *lactis*, *L. pentosus*, and *L. salivarius* with profile status ranging from 'very good to genus' to 'doubtful'. *L. pentosus* was more frequently isolated than other species in sourdough. The number of *Lactobacillus* strains (80% of isolates) (*L. salivarius, L. brevis, L. pentosus,* and *L. crispatus*) suggests that lactobacilli were predominant in sourdough.

In previous studies, Catzeddu *et al.* (19) reported that typical sourdough prokaryotes are members of the LAB genus *Lactobacillus* with obligately homofermentative, and facultatively or obligately heterofermentative strains. These results were in accordance with Catzeddu *et al.* (19). However, there have been some discussions of the classification of 3 of DU-10, DU-12, and DU-21. Although carbohydrate fermentation study of strain DU-10 suggested it was *L. pentosus*, microscopic observation was consistent with the sequencing results. A notable discrepancy also occurred in the taxonomic classification of strain DU-12, where 16S rDNA results suggested it was a different genera than indicated by the carbohydrate fermentation

Table 1. Speciation results from cell form, Gram-stain, catalase reaction, carbohydrate fermentation, and partial 16S rDNA sequencing

Strain No.	Cell form	Gram-stain	Catalase reaction	Carbohydrate fermentation	Partial 16S rDNA sequencing (GenBank)
DU-01	Rods	+	-	*Lactobacillus salivarius*	NT[1)]
DU-02	Rods	+	-	*Lactobacillus brevis* 1	NT
DU-03	Rods	+	-	*Lactobacillus brevis* 1	NT
DU-04	Cocci	+	-	*Lactobacillus pentosus*	NT
DU-05	Cocci	+	-	*Lactobacillus pentosus*	NT
DU-06	Cocci	+	-	*Lactococcus lactis* ssp. *lactis* 1	NT
DU-07	Rods	+	-	*Lactobacillus salivarius*	NT
DU-08	Rods	+	-	*Lactobacillus salivarius*	NT
DU-09	Cocci	+	-	*Lactobacillus pentosus*	NT
DU-10	Cocci	+	-	*Lactobacillus pentosus*	*Enterococcus falvescens*
DU-11	Cocci	+	-	*Lactococcus lactis* ssp. *lactis* 1	NT
DU-12	Cocci	+	-	*Lactococcus lactis* ssp. *lactis* 1	*Enterococcus faecium*
DU-13	Cocci	+	-	*Lactococcus lactis* ssp. *lactis* 1	NT
DU-14	Cocci	+	-	*Lactobacillus pentosus*	NT
DU-15	Cocci	+	-	*Lactobacillus pentosus*	NT
DU-16	Rods	+	-	*Lactobacillus salivarius*	NT
DU-17	Rods	+	-	*Lactobacillus crispatus*	NT
DU-18	Rods	+	-	*Lactobacillus brevis* 1	NT
DU-19	Rods	+	-	*Lactobacillus brevis* 1	NT
DU-20	Rods	+	-	*Lactobacillus brevis* 1	NT
DU-21	Rods	+	-	*Lactobacillus crispatus*	*Lactobacillus amylovorus*

[1)]Not tested.

study. Identification of sourdough LAB by phenotypic methods, such as sugar fermentation patterns, is considered not reliable and a polyphasic approach has been used to identify strains isolated from sourdoughs and to study their biological diversity (11,20). The most frequently used methods are hybridization or species-specific amplification using probes and primers targeted towards the 16S rDNA sequence, amplification and sequence analysis of the 16S rDNA and 16S/23S rDNA spacer region, cluster analysis of the electrophoretic fragments obtained by randomly amplified polymorphic DNA (RAPD-PCR analysis) (9, 21). Speciation by the carbohydrate fermentation study exhibited some discrepancies compared with those by the partial 16S rDNA sequencing. All LAB were identified as *L. brevis*, *L. crispatus*, *Lc. lactis* subsp. *lactis*, *L. pentosus*, and *L. salivarius* with profile status ranging from 'very good to genus' to 'doubtful'. This identification method, therefore, lacks the ability to differentiate some closely related microorganisms. This implied these LAB exhibited carbohydrate fermentation profiles rather dissimilar to the genus *Lactobacillus*. This method might, then, be useful to distinguish these strains if the database were more comprehensive.

Determination of EPS dry weight To select the most suitable strains for EPS production, strains DU-07, DU-10, DU-12, DU-19, and DU-21 were grown in MRS broth at 37°C for 48 hr. Results are presented in Fig. 1. The viscometric analysis of a culture medium during or after fermentation has been applied to screen for EPS production. However, this is valid as far as the increase in viscosity can be ascribed to the production of EPS solely and not to other effects such as an increase in biomass (22). The partial purification of EPS through precipitation with ethanol or acetone and its spectrophotometrical or gravimetrical quantification is another screening method. For the better comparison of the effect of strains on EPS synthesis, we determined the dry weight of EPS at the end of an experiment.

As shown in Fig. 1, there were significant differences ($p<0.05$) in EPS production ability of isolates from sourdough. The highest amount of EPS was detected from strain DU-21 (13.32±0.094 g/L) and the minimum production was detected from strain DU-07 (11.51±0.167 g/L). These results were higher than those reported by Cerning *et al.* (23), and Bae and Huh (24). However, results were lower than those reported by Kim and Chang (25).

Studies of the application of EPS produced by sourdough LAB have primarily focused on HePS from lactobacilli in dairy fermentations. HePS are produced in small amounts usually below 0.5 g/L (17).

Traditional yoghurt production includes the fermentation of milk with a mix of thermophilic cultures consisting of strains of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*. The current industrial practice prefers the use of *S. thermophilus* alone, since it results in a mild flavor of the final product. In addition to the pH lowering effect and flavor formation, *S. thermophilus* plays a major role in the creation of yoghurt texture through *in situ* EPS production (26). *S. thermophilus* St-body 1 (Chr. Hansen, Denmark) used as the EPS-producing indicator strain in this study was obtained from the Seoul Milk Research Institute

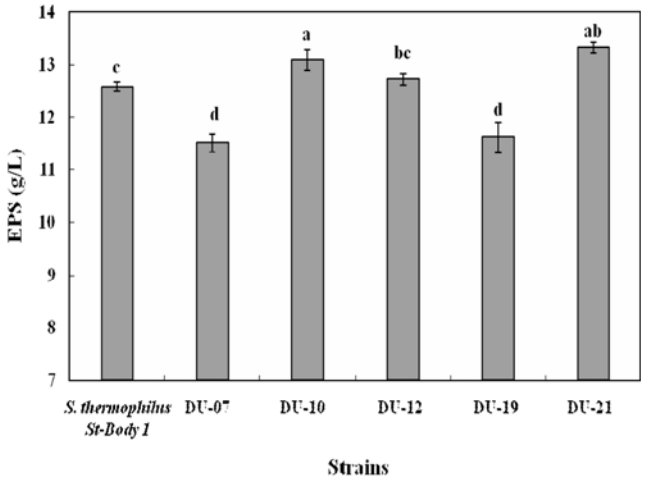

Fig. 1. Dry weigh (g/L) of exopolysaccharides (EPS) produced by various lactic acid bacteria isolated from sourdough. [a-d]Means with the different letter are significantly different $\alpha=0.05$.

(Ansan, Korea), and is already responsible for manufacture of set-type yoghurt. The maximum quantity of EPS produced by *S. thermophilus* St-body 1 and *L. amylovorus* DU-21 differed significantly ($p<0.05$), whit *S. thermophilus* St-body 1 producing 12.58±0.091 g/L at 38°C for 48 hr, compared with *L. amylovorus* DU-21 that generated 13.32±0.094 g/L at 38°C for 48 hr. An economically viable titre of EPS produced in fermentation for use as a food additive has been quoted to be in the range of 10-15 g/L (27). More importantly, all isolates tested in this study can produce EPS efficiently. Currently, we are testing various carbon sources that might influence the production of EPS from the culture fluid of LAB, determined optimal concentration for cell growth and EPS production.

Phylogenetic analysis Three of 50 lactic acid bacteria isolated from sourdough were selected according to their production of high level of EPS. As with bacteria in general, analysis of 16S rDNA sequences has been applied to the speciation of probiotic lactobacilli and bifidobacteria (28). Yeung *et al*. (29) used partial 16S rDNA sequencing to identify named commercial strains obtained directly from the manufacturer and found discrepancies in 14 of 29 species designations. In a recent study, a polyphasic approach was used to characterize LAB from Greek traditional sourdoughs and isolates were identified as *L. sanfranciscensis*, *L. brevis*, *L. paralimentarius*, and *Weissella cibaria* (20).

The taxonomical positions of 3°C of the 21 phenotypically identified isolates were confirmed by sequence analysis of at least 400 bp of the 5' region of the 16S rDNA gene. The lengths of partial 16S rDNA gene sequences of strains DU-10 and DU-12 were 427 and 551 bp, respectively. Phylogenetic analysis based on 16S rDNA gene sequences indicated that both strains belong to genus *Enterococcus*. Strain DU-10 showed the highest 16S rDNA gene sequence similarity with *E. flavescens* AJ420802 (99.0%). The 16S rDNA gene sequence similarity between strains DU-12 and *E. faecium* AY172570 was 99.0%.

The enterococci are LAB that are important in environmental, food, and clinical microbiology. They are ubiquitous microorganisms, but have a predominant habitat in the gastrointestinal tracts of humans and animals (30). Although enterococci occur as commensals of the gastrointestinal tract of warm-blooded animals, they may also display subtle virulence traits and certain strains of enterococci have emerged as leading causes of nosocomial infection, including urinary tract infections, wound infections, and bacteraemia (31). The use of certain *Enterococcus* strains as starters in food fermentation must consider the absence of virulence and the inability to transfer antibiotic resistance to other microorganisms and to acquire resistance to vancomycin themselves (32). The use of enterococci as probiotics remains a controversial issue.

The lengths of partial 16S rDNA gene sequences of strain DU-21 was 785 bp (Fig. 2). Strain DU-21 showed the highest 16S rDNA gene sequence similarity with *L. amylovorus* M58805 (99.0%) (Fig. 3). These value was not low enough to place strain DU-21 as novel species in the genus *Lactobacillus*. Therefore, on the basis of the data presented, strain DU-21 should be tentatively identified as *L. amylovorus* DU-21.

CTATACATGCAAGTCGAGCGAGCGGAACCAACAGATTTACTTCGGTAATG
ACGTTGGGAAAGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGC
CCCTAAGTCTGGGATACCATTTGGAAACAGGTGCTAATACCGGATAATAA
AGCAGATCGCATGATCAGCTTTTGAAAGGCGGCGTAAGCTGTCGCTAAGG
GATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGG
CGACGATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAG
ACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATG
GACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGAT
CGTAAAGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTT
ATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGC
GAGCGCAGGCGGAAAAATAAGTCTAATGTGAAAGCCCTCGGCTTAACCGA
GGAACTGCATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAA
CTCCNTGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGC
GAAGGCGGCTCTCTGTTTGCAACTGACGCTGAGGCTCGAAGCCTGGGTTA
GCGAACAGGATTATATACCCCTCGTAGCCATGCCG

Fig. 2. 16S rDNA partial sequence of *Lactobacillus amylovorus* DU-21.

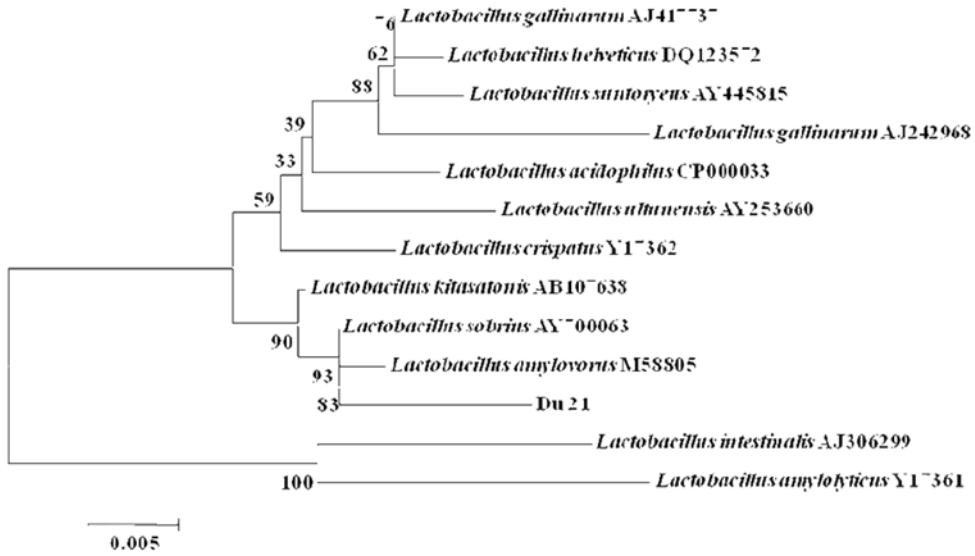

Fig. 3. Phylogenetic tree based on 16S rDNA partial sequences of *L. amylovorus* DU-21 isolated from sourdough. Numbers at nodes indicate bootstrap values obtained with 1,000 resamplings. Scale bar represents 0.5% sequence divergence.

New species and more specific strains of probiotic bacteria are constantly being sought for novel probiotic products. Prior to the incorporation of novel strains into products, their efficacy should be carefully assessed and an evaluation made as to whether they share the safety status of traditional food grade organisms. The safety of a putative novel probiotic bacteria is contingent on its accurate identification. Because probiotic effects are known to be strain specific, unequivocal identification of the probiotic bacteria at the genus, species, and strain level is essential. Correct taxonomic identification of both species and strain is a safety issue for quality control of the product, consumer or prescriber information, diagnosis, and appropriate treatment of suspected clinical cases, and epidemiological surveillance of the exposed population. Currently, we are working on more detailed analysis methods such as random amplified polymorphic DNA (RAPD) and denaturing gradient gel electrophoresis (DGGE) to identify strain DU-21 at the species level (33,34).

In conclusion, the *L. amylovorus* DU-21 isolated from sourdough had no pathogenic properties and possessed adequate properties are functional starter cultures. Interestingly, the *L. amylovorus* DU-21 seem to be endemic to sourdough fermentations, as they have not been isolated from other environments. These studies show that EPS produced by LAB may be used to replace or reduce more expensive hydrocolloids used to improve yoghurt reology. Although genetic modifications have been shown to be successful in enhancing the production of EPS (35), the consumer demand and countries legislations restrict the use of genetically modified organisms for food applications. Therefore, screening of LAB, concerning their EPS production, seems the most suitable approach. Further studies will be required to determine and compare some probiotic characteristics and resistance to biological barriers of *L. amylovorus* DU-21. In addition, we will test various carbon sources that might influence the production of EPS from the culture fluid of LAB, determined optimal concentration for cell growth and EPS production.

References

1. De Vuyst L, De Vin F, Vaningelgem F, Degeest B. Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. Int. Dairy J. 11: 687-707 (2001)
2. Sutherland IW. Novel and established applications of microbial polysaccharides. Trends Biotechnol. 16: 41-46 (1998)
3. De Vuyst L, Degeest B. Exopolysaccharides from lactic acid bacteria. Technological bottlenecks and practical solutions. Macromol. Symp. 140: 31-41 (1999)
4. German B, Schiffrin ED, Reniero R, Mollet B, Pfecfer A, Neeser JR. The development of functional foods: Lessons from the gut. Trends Biotechnol. 17: 492-499 (1990)
5. Kitazawa H, Harata T, Uemura J, Saito T, Kaneko T, Itoh T. Phosphate group requirement for mitogenic activation of lymphocytes by an extracellular phosphopolysaccharide from *Lactobacillus delbrueckii* spp. *bulgaricus*. Int. J. Food Microbiol. 40: 169-175 (1998)
6. Chabot S, Yu HL, De Léséleuc L, Cloutier D, Van Calsteren MR, Lessard M, Roy D, Lacroix M, Oth D. Exopolysaccharides from *Lactobacillus rhamnosus* RW-9595M stimulate TNF, IL-6, and IL-12 in human and mouse cultured immunocompetent cells, and IFN-γ in mouse splenocytes. Lait 81: 683-697 (2001)
7. Nakajima H, Suzuki Y, Kaizu H, Hirota T. Cholesterol lowering activity of ropy fermented milk. J. Food Sci. 57: 1327-1329 (1992)
8. De Vuyst L, Gänzle MG. Second International Symposium on Sourdough: From Fundamentals to Applications. Trends Food Sci. Tech. 16: 2-3 (2005)
9. Vogel RF, Knorr R, Müller MRA, Steudel U, Gänzle MG, Ehrmann M. Non-dairy lactic fermentations: The cereal world. Anton. Leeuw. Int. J.G. 76: 403-411 (1999)
10. Gobbetti M, Corsetti A, Rossi J, La Rosa F, De Vincenzi S. Identification and clustering of lactic acid bacteria and yeasts from wheat sourdoughs of central Italy. Ital. J. Food Sci. 1: 85-94 (1994)
11. Ehrmann MA, Vogel RF. Molecular taxonomy and genetics of sourdough lactic acid bacteria. Trends Food Sci. Tech. 16: 31-42 (2005)
12. Paramithiotis S, Chouliaras Y, Tsakalidous E, Kalantzopoulos G. Application of selected starter cultures for the production of wheat sourdough bread using a traditional three-stage procedure. Process Biochem. 40: 2813-2819 (2005)
13. Yang Z, Huttunen E, Staaf M, Widmalm G, Tenhu H. Separation, purification, and characterization of extracellular polysaccharides produced by slime-forming *Lactococcus lactis* ssp. *cremoris* strains.

Int. Dairy J. 9: 631-638 (1999)
14. Yoon JH, Lee ST, Park YH. Inter- and intraspecific phylogenetic analysis of genus nocardioides and related taxa based on 16S rDNA sequences. Int. J. Syst. Bacteriol. 48: 187-194 (1996)
15. Thompson JD, Higgins DG, Gibson TJ, Clustal W. Improving the sensitivity of progressive multiple sequence alignment through sequence weighing, position-specific gap penalties, and weight matrix choice. Nucleic Acids Res. 22: 4673-4680 (1994)
16. Monchois V, Willwmot RM, Monsan P. Glucansucrases: Mechanism of action and structure-function relationships. FEMS Microbiol. Rev. 23: 131-151 (1999)
17. Laws A, Marshall VM. The relevance of exopolysaccharides to the rheological properties in milk fermented with ropy strains of lactic acid bacteria. Int. Dairy J. 11: 709-721 (2001)
18. Tieking M, Kakitzky S, Valcheva R, Korakli M, Vogel RF, Gänzle MG. Extracellular homopolysaccharides and oligosaccharides from intestinal lactobacilli. J. Appl. Microbiol. 99: 692-702 (2005)
19. Catzeddu P, Mura E, Parente E, Sanna M, Farris GA. Molecular characterization of lactic acid bacteria from sourdough breads produced in Sardinia (Italy) and multivariable statistical analyses of results. Syst. Appl. Microbiol. 29: 138-144 (2006)
20. De Vuyst L, Schrijvers V, Paramithiotis S, Hoste B, Vancanneyt M, Swings J, Kalantzopoulos G, Tsakalidou E, Messens W. The biodiversity of lactic acid bacteria in Greek traditional wheat sourdoughs is reflected in both composition and metabolite formation. Appl. Environ. Microb. 68: 6059-6069 (2002)
21. Müller MRA, Wolfrum G, Stolz P, Ehrmann MA, Vogel RF. Monitoring the growth of *Lactobacillus* species during a rye flour fermentation. Food Microbiol. 18: 217-227 (2001)
22. Sanni AI, Onilude AA, Ogunbanwo ST, Fadahunsi IF, Afolabi RO. Production of exopolysaccharides by lactic acid bacteria isolated from traditional fermented foods in Nigeria. Eur. Food Res. Technol. 214: 405-407 (2002)
23. Cerning J, Bouillanne C, Landom M, Mesmazeaud M. Isolation and characterization of exopolysaccharides from skim-forming mesophilic lactic acid bacteria. J. Dairy Sci. 75: 692-699 (1992)
24. Bae IH, Huh JW. Isolation of *Lactobacillus* spp. producing exopolysaccharide and optimization of its production. Korean J. Biotechnol. Bioeng. 17: 169-175 (2002)
25. Kim HJ, Chang HC. Isolation and characterization of exopolysaccharide producing lactic acid bacteria from *kimchi*. Korean J. Microbiol. Biotechnol. 34: 196-203 (2006)
26. Purwandari U, Shah NP, Vasiljevic T. Effects of exopolysaccharide-producing strains of *Streptococcus thermophilus* on technological and rheological properties of set-type yoghurt. Int. Dairy J. 17: 1344-1352 (2007)
27. Van den Berg DJC. Production of a novel extracellular polysaccharide by *Lactobacillus sake* 0-1 and characterization of the polysaccharide. Appl. Environ. Microb. 61: 2840-2844 (1995)
28. Tannock G. Identification of lactobacilli and bifidobacteria. pp. 45-56. In: Probiotics. A Critical Review. Tannock G (ed). Horizon Scientific Press, Norfolk, England (1999)
29. Yeung PSM, Sanders ME, Kitts CL, Cano R, Tong PS. Species-specific identification of commercial probiotic strains. J. Dairy Sci. 85: 1039-1051 (2002)
30. Gelsomino R, Vancanneyt M, Condon S, Swings J, Cogan TM. Enterococcal diversity in the environment of an Irish cheddar-type cheesemaking factory. Int. J. Food Microbiol. 71: 177-188 (2001)
31. Franz CMAP, Holzapfel WH, Stiles ME. Enterococci at the crossroads of food safety? Int. J. Food Microbiol. 47: 1-24 (1999)
32. Mundy LM, Sahn DF, Gilmore M. Relationships between enterococcal virulence and antimicrobial resistance. Clin. Microbiol. Rev. 13: 513-522 (2000)
33. Innis MA, Gelfand DH, Sninsky JJ. PCR Strategies. Academic Press, San Diego, CA, USA. pp. 249-276 (1995)
34. Ampe F, Omar NB, Moizan C, Wacher C, Guyot JP. Polyphasic study of the spatial distribution of microorganisms in Mexican pozol, a fermented maize dough, demonstrates the need for cultivation-independent methods to investigate traditional fermentations. Appl. Environ. Microb. 65: 5464-5473 (1999)
35. Wlman AD, Maddox IS. Exopolysaccharides from lactic acid bacteria: Perspectives and challenges. Trends Biotechnol. 21: 269-274 (2003)