

Selection of autolytic lactic acid bacteria as potential adjunct cultures to accelerate ripening of white-brined cheeses

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Abstract

The aim of this study was to select lactic acid bacteria (LAB) having autolytic activity on the basis of the technological aspects to accelerate the ripening of white-brined cheeses. For this purpose, mesophilic (n=526) and thermophilic (n=413) LAB isolates were collected from 34 raw milk and 16 cheese samples. 27 LAB isolates with a high autolytic activity ranging from 34 to 54 % were identified by (GTG)₅ fingerprint and 16S rDNA sequence analyses, which showed that *Lactococcus lactis* and *Enterococcus faecium* were abundant among the autolytic isolates in raw milk while *E. faecium* and *Lactobacillus casei* were in white-brined cheese. The autolysis rate of LAB strains increased by raising the temperature and pH; however, decreased somewhat at high NaCl concentrations. Additionally, the highest autolysis was observed in the presence of glucose for most of the strains. On the other hand, *Lb. plantarum* PFC231 was autolyzed differently, being the highest in the presence of lactose. *E. durans* PFC235, *E. faecium* PFC232 and *Lb. plantarum* PFC231 were autolyzed more than other LAB strains, when exposed to low pH, high NaCl concentration at 10 °C simultaneously. However, only *Lb. plantarum* PFC231, *Leuconostoc mesenteroides* PFC234, *Lc. lactis* subsp. *cremoris* PFC233, *Pediococcus acidilactici* PFC237 and *Lb. rhamnosus* PFC238 were able to show intracellular caseinolytic activity. In conclusion, *Lb. plantarum* PFC231 has the potential to be used as an adjunct starter culture in the production of white-brined cheeses because of its autolysis at low temperature, pH and high salt concentrations, as well as intracellular caseinolytic activity.

Key words: lactic acid bacteria, *Lactobacillus plantarum*, autolysis, white-brined cheese, cheese ripening

Introduction

The main function of lactic acid bacteria (LAB) used as starters in fermented foods is to produce lactic acid to give the characteristic nature of foods. However, various physiological behaviors of LAB can also provide desirable characteristics

in fermented food products (De Vuyst and Leroy, 2007). For instance, LAB strains contribute to the development of desired flavor aroma in cheese by autolysis during ripening (McSweeney, 2004; Hannon et al., 2007). In this process, peptides are cleaved into smaller units and free amino acids by intracellular peptidases released during LAB autoly-

sis. Thereby aromatic precursors such as branched-chain and sulfur-containing amino acids responsible for the formation of volatile aroma compounds are substantially released (Beresford et al., 2001). Activity of lipolytic enzymes, which are hydrolases that cleave the ester linkage between a fatty acid and the glycerol core of the triacylglyceride, is also crucial for flavor development in some cheese varieties (Collins et al., 2003).

Autolysis is the enzymatic cleavage of cell wall peptidoglycans by peptidoglycan hydrolases present in bacterial cells called autolysin. In fact, bacterial peptidoglycan hydrolases act in different cellular functions that are necessary for the modification of the rigid peptidoglycan network during the cell growth and division. It also allows for the elimination of weakened or impaired cells in the cell population (Shockman et al., 1996). Peptidoglycan hydrolases with different specificity and/or structure may be present in a bacterial cell at the same time and these relevant hydrolases constitute a complex enzymatic system (Chapot-Chartier and Kulakauskas, 2014).

LAB may exhibit autolytic activity at different levels depending on the cell wall composition, the presence of autolysis-stimulating conditions and genomic defects (Lortal and Chapot-Chartier, 2005). Apart from these, differences in peptidoglycan structure during cell division can alter the autolysis behavior of the same species (Smith et al., 2000). The level of LAB autolysis is dependent on factors such as carbon source, temperature, osmotic concentration, growth phase and pH, and the autolytic activity of LAB may change from 32 to 94 % (Dako et al., 1995; Lortal and Chapot-Chartier, 2005; Boutrou et al., 1998; Çibik and Chabot-Chartier, 2004).

Autolytic LAB have been isolated to improve the aroma properties of various cheeses (Collins et al., 2003; Lortal and Chapot-Chartier, 2005; Lazzi et al., 2016; Meng et al., 2018). However, to the best of our knowledge, the isolation of autolytic LAB with a potential for accelerating the ripening of white-brined cheeses has not been studied yet. White-brined (or pickled) cheeses are ripened and stored in brine, and produced worldwide under various names and by different processing methods. Bovine or mixtures of other milk types can be used at different ratios in the production (Gursoy et al., 2013). Some of the white-brined cheeses are pro-

duced with pasteurized milk, and cheese blocks are ripened in brine (3 to 15 % NaCl) up to 6 months. In the traditional production of white-brined cheeses, raw milk is used without any lactic culture; however, in modern dairy plants, commercial lactic cultures have been used (Gursoy et al., 2013). Cheese ripening is influenced by various factors including the endogenous and exogenous enzymes and microflora derived from the raw milk, starter cultures, coagulants, manufacturing and ripening conditions (Fox and McSweeney, 1996; Sousa et al., 2001; Wilkinson and Kilcawley 2005). Proteolysis is usually regarded as the main biochemical process during cheese ripening and one of the most important factors for the development of typical cheese flavor and texture. However, high salt concentration and acidic conditions cause long ripening of white cheese.

In this study, LAB with high autolytic activity were selected on the basis of the technological aspects of white-brined cheese ripening. The potential of LAB with both autolytic and intracellular caseinolytic activities as an adjunct starter culture was determined in white-brined cheese production.

Materials and methods

Materials

In this present study, raw milk (n=34) and white-brined cheese samples (n=16) produced without any starter culture were collected from local farms, producers and open markets of Denizli (Turkey) and used for the isolation of autolytic LAB. Isolates were stored at -20 °C in culture medium containing glycerol (20 %) throughout the study.

Isolation, identification and determination of autolytic activity of LAB

Isolates were collected on MRS and M17 agars supplemented with 0.5 % glucose, which were incubated at 43 °C (thermophilic) and 30 °C (mesophilic) under aerobic conditions that were seeded from appropriate dilutions prepared from the obtained raw milk and cheese samples.

Prior to the identification of collected isolates, autolytic activities were determined according to Çibik and Chapot-Chartier, (2004). Accordingly, the optical density (OD) of each isolate at 620 nm

was adjusted to a value between 0.6 to 0.8 with potassium phosphate buffer (100 mM, pH 7.0), and changes in optical density at 30 °C monitored for 24 hours. Autolysis rates of the isolates were calculated by the following equation.

$$\text{Autolysis Rate (\%)} = 100 - [(\text{OD}_{\text{final}} / \text{OD}_{\text{initial}}) \times 100]$$

To amplify (GTG)₅ repetitive regions on the genomes of the isolates, master mix (4 µL) (FIRE-POL/ SOLIS BioDyne), primer (5-GTGGTGGTGGTGGT-3) (1 µL) and template DNA (1 µL) were used in a PCR reaction of 20 µL. In the PCR, 95 °C/3 min initial denaturation, 30 cycles of 95 °C/1 min, 45 °C/30 s, 72 °C/5 min reactions were applied and finalized with 72 °C/10 min final extension.

For the identification of the isolates, 1464 bp of the 16S rDNA gene was amplified by PCR using two different primer pairs (529F-1491R and 27F-780R). PCR mixture was prepared with 5 µL buffer, 2 µL dNTP mix (Fermentas, Massachusetts, USA), 1 µL each forward and reverse primer (529F 5'GTGC-CAGCMGCCGCGG 3'-1491R 5'ACGGCTACCTGT-TACGACTT 3' and 27F 5'AGAGTTTGATCCTGGCT-CAG 3'-780R 5'TACCAGGTATCTAATCCTGTT 3') 1 µL Hi-Fi Taq DNA polymerase (Fermentas, Massachusetts, USA) and 2 µL genomic DNA, and total volume was completed to 50 µL. An amplification program was applied including initial denaturation at 95 °C/5 min, 30 cycles of 95 °C /30 s denaturation, 57 °C/30 s annealing, 72 °C/1 min extension and 72 °C/5 min final extension in the PCR equipment (Techne, Staffordshire, UK). The 16S rDNA fragments were DNA sequenced which was subsequently searched for ultimate identification at BLAST (<https://blast.ncbi.nlm.nih.gov/>).

Effect of environmental factors on autolytic activity of LAB

To determine the autolytic activity of the LAB strains under different conditions such as pH, salt concentration, incubation time, temperature and the presence of sugar, each LAB strains, which were initially collected at 6000 g for 15 min after grown in MRS and M17G, were suspended to an OD value between 0.6-0.8 by using either potassium phosphate-buffered saline (PBS) (100 mM) with a pH adjusted by 0.1N HCl to 3.5, 4.0, 5.0 and 7.0 or PBS (100 mM, pH 7.0) containing 3.0, 5.0, 7.0 and 10.0 % NaCl. Autolytic activity was deter-

mined after incubation at 30 °C for 24 h. In another group, LAB isolates were suspended in PBS (100 mM, pH 7.0) to an OD value between 0.6-0.8 and kept at 10, 20, 30 and 40 °C followed by the determination of autolytic activity after 12 and 24 hours. To determine the effect of a carbon source used in growth medium on autolysis, LAB strains were first cultivated in modified MRS and M17 media containing 1 % either glucose, lactose, sucrose or maltose, then centrifuged and re-suspended in PBS buffer (100 mM, pH 7.0). Autolysis percentages were determined at 30 °C after 24 h. To determine the combined effect of the relevant factors on autolysis, cells were suspended in four different PBS buffers (100 mM) with a pH adjusted to 4 or 5 and salt concentration of 3 or 5 % to a final OD value between 0.6-0.8, and autolysis percentages were determined subsequently.

Determination of intracellular proteolytic activity of LAB after autolysis

The intracellular proteolytic activity of autolytic LAB was determined by screening the casein fragmentation by cellular enzymes released after autolysis. Accordingly, LAB cells were first adjusted to an OD value between 0.6-0.8 with PBS (100 mM, pH 7.0). Therefore, cells were allowed to autolyze at 30 °C for 24 h. Subsequently, cell debris was separated by centrifugation at 9000 g for 15 min, and supernatant was filtered through a 0.45 µm syringe filter. Casein mixture (Sigma, St. Louis, MO, USA, Product No: 22078) (0.1%) was added to each filtered supernatant, and aliquots were taken after 4, 24, 48 and 72 h.

The fragmentation of casein by the enzymes obtained after autolysis of the selected LAB strains was screened with the SDS-polyacrylamide gel electrophoresis (SDS-PAGE) performed on 8 and 12 % slab gel according to Schägger and von Jagow (1987). Samples were run at 75 V at stack gel and 100 V for separating gel until the marker staining reached the end of gel. Afterwards, gel was stained with the 0.23 % solution of Coomassie Blue R-250 for 90 min, and de-stained in the methanol/acetic acid solution (5 % methanol, 7 % acetic acid). De-stained gels were scanned using the Scanexpress 12000 (Mustac, Germany). Casein (Sigma, St. Louis, MO, USA, Product No: 22078) was run on gel after treated as above in only PBS as control to compare.

Statistical analysis

One-way ANOVA in the Minitab 14.0 package program (Minitab Inc., State College, PA, USA) were used to determine the effect of environmental factors on autolysis of LAB strains. Tukey's test was used as a post-ANOVA test to compare the significant differences among samples at $\alpha=0.05$ level.

Results and discussion

Autolytic behavior of LAB isolates in microflora of raw milk and white-brined cheese

In this study, 515 and 424 isolates were collected from 34 different raw milk and 16 cheeses, respectively, among the colonies grown on the MRS and M17G agars at thermophilic and mesophilic conditions. Subsequently, the autolysis rates of all isolates (a total of 939) were determined every 6 h during 24 h.

In terms of the distribution of autolysis rates of LAB isolates, 1.4 % of the mesophilic isolates (1M11, 12M15, 19M04 and 24M05) from raw milk showed an autolysis rate more than 50 % while 12.3 % of the isolates had an autolysis rate between 30 and 50 % after 24 h (Figure 1a). Additionally, 2.6 % of the thermophilic isolates (3T17, 5T08, 8T05, 11T13, 12T08 and 17T17) autolyzed more than 40 %, and 27.7 % of the isolates between 20 and 40 % (Figure 1b). The autolysis rate was higher than 30 % for 5.37 % of the cheese mesophilic isolates (36M14, 42M07, 42M11, 42M13, 42M15, 42M19, 44M03, 47M01, 47M07, 47M09, 49M03, 50M08 and 50M09), and 11.15 % of the thermophilic isolates autolyzed 20 to 30 % (Figure 1c). It was also found that 3.85 % (45T20, 46T02, 46T04, 46T06, 46T20, 50T07 and 50T09) of the thermophilic isolates had an autolysis rate more than 30 %, and 14.83 % of the thermophilic isolates autolyzed 20 to 30 % (Figure 1d).

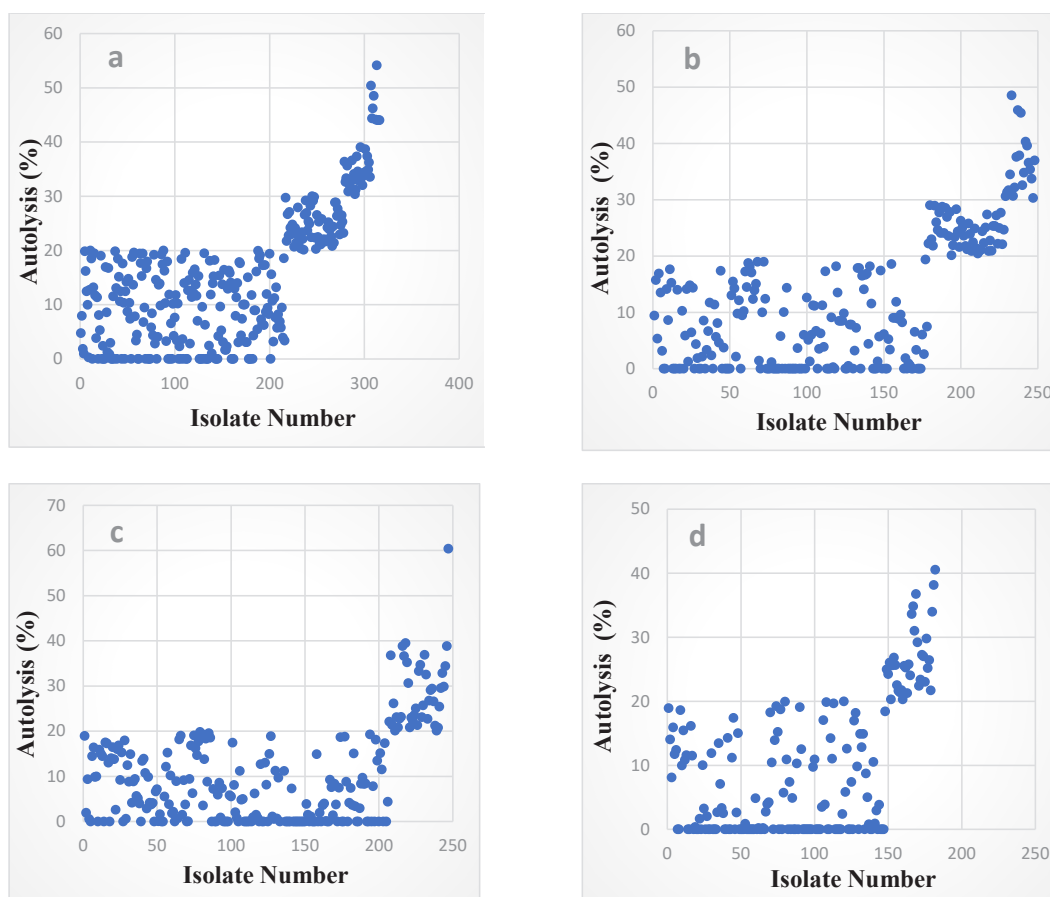


FIGURE 1. Distribution of autolysis rates as estimated at 30 °C or 43 °C after 24h incubation of cells in phosphate buffer (100 mM, pH=7.0) for (a) mesophilic and (b) thermophilic isolates from raw milk and (c) mesophilic and (d) thermophilic isolates from white-brined cheese samples

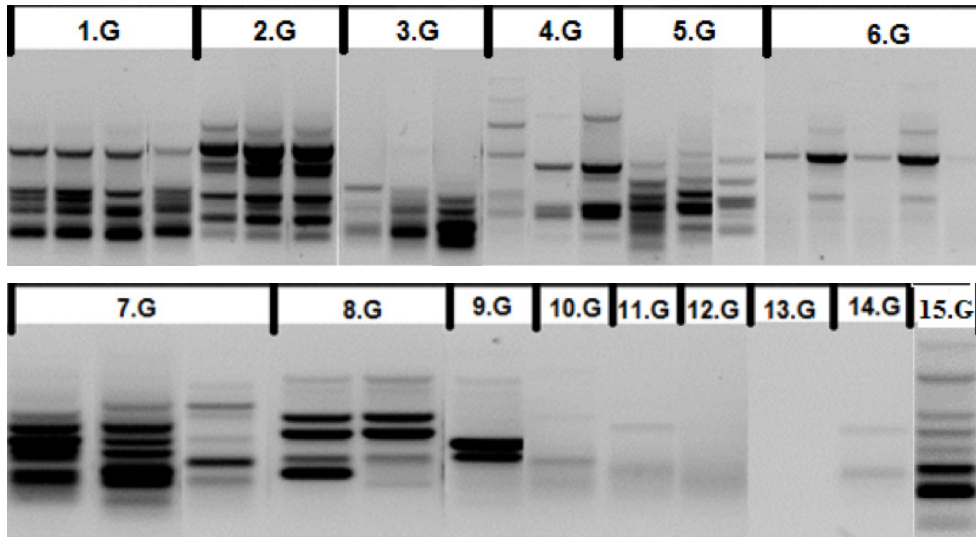


FIGURE 2. The (GTG)5 profile of isolates with high autolytic activity obtained from raw milk and white-brined cheese samples

TABLE 1. Autolysis rates as estimated at 30 °C (mesophilic, M) or 43°C (thermophilic, T) after 24h incubation of cells in phosphate buffer (100 mM, pH=7.0) and homological characteristics of identified LAB isolates with high autolytic activity

Source	Culture Code	Isolate Code*	Species**	Homology (%)	Autolysis Rate (%)***
Raw Milk	PFC229	01M09	<i>Lc. lactis</i> subsp. <i>lactis</i>	100	54.00
	PFC236	05T08	<i>Lb. helveticus</i>	100	48.85
	PFC235	03T17	<i>E. durans</i>	97	48.54
	PFC255	08M13	<i>Str. macedonicus</i>	98	44.10
	PFC234	10M19	<i>Leu. mesenteroides</i>	98	46.76
	PFC233	17M20	<i>Lc. lactis</i> subsp. <i>cremoris</i>	97	44.20
	PFC239	24M08	<i>Lc. lactis</i> subsp. <i>lactis</i>	100	30.09
	PFC232	24M05	<i>E. faecium</i>	98	53.71
	PFC247	24M07	<i>E. faecium</i>	99	39.45
	PFC254	19M02	<i>E. faecalis</i>	100	40.07
White-Brined Cheese	PFC240	42M07	<i>Lb. casei</i>	100	38.87
	PFC241	42M11	<i>Lb. casei</i>	98	36.62
	PFC230	42M13	<i>Lb. casei</i>	100	39.50
	PFC242	42M15	<i>Lb. casei</i>	99	35.22
	PFC243	42M19	<i>Lb. casei</i>	99	30.64
	PFC249	45T20	<i>E. faecium</i>	99	33.64
	PFC250	46T02	<i>E. faecium</i>	99	34.84
	PFC251	46T04	<i>E. faecium</i>	99	31.01
	PFC252	46T06	<i>E. faecium</i>	99	36.76
	PFC253	46T20	<i>E. faecium</i>	99	40.53
	PFC244	49M03	<i>Lb. casei</i>	96	32.87
	PFC245	47M01	<i>Lb. plantarum</i>	97	34.69
	PFC231	47M07	<i>Lb. plantarum</i>	97	36.88
	PFC246	47M09	<i>Lb. pentosus</i>	98	32.55
	PFC248	50M08	<i>E. faecium</i>	98	38.83
	PFC237	50T07	<i>P. acidilactici</i>	99	33.99
	PFC238	50T09	<i>Lb. rhamnosus</i>	99	38.18

* M and T indicate mesophilic and thermophilic, respectively.

**Lc = *Lactococcus*, Lb = *Lactobacillus*, E = *Enterococcus*, Str = *Streptococcus*, Leu = *Leuconostoc*, P = *Pediococcus*

***rates in phosphate buffer (100 mM) at pH 7.0

Autolytic LAB diversity of raw milk and white-brined cheese microflora

A total of 33 LAB isolates selected from raw milk and cheese samples, which had autolysis rates more than 30 %, were ultimately identified. These isolates were clustered into 15 groups according to (GTG)₅ profiles (Figure 2). Subsequently, two distinct regions of the 16S rRNA gene overlapping each other were amplified for final identification of the selected 33 LAB isolates and therefore, DNA sequence of 1464 bp of 16S rDNA gene was taken into consideration. Accordingly, 27 isolates with

high autolytic activity were 97 % homolog with one LAB species (Table 1); however, remaining 6 isolates were not identified as LAB species. Results indicated that *Lc. lactis* and *E. faecium* were abundance among the autolytic isolates in raw milk while *E. faecium* and *Lb. casei* were in white-brined cheese. The identification of *E. faecium* as high autolytic activity in both raw milk and white-brined cheese was in good agreement with microflora studies previously on these food samples. This is especially the case for *Enterococcus* spp., which can tolerate high salt concentrations (Amaral et al., 2017; Karabey et al., 2018). Recent studies have

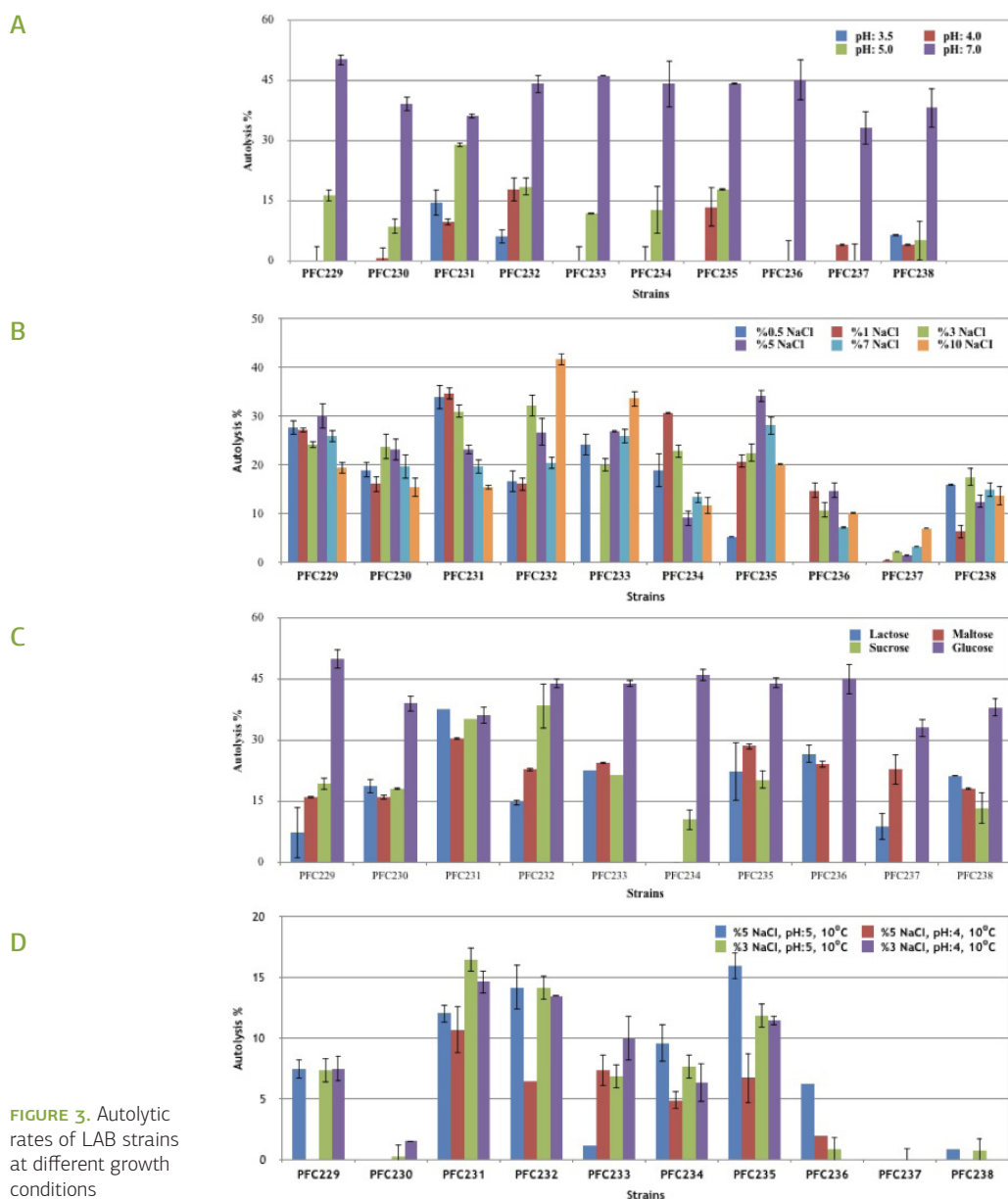


FIGURE 3. Autolytic rates of LAB strains at different growth conditions

revealed that *E. faecium* has a potential as starter or adjunct culture in production of different cheese types (Ogier and Serror, 2008; Aspri et al., 2017). On the other hand, *Lb. casei*, *Lb. plantarum* and *Lc. lactis* subsp. *lactis* strains have been reported as

predominant in terms of autolytic activity in many studies (Dako et al., 1995; Boutrou et al., 1998; Cibik and Chapot-Chartier, 2004; Nunez et al., 2011; Piraino et al., 2008; Zuo et al., 2014).

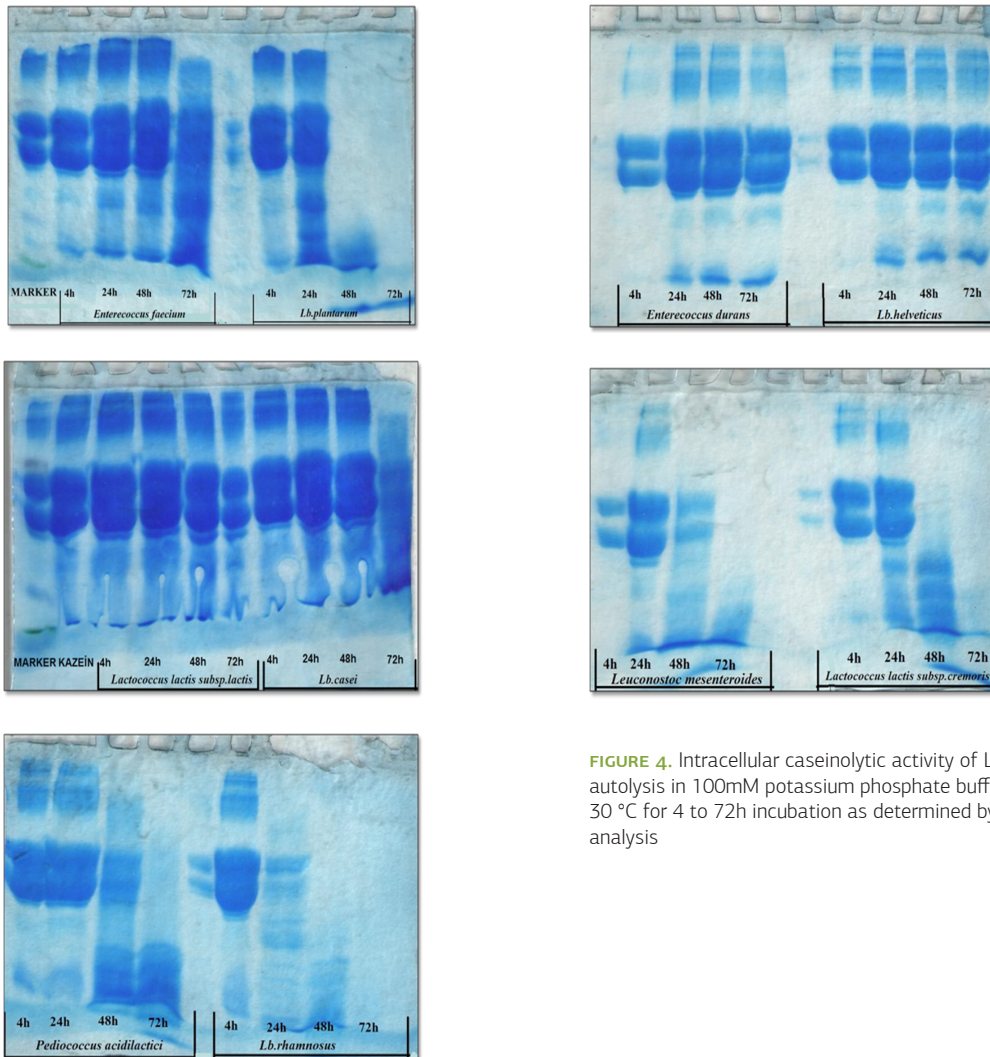


FIGURE 4. Intracellular caseinolytic activity of LAB strains after autolysis in 100mM potassium phosphate buffer (pH=7.0) at 30 °C for 4 to 72h incubation as determined by SDS-PAGE analysis

Autolytic behavior of LAB strains under different conditions

The effect of different pH values, salt concentrations and carbon sources on the autolytic behavior of 10 LAB strains was determined under conditions similar to the production of white-brined cheese. The autolysis rates of LAB strains decreased by a reduction in buffer pH as shown in Fig. 3a ($p < 0.05$). The LAB strains except *E. faeci-*

um PFC232, *E. durans* PFC235 and *Lb. rhamnosus* PFC238 did not autolyze at pH values of 3.5 and 4. Significant reduction in the autolysis rates of LAB isolates associated with a pH drop might be related with the optimum pH required for the activity of peptidoglycan hydrolase (Chapot-Chartier and Kulakauskas, 2014). Similar results were reported by Ostlie et al. (1995), but Huard et al. (2004) and Nunez et al. (2011) reported that the autolysis rates increased with a decrease in pH.

Among LAB strains, *Lb. plantarum* PFC231, *E. faecium* PFC232, *E. durans* PFC235 and *Lc. lactis* subsp. *lactis* PFC229 were distinctive because of their high autolytic activity around pH 5.0, which was similar to that of white-brined cheese. The effect of NaCl concentration on autolysis behavior of LAB strains was insignificant ($p > 0.05$). The maximum autolysis for each strain was found at a different NaCl concentration. Among all strains, the highest autolysis rate was determined in *E. faecium* PFC232 with 41 % at a NaCl concentration of 10 % (Fig. 3b). On the other hand, the autolysis rates of *Lc. lactis* subsp. *lactis* PFC229, *Lb. casei* PFC230 and *Lb. plantarum* PFC231 were not influenced by NaCl concentration ($p > 0.05$). Brine concentration of white-brined cheeses varies from 3 to 15 %, and autolytic characteristics of *Lb. plantarum* PFC231 (30-33 %) and *Lc. lactis* subsp. *lactis* PFC229 (24-29 %) strains could be significant for the production of this type of cheeses. The autolytic activity of these strains was not influenced by NaCl concentration.

The highest autolytic activity was determined in growth medium with glucose, except for *Lb. plantarum* PFC231 ($p < 0.05$) which was the highest (37 %) in the presence of lactose (Fig. 3c). Insignificant differences in autolysis rates were found for LAB strains in growth media containing sugars other than glucose. Vegarud et al. (1983) noted that the use of glucose instead of lactose as a carbon source in growth medium promotes the development of autolysis. On the other hand, high autolytic activity of *Lb. plantarum* PFC231 in the presence of lactose may have a technological importance for white-brined cheese production in which lactose is the main carbon source for such strains.

Since starter cultures used in white-brined cheeses may encounter with low pH, high salt concentration and cold conditions during ripening, the simultaneous effect of these factors on the autolysis of LAB strains was also studied. High autolysis rates in 6 of 10 LAB strains were observed in medium containing 5 % NaCl at pH 5. In 3 of these ten strains, the highest autolysis rate was found in medium with 3 % NaCl at pH 4 (Fig. 3d). *Lb. plantarum* PFC231 strain had the highest autolysis rate (16.48 %) at pH 5 and 3 % NaCl among all experimental groups. In fact, the autolysis rate of the same strain in PBS buffer was higher than this rate. These results showed that the combined effect of

pH, temperature and NaCl may reduce the rate of autolysis of LAB strains. Nunez et al. (2011) reported that the pH of medium was more effective than NaCl concentration to the extent of the autolysis of LAB strains in such a simultaneous model system. On the other hand, *E. durans* PFC235 and *E. faecium* PFC232 strains as well as *Lb. plantarum* PFC231 may contribute to cheese ripening through autolysis.

Intracellular enzymes of starters used as an adjunct culture are expected to exhibit proteolytic activity as well as high autolytic character for successful ripening of white-brined cheeses. With respect to that, the ability of intracellular proteolytic activity on casein fragmentation was studied after the autolysis of LAB cells. *E. faecium* PFC232 cell lysate disrupted casein at the end of 24 h and eventually formed low molecular weight casein fragments in 72 h. The *Lb. plantarum* PFC231 cell lysate disrupted casein after 24 h while casein bands disappeared after 48 h (Fig. 4). The cell lysates of *E. durans* PFC235, *Lc. lactis* subsp. *lactis* PFC229 and *Lb. helveticus* PFC236 strains had no proteolytic activity on casein. *Leu. mesenteroides* PFC234 and *P. acidilactici* PFC237 showed high proteolytic activity after 48 h, and there were not any casein fragments after 72 h. *Lb. casei* PFC230 cell lysate was able to disrupt casein in 72 h. The proteolytic activities of cell lysates of the *Lc. lactis* subsp. *cremoris* PFC233 and *Lb. rhamnosus* PFC238 were first detected at 24 h, and low molecular weight casein fragments remained after 48 h.

The intracellular proteolytic activity of *Lb. plantarum* PFC231 strain was high due to the complete degradation of casein at the end of 48 h. Additionally, *Leu. mesenteroides* PFC234, *Lc. lactis* subsp. *cremoris* PFC233, *P. acidilactici* PFC237 and *Lb. rhamnosus* PFC238 also showed high caseinolytic activity (Fig. 4). These results indicated that these LAB strains, especially *Lb. plantarum* PFC231, may degrade casein and thus contribute to cheese ripening by their intracellular proteolytic enzymes after autolysis. In fact, peptidases released as a result of autolysis of cells and existed in cheese matrices have been previously reported to have the ability to degrade proteins into peptides and amino acids, which may have a significant role in the development of cheese aroma (Boutrou et al., 1998; Sheehan et al., 2005; Hannon et al. 2007).

Conclusions

In this study, the isolation, identification and characterization of autolytic LAB was examined for the first time on the basis of technological aspects of white-brined cheese production for accelerated ripening. Raw milk was an important source for more autolytic LAB isolates than cheese samples, and the abundant autolytic strains were *E. faecium* and *Lc. lactis* in the microflora of raw milk and cheese samples (Fig. 1, Table 1). Furthermore, the autolytic behavior of LAB strains decreased at low pH values. Although the overall effect of salt concentration on the autolytic activity of LAB strains was insignificant, each strain exhibited a maximum autolytic rate at different salt concentration. The use of glucose as a carbon source increased the autolytic rates of LAB strains significantly; however, the highest activity was found for *Lb. plantarum* PFC231 strain in the presence of lactose. The proteolytic activity of the intracellular content of LAB strains should be also considered besides their high autolytic activity. Results indicated that for the autolytic behavior at low temperature, pH and high

salt concentrations, which are influential factors for cheese ripening, *Lb. plantarum* PFC231 has the potential to be used as an adjunct starter culture in the production of white-brined cheeses. Further studies are needed to test the actual potential of the LAB strains with high autolytic and intracellular proteolytic activity in white-brined cheese production. Moreover, the intracellular proteolytic activity of the selected LAB strains with high autolytic activity should be further studied individual casein fractions in details.

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Odabir autolitičkih bakterija mliječne kiseline kao mogućih starter kultura za ubrzavanje zrenja sireva u salamuri

Sažetak

Cilj ovog rada bio je odabrati bakterije mliječne kiseline (LAB) koje imaju autolitičku aktivnost, kako bi se upotrebljavale za ubrzavanje procesa zrenja sireva u salamuri. U tu svrhu je iz 34 uzorka sirovog mlijeka te 16 uzoraka sira izolirano 526 mezofilnih i 413 termofilnih sojeva LAB. Primjenom 16SrDNA analize sekvenci i (GTG)₅ metode otiska prsta izolirano je ukupno 27 sojeva s autolitičkom aktivnošću koja se kretala između 34 i 54 %. Pritom su se najdominantnijim autolitičkim vrstama u sirovom mlijeku pokazali *Lactococcus lactis* i *Enterococcus faecium*, dok su u siru prevladavali *Enterococcus faecium* i *Lactobacillus casei*. Stupanj autolize sojeva LAB rastao je s porastom temperature i pH, dok je opadao pri povišenim koncentracijama NaCl. Nadalje, kod svih sojeva je najveća autoliza zabilježena uz prisutnost glukoze. Međutim, soj *Lb. plantarum* PFC231 ovdje se izdvajao, pokazujući najveću autolizu u prisutnosti laktoze. Sojevi *E. durans* PFC235, *E. faecium* PFC232 i *Lb. plantarum* PFC231 su pri izloženosti niskom pH i visokoj koncentraciji NaCl pri 10 °C pokazivali viši stupanj autolize u odnosu na ostale sojeve. Međutim, samo su sojevi *Lb. plantarum* PFC231, *Leuconostoc mesenteroides* PFC234, *Lc. lactis* subsp. *cremoris* PFC233, *Pediococcus acidilactici* PFC237 i *Lb. rhamnosus* PFC238 pokazali sposobnost unutarstanične hidrolize kazeina. Zaključno, soj *Lb. plantarum* PFC231 jedini je pokazao potencijal za upotrebu kao starter kultura u proizvodnji sireva u salamuri, budući da ima autolitičku aktivnost pri niskim temperaturama i pH vrijednosti, te visokoj koncentraciji soli, a pokazao je i sposobnost unutarstanične hidrolize kazeina.

Ključne riječi: bakterije mliječne kiseline, *Lactobacillus plantarum*, autoliza, sir u salamuri, zrenje sira

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