Effect of some environmental factors on autolysis of lactococci used for cheese production

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Summary

About 300 strains were isolated from different sources and phenotypically identified. Twenty-three of them, identified as *Lactococcus lactis,* were subjected to genotypic characterization and tested for autolytic activity. These strains were subdivided by the plasmide profile technique into 4 clusters, each containing the isolates that shared the same plasmid pattern. Three strains showed completely different plasmid profiles. Three highly autolytic strains were found among 23 isolates. One of them, *Lactococcus lactis* HMM81, which exhibited the highest autolytic activity in citrate buffer (approximately 48%), was chosen for further investigation of factors influencing the autolysis. These comprised extrinsic factors (incubation temperature) and intrinsic factors (NaCl concentration, pH of the medium, citrate buffer concentration, growth phase). As a reference strain, a non–autolytic strain *Lactococcus lactis* subsp. *lactis* NIZO B643 was used. Based on the investigation of the extrisic and intrinsic factors, the most suitable conditions for fast prediction of autolytic activity of a strain were suggested: pre-incubation for 6 h at 30 °C, re-suspendation of cells in 50 mmol·l^{-1} sodium citrate buffer (pH 5.0, containing 15 g·l^{-1} NaCl) and cultivation at 30 °C for 6 days.

Keywords

Lactococcus, autolysis, prediction, citrate buffer

Strains of *Lactococcus* sp. are used worldwide for the production of fermented dairy products. Important characteristics of starter cultures related to cheese making are acidification activity, proteolytic activity and flavour production. Proteolysis is one of the most important attributes of cheese making. It has crucial importance during cheese ripening because it contributes to texture and flavour development. An important factor controlling cheese ripening and flavour development is the rate of starter autolysis, due to the release of intracellular enzymes into the curd and their activity in flavour development [1–4]. During last 30 years, many authors have studied the relationship between autolysis and proteolysis in Cheddar cheeses [5–10]. A theory was formulated that starter lysis is associated with an increased rate of formation of small peptides and free amino acids in the cheese matrix. HANNON et al. [8] reported that starter autolysis accelerated the development of cheese flavour and improved its organoleptic characteristics. The extent of starter lysis also influences the level of bitterness in cheese. CROW et al. [6] found that bitterness was not detected in the cheese where lysis and associated amino acid production were the greatest. This can be viewed as a combination of the effect of the removal of bitter peptides and the possible flavour-masking of any remaining bitter peptides by the base flavour of amino acids [6]. Recently, BOURDAT-DESCHAMPS et al. [9] reported that starter lysis by a highly autolytic strain *L. lactis* subsp. *cremoris* AM2 also clearly stimulated transamination of aromatic amino acids, which was the first step of the conversion of these amino acids to aroma compounds. Stimulation of transamination reactions was probably due to a better accessibility of the aminotransferases resulting from cell wall damage by autolysis [9].

The rate and extent of autolysis and concomitant enzyme released are essentially uncontrolled. Individual starter strains lyse at different rates beyond the influence of the cheese manufacturer. Since the ripening of cheese can be both a slow and a costly process, controlling the rate and extent of

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lysis would be beneficial from the point of view of cheese manufacturers [11]. Several approaches have been proposed to accelerate cheese ripening by using genetically modified starters or selection of naturally highly autolytic strains. However, this approach may be limited by legal barriers since genetically modified starters are not allowed in all countries. There is also a widespread lack of their acceptance by consumers [12]. Thus, selection of natural highly autolytic lactococcal strains from different sources has recently been suggested and the activity of peptidoglycan hydrolases has been evaluated [13, 14].

The aim of the presented paper was to genotypically and phenotypically screen the collection of lactococci of different origin, to compare their autolytic activity, to choose the most active strain, to evaluate the effect of different factors of important for cheese production and ripening and to find a method for fast and simple screening of autolytic activity of lactococcal strains intended to be used as starters or adjunct cultures for cheese production.

MATERIALS AND METHODS

Baterial strains

Lactococci used for the investigation were isolated from different sources (Tab. 1). As a control non-autolytic strain, *Lactococcus lactis* subsp. *lactis* NIZO B643 (National Collection of Dairy Organism, Reading, United Kingdom) was used.

Determination of growth at 30 °C and 45 °C

The strains tested were inoculated on the surface of M17 agar medium (Oxoid, Basingstoke, United Kingdom) with lactose (Pliva – Lachema,

Tab. 1. Identification, plasmid profile and phenotypic characteristics of *Lactococcus* sp. isolates.

Strain	Subspecies	Plasmid profile group	Phenotypic characteristics					
			Growth in LM17 at 30 °C	Growth in LM17 at 45 °C	Growth in LM17 at 30 °C + 4% (w/w) NaCl	Arginine hydrolysis	Citrate fermentation	Autolytic activity
Strains from raw cow mastitis milk								
HMM21	lactis	1	+	–	+	+	–	M
HMM22	lactis	4	+	–	+	+	–	H
HMM31	lactis	2	+	–	+	+	–	VH
HMM32	cremoris	2	+	–	+	–	–	H
HMM4	lactis	2	+	–	+	+	–	H
HMM5	lactis	2	+	–	+	+	–	M
HMM51	lactis	1	+	–	+	+	–	L
HMM52	lactis	1	+	–	+	+	–	M
HMM61	cremoris	1	+	–	–	–	–	H
HMM62	cremoris	1	+	–	–	–	–	M
HMM72	cremoris	1	+	–	–	–	–	L
HMM81	cremoris	U	+	–	–	–	–	VH
HMM82	lactis	3	+	–	–	+	–	VH
HMM91	cremoris	4	+	–	–	–	–	M
HMM92	lactis	3	+	–	+	+	–	M
Strain from cow udder								
NZS1	*lactis*	4	+	–	–	+	–	M
Strains from Tome Fermier cheese (French cheese made from raw cow milk)								
S12A1	*lactis*	4	+	–	+	+	–	M
S12A2	*lactis*	4	+	–	+	+	–	M
S12B1	*lactis*	4	+	–	+	+	–	M
S12B2	*lactis*	4	+	–	+	+	–	H
S12C2	*lactis*	U	+	–	–	+	–	L
S12E1	*lactis*	4	+	–	+	+	–	M
Strain from Grande Ribeaupierre cheese (French cheese made from raw cow milk)								
S32	*lactis*	U	+	–	+	+	–	L

L – low autolytic activity (< 10% cell lysis), M – medium autolytic activity (10–20% cell lysis), H – high autolytic activity (20–30% cell lysis), VH – very high autolytic activity (> 30% cell lysis), U – unique plasmid profile, (+) – positive, (–) – negative.

Brno, Czech Republic; 0.5% w/w; LM17 agar) and incubated at 30 °C or 45 °C for 72 h. If a significant growth was observed, the test was considered positive. If the growth of the strain tested was weak or absent, the test was considered negative [15].

Salt tolerance test

The strains tested were inoculated on the surface of LM17 agar medium with 4% (w/w) NaCl (Pliva – Lachema). The plates were incubated at 30 °C for 72 h. If the significant growth of strain tested was observed, the test was considered positive. If the growth of the strain tested was weak or absent, the test was considered negative [15].

Determination of citrate fermentation

The determination of citrate fermentation ability was done according to the method of REDDY et al. [16].

Determination of arginine hydrolysis using TTC agar medium

Arginine agar was prepared according to TURNER et al. [17]. The medium was poured into Petri dishes and dried in the dark at 35 °C for 24 h. Cultures of all strains tested were diluted to 10^{-7} and aliquots of 0.1 ml were spread on the surface of the plates, using sterile glass spreaders. Plates were incubated in the atmosphere of approx. 100% relative humidity at 30 °C and examined at 24 h and 48 h intervals. Colonies of *Lactococcus lactis* subsp. *lactis* developing on media containing 2,3,5-trifenyltetrazolium chloride (TTC; Sigma, St. Louis, Missouri, USA) were bright-red and colonies of *Lactococcus lactis* subsp. *cremoris* were white [17].

Plasmid profiles detection

Plasmid DNA was extracted using QiaPrep Spin Kit (Qiagen, Hilden, Germany). Gel electrophoresis was carried out in 0.8% (w/w) agarose (Sigma) gel in TBE buffer (Sigma) at a constant voltage of 80 V.

Determination of autolytic activity

Autolytic activity of lactococci strains was determined in a buffer system using the method of BOUTROU et al. [18] with the following modification: All strains were inoculated (1%) into LM17 broth (50 ml) and incubated at 30 °C for 6 h. Bacterial cells were harvested by centrifugation at 8000 *g* for 15 min at 4 °C. The pellets were washed twice with 40 ml of 50 mmol·l^{-1} sodium β-glycerophosphate buffer (sodium β-glycerophosphate, Sigma) at pH 7.0. The washed pellets were resuspended in 40 ml of 50 mmol·l^{-1} sodium citrate buffer (sodium citrate, Pliva – Lachema), pH 5.0, containing 15 g·l^{-1} NaCl and incubated at 13 °C for 12 days. Cell lysis was evaluated by absorbance measurement of the suspension at 650 nm with a spectrophotometer (Unicam Helios Gamma & Delta, Thermo Spectronic, Cambridge, United Kingdom). Cell autolysis was characterized by percentage of cell lysis, which was defined as follows:

$$(A_0 - A_t) \times 100/A_0. \quad (1)$$

where A_0 is initial absorbance and A_t is absorbance measured after 12 days of incubation [18].

Determination of autolytic activity under different conditions

The autolytic activity of the strains tested in citrate buffer was determined under different conditions (Tab. 2) using the method of BOUTROU et al. [18] with the following modification: All strains were inoculated (1%) into LM17 broth (50 ml) and incubated at 30 °C for 6 h. Bacterial cells were harvested by centrifugation at 8000 *g* for 15 min at 4 °C. The pellets were washed twice with 40 ml of 50 mmol·l^{-1} sodium β-glycerophosphate buffer at pH 7.0. The washed pellets were resuspended in 40 ml of sodium citrate buffer (concentrations: 10; 50; 100; 200; 1000 mmol·l^{-1}), pH values 4.0; 5.0; 6.0; 6.5; 7.0; 8.0 containing different NaCl amounts (0.0; 2.0; 6.5; 10.0; 15.0 g·l^{-1}) and were incubated at different temperatures (13; 30; 45; 56 °C) for 12 days (Tab. 2). Cell lysis was evaluated

Tab. 2. Conditions used for determination of the autolytic activity.

Temperature [°C]	pH of citrate buffer	NaCl concentration [g·l^{-1}]	Citrate buffer concentration [mmol·l^{-1}]
13	5.0	15.0	50
30	5.0	15.0	50
45	5.0	15.0	50
56	5.0	15.0	50
13	4.0	15.0	50
13	6.0	15.0	50
13	6.5	15.0	50
13	7.0	15.0	50
13	8.0	15.0	50
13	5.0	2.0	50
13	5.0	6.5	50
13	5.0	10.0	50
13	5.0	15.0	10
13	5.0	15.0	100
13	5.0	15.0	200
13	5.0	15.0	1000

by absorbance measurement of the suspension at 650 nm. Cell autolysis was characterized by percentage of cell lysis according to eq. 1.

RESULTS AND DISCUSSION

About 300 strains were isolated from different sources (cheeses, raw milk, raw mastitis milk) and phenotypically identified. A number of 23 of them, phenotypically identified as *Lactococcus lactis,* were subjected to further investigation: genotypic characterization and determination of autolytic activity.

Phenotypic characterization

The main phenotypic characteristics of the isolates are summarized in Tab. 1. All the isolates tested grew well at 30 °C, none of them grew at 45 °C. Six strains were unable to produce ammonia from arginine on the arginine medium with TTC indicator and eight strains did not grow at 30 °C in the presence of 4% (w/w) NaCl. All these strains could therefore belong to the *cremoris* subspecies. None of the isolates was able to hydrolyse citrate on the citrate medium. Thus it was assumed that no *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* was presented among the strains isolated.

Plasmid profile analysis

Lactococcus lactis plasmids range in size from less than 2 kb to greater than 100 kb and are present in numbers from 1 to 12 per cell. Most strains contain 4–8 plasmids. Many of the functions encoded on plasmids are related to the growth of lactococci in milk [19].

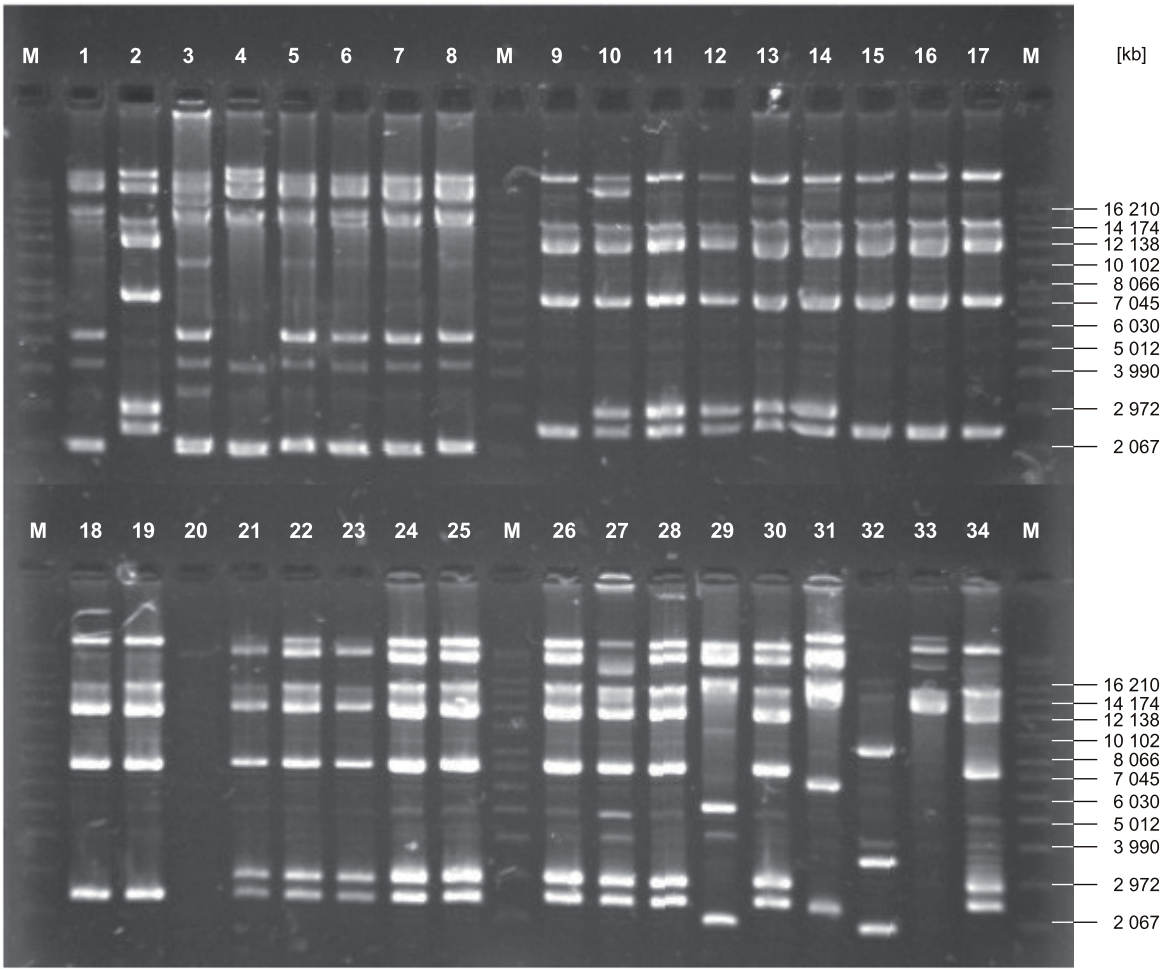

Fig. 1. Plasmid profiles of lactococci isolates.
Track M – DNA Ladder, Supercoiled, 2-16 kb (SIGMA); tracks 1–8, 32–34 – commercial cultures; tracks 9–31: 9 – *L. lactis* HMM 21, 10 – *L. lactis* HMM 22, 11 – *L. lactis* HMM 31, 12 – *L. lactis* HMM 32, 13 – *L. lactis* HMM 4, 14 – *L. lactis* HMM 5, 15 – *L. lactis* HMM 51, 16 – *L. lactis* HMM 52, 17 – *L. lactis* HMM 61, 18 – *L. lactis* HMM 62, 19 – *L. lactis* HMM 72, 20 – *L. lactis* HMM 81, 21 – *L. lactis* HMM 82, 22 – *L. lactis* HMM 91, 23 – *L. lactis* HMM 92, 24 – *L. lactis* NZS 1, 25 – *L. lactis* S12A1, 26 – *L. lactis* S12A2, 27 – *L. lactis* S12B1, 28 – *L. lactis* S12B2, 29 – *L. lactis* S12C2, 30 – *L. lactis* S12E1, 31 – *L. lactis* S32.

All our strains harboured plasmids in numbers and masses typical for lactococci [19–20]. The 23 isolates were subdivided according to the plasmid profile into 4 groups, each containing the isolates that shared the same plasmid pattern. The small differences in each cluster, evident in particular in cluster 4, were probably caused by unavoidable mechanical stress to which plasmid DNA was exposed during preparation.

The isolates belonging to clusters 1 and 2 displayed very similar plasmid profiles, differing only in the presence of one 2972 kb plasmid (Fig. 1) and suggesting that they could be derivates of the same parental strain [20]. The same could be said about clusters 2 and 4. They differed only in the presence of a plasmid greater than 16210 kb. The isolates belonging to the third cluster were found to contain three large plasmids and two small plasmids, of about 2972 kb (Fig. 1). Three strains isolated showed completely different plasmid profiles – *Lactococcus lactis* HMM81, *Lactococcus lactis* S12C2, *Lactococcus lactis* S32 (Fig. 1). These strains were not classified into any group.

Autolytic activity

In order to evaluate lactococcal lysis, a simple test under conditions similar to those used to make semi-hard cheese, was used. This method was based on the method of BOUTROU et al. [18], but the cultivation time was shortened from 30 to 12 days. Because of the very fast lysis of highly autolytic strains, 12 days were found sufficient to predict the lytic capacity.

Only three strains exhibited a very high autolytic activity in the citrate buffer (pH 5, cultivation at 13 °C; Tab. 1): *Lactococcus lactis* HMM31, *Lactococcus lactis* HMM 81 and *Lactococcus lactis* HMM82, but the highest autolytic activity was exhibited by *Lactococcus lactis* HMM81. *Lactococcus lactis* HMM81 (HMM81) showed approximately 48% of cell lysis after incubation at 13 °C for 12 days (Fig. 2). Moreover, the lytic capacity of HMM81 was higher in comparison to *Lactococcus lactis* subsp. *cremoris* AM2, the reference strain whose lytic activity was previously described (*Lactococcus lactis* subsp. *cremoris* AM2 showed approximately 32% of cell lysis during incubation at 13 °C for 12 days [18]). In accordance with these results, the strain HMM 81 was chosen for the investigation of the impact of some extrinsic and intrinsic factors [21] on the autolysis.

Determination of autolytic activity under different conditions

Autolysis of lactococcal strains is a complex biochemical process affected by different factors.

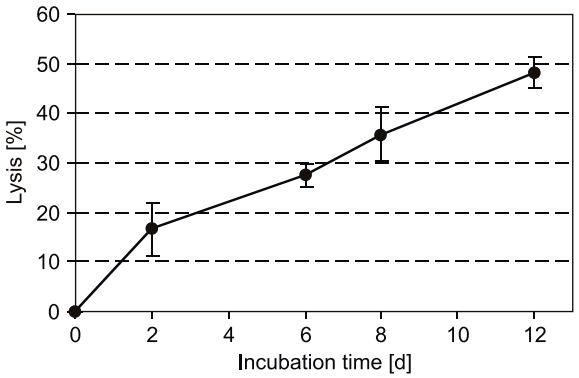

Fig. 2. Cell lysis of *Lactococcus lactis* HMM81 in the citrate buffer system (50 mmol·l^{-1}; pH 5.0; 15 g·l^{-1} NaCl) during 12 days of incubation at 13 °C.
The error bars represent a 95% confidence interval.

The effect of different conditions on autolytic activity of lactococal strains was described for the first time by MOU et al. [22]. This study was concentrated on studying the effect of the growth phase, effect of pH of the medium, and the effect of incubation temperature [22]. Subsequently, WILKINSON et al. [23] investigated some factors (salt-in-moisture levels in the cheese, temperature of cheese ripening and the concentration in salt in the cheese matrix) that may affect autolysis in the cheese during ripening. In this context, the present study was directed at determination of the citrate buffer concentration, growth phase of the strain, NaCl concentration, pH of the medium, incubation temperature and their effects on the autolysis of two lactococci strains of different autolytic activity level, *Lactococcus lactis* subsp. *lactis* HMM81 (a) and *Lactococcus lactis* subsp. *lactis* NIZO B643 (B643) (b) (non–autolytic strain).

The effect of the citrate buffer concentration on the autolytic activity of strains HMM81 and B643 is shown in Fig. 3. Within the range of concentrations tested (0.01–1.0 mol·l^{-1}), the maximal cell lysis of HMM81 (approximately 70% of cell lysis after 12 days incubation) was detected in the buffer with a concentration of sodium citrate of 0.2 mol·l^{-1}. A high extent of cell lysis of HMM81 (approximately 50% of cell lysis after 12 days incubation) was also detected in a buffer with sodium citrate concentration of 0.05 mol·l^{-1}. However, in the citrate buffer with a concentration of 0.2 mol·l^{-1}, a high cell lysis of the non-autolytic strain B643 was also observed. In contrast, citrate buffer with a concentration of 0.05 mol·l^{-1} did not cause lysis of the non-autolytic strain B643. This sodium citrate concentration, 0.05 mol·l^{-1}, was therefore taken as suitable for prediction of autolytic activity in lactococci strains.

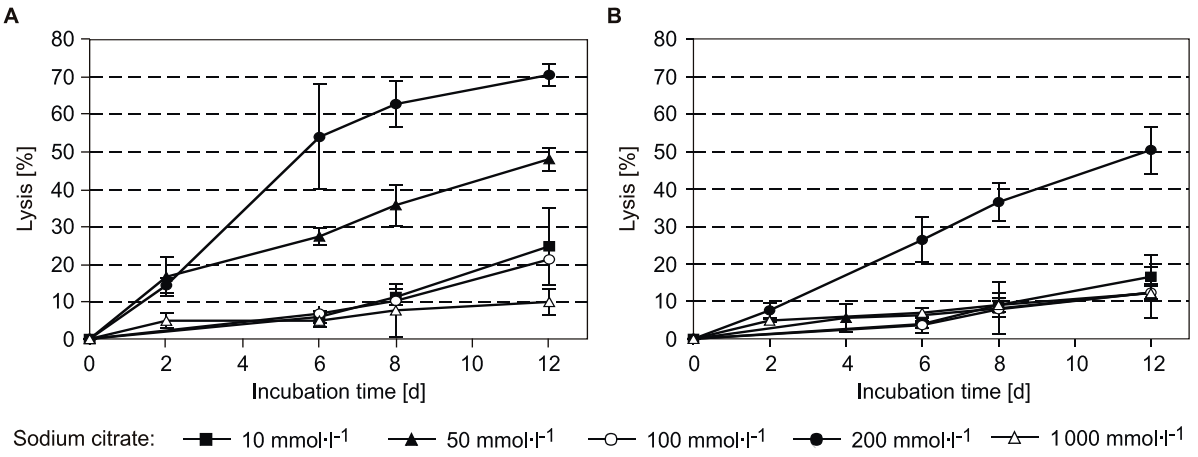

Fig. 3. Effect of sodium citrate concentration on autolytic activity of *Lactococcus lactis* HMM81 (A) and *Lactococcus lactis* subsp. *lactis* NIZO B643 (B).
Lactococcus lactis HMM81 and *Lactococcus lactis* subsp. *lactis* NIZO B643 were grown in the LM17 broth for 6 h at 30 °C, suspended in the sodium citrate buffer of various concentrations (0.01, 0.05, 0.1, 0.2, 1.0 mol·l^{-1}) containing 15 g·l^{-1} NaCl, pH 5 and incubated for 12 days at 13 °C. The error bars represent a 95% confidence interval.

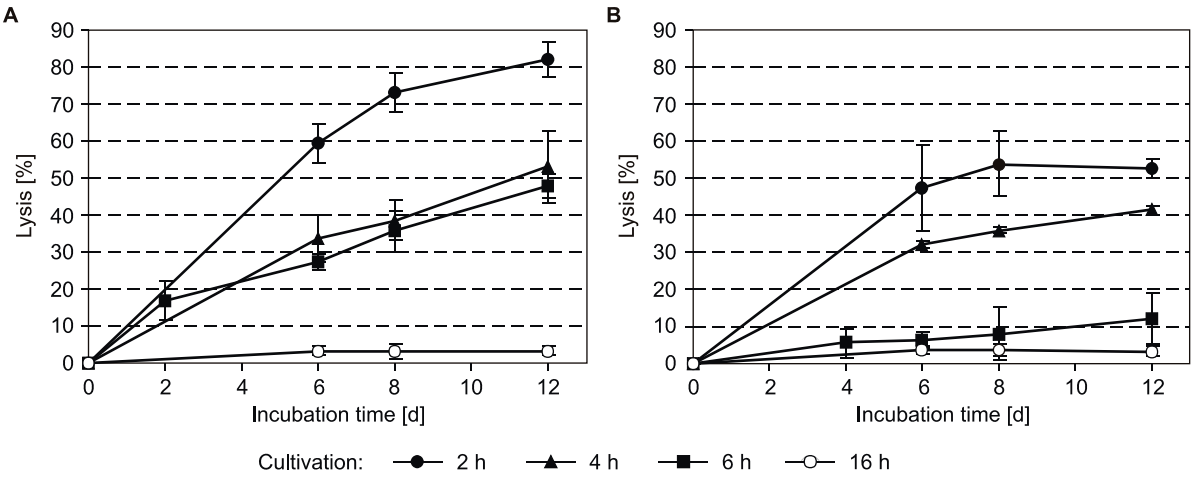

Fig. 4. Effect of the growth phase on autolytic activity of *Lactococcus lactis* HMM81 (A) and *Lactococcus lactis* subsp. *lactis* NIZO B643 (B).
Lactococcus lactis HMM81 and *Lactococcus lactis* subsp. *lactis* NIZO B643 were grown in LM17 broth at 30 °C. At various times (2, 4, 6, and 16 h) samples were withdrawn, harvested, washed, suspended in 0.05 mol·l^{-1} sodium citrate buffer containing 15 g·l^{-1} NaCl, pH 5 and incubated for 12 days at 13 °C. The error bars represent a 95% confidence interval.

The effect of growth phase on autolysis was determined by harvesting of bacterial cells from LM17 broth after 2, 4, 6 and 16 h and detecting the autolytic activity in a sodium citrate buffer (Tab. 2). The autolytic activity of HMM81 was highest after 2 h of cultivation (reaching approximately 80% of cell lysis after 12 days of incubation at 13 °C). A high autolytic activity was detected after 4 h and 6 h (exponential phase of growth of HMM81) of cultivation as well, reaching approximately 55% of cell lysis after 12 days of incubation at 13 °C. A low autolytic activity was detected after transition of HMM81 culture to the stationary phase of growth (16 h of cultivation). A high autolytic activity of the non-autolytic strain B643 was detected after 2 h and 4 h of cultivation. Based on these results it can be concluded that exponential phase of growth (6 h in case of HMM81) is the most suitable growth phase for prediction of autolytic activity in lactococci strains (Fig. 4). These results are in agreement with Mou et al. [22] and Boutrou et al. [18].

The effect of NaCl concentration on the autolytic activity of HMM81 and B643 is shown

in Fig. 5. The NaCl concentration range of 0–15 g·l^{-1} was tested. A high autolytic activity of HMM81 was shown in the buffer system containing 6.5 g·l^{-1} NaCl (reaching approximately 60% of cell lysis after 12 days of incubation at 13 °C) and 15.0 g·l^{-1} NaCl (reaching approximately 50% of cell lysis after 12 days of incubation at 13 °C). With the non-autolytic strain B643, a high lytic activity was detected in the buffer system containing 6.5 g·l^{-1} and 10.0 g·l^{-1} NaCl. The autolytic activity of the non-autolytic strain was weak in the buffer system containing 15.0 g·l^{-1} NaCl. Based on these results it can be concluded that the buffer system containing 15.0 g·l^{-1} NaCl is the most suitable for prediction of autolytic activity in lactococci strains.

The autolytic activity of HMM81 and B643 at various pH values is shown in Fig. 6. A high autolytic activity of HMM81 was detected at pH 5.0 (reaching approximately 50% of cell lysis after 12 days of incubation at 13 °C) and pH 6.0 (reaching approximately 72% of cell lysis after 12 days of incubation at 13 °C). Because of the high auto-

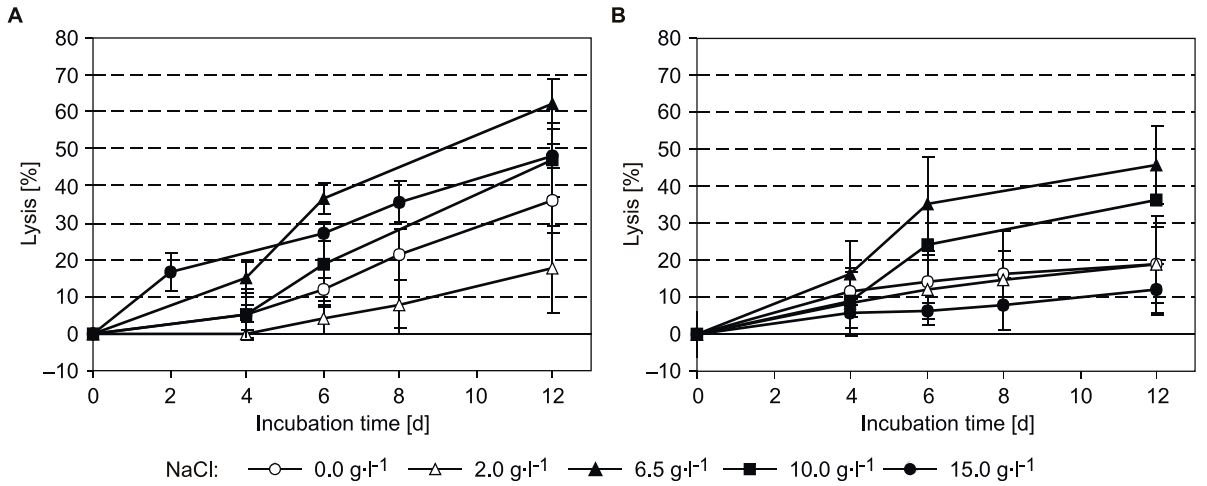

Fig. 5. Effect of NaCl concentration on autolytic activity of *Lactococcus lactis* HMM81 (A) and *Lactococcus lactis* subsp. *lactis* NIZO B643 (B).

Lactococcus lactis HMM81 and *Lactococcus lactis* subsp. *lactis* NIZO B643 were grown in LM17 broth for 6 h at 30 °C, suspended in sodium citrate buffer (pH 5) containing different NaCl amounts (0.0; 2.0; 6.5; 10.0; 15.0 g·l^{-1}) and incubated for 12 days at 13 °C. The error bars represent a 95% confidence interval.

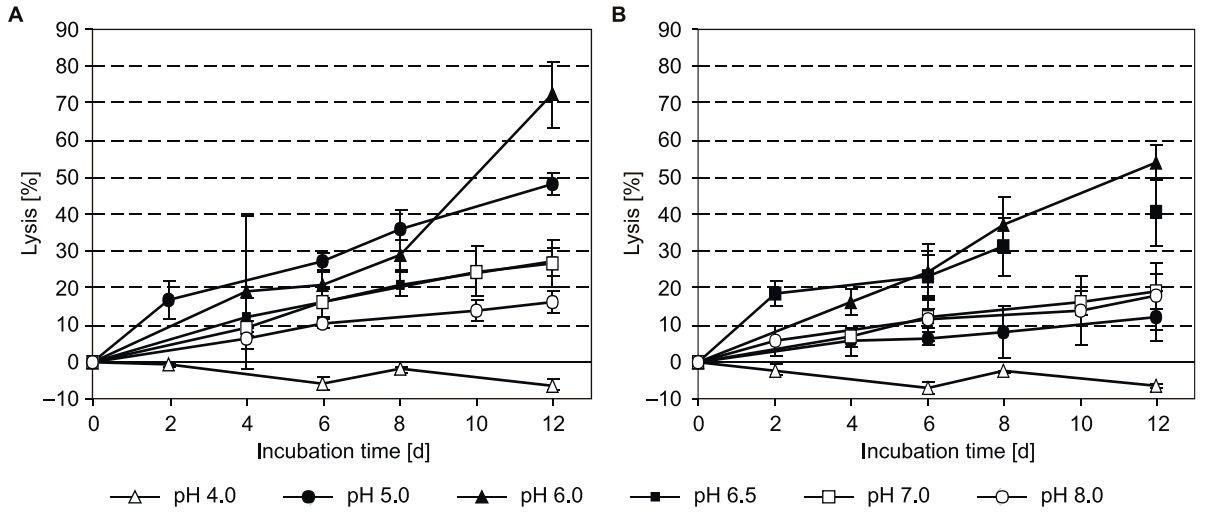

Fig. 6. Effect of pH of the medium on autolytic activity of *Lactococcus lactis* HMM81 (A) and *Lactococcus lactis* subsp. *lactis* NIZO B643 (B).

Lactococcus lactis HMM81 and *Lactococcus lactis* subsp. *lactis* NIZO B643 were grown in LM17 broth for 6 h at 30 °C, suspended in the sodium citrate buffer (pH 4.0, 5.0, 6.0, 6.5, 7.0, or 8.0) and incubated for 12 days at 13 °C. The error bars represent a 95% confidence interval.

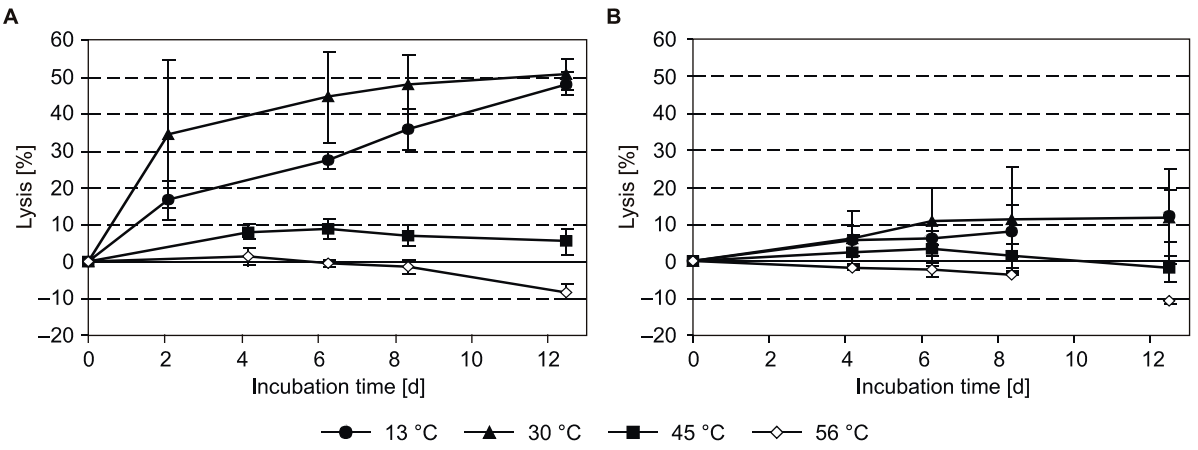

Fig. 7. Effect of the incubation temperature on autolytic activity of *Lactococcus lactis* HMM81 (A) and *Lactococcus lactis* subsp. *lactis* NIZO B643 (B).
Lactococcus lactis HMM81 and *Lactococcus lactis* subsp. *lactis* NIZO B643 were grown in LM17 broth for 6 h at 30 °C, suspended in sodium citrate buffer containing 15 $g \cdot l^{-1}$ NaCl, pH 5 and incubated for 12 days at 13, 30, 45 and 56 °C. The error bars represent a 95% confidence interval.

lytic activity of the non-autolytic strain B643 in the buffer at pH 6.0, pH 5.0 was taken as optimal for prediction of autolytic activity in lactococci strains.

The effect of the temperature of the citrate buffer on the autolytic activity of HMM81 and B643 is shown in Fig. 7. Approximatelly the same and high autolytic activity of HMM81 was detected at both 13 °C and 30 °C after incubation for 12 days. A difference between these two temperatures was in the rate of autolysis. The maximal lysis was detected earlier at 30 °C (approximatelly after 6 days of incubation) than at 13 °C (after 12 days of incubation). Finally, both temperatures, 13 °C and 30 °C, were evaluated as suitable for prediction of autolytic activity in lactococci strains. The temperature 30 °C may be used when a shorter incubation time is preferred. A fast decrease in the autolytic activity was observed during the incubation at 45 °C and 56 °C. The same phenomenon was shown in the non-autolytic strain B643. The explanation of this fact was suggested by Mou et al. [22]. The complete loss of autolytic activity observed at high temperatures resulted probably from the inactivation of autolysins [22].

CONCLUSIONS

Many different methods could be used for the selection of new lactococcal dairy starters and adjunct cultures, and determination of their autolytic ability is one of them. According to the results obtained for autolytic strain HMM81 and non-autolytic strain B643, we recommend the determination of autolytic activity of lactococci strains in citrate buffer (pH 5), containing 15 $g \cdot l^{-1}$ NaCl. Bacteria have to be washed twice in 40 ml of 50 $mmol^{-1}$ β-glycerophosphate buffer (pH 7) before incubation in the citrate buffer. Cell lysis should be measured spectrophotometrically at 650 nm during incubation.

The technique of autolytic activity determination in the citrate buffer was found suitable for fast screening of autolytic activity of the large group isolates. The method presented was also found relatively cheap, equipment undemanding and could be used by small dairy factories for selecting new lactococcal strarters and adjunct cultures. However, the autolytic properties of these technologically interesting strains isolated from different sources have to be also verified during cheese making or cheese ripening, because the autolytic ability observed in the buffer system does not guarantee that these strains will be able to lyse in the cheese environment.

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