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Technological characterization and antibacterial activity of Lactococcus lactis subsp. cremoris strains for potential use as starter culture for cheddar cheese manufacture

Tianfu CHENG[1,2#], Lin WANG[1#], Zengwang GUO[1*], Bailiang LI[1,3*]

Abstract

Several technological applications of *Lactococcus lactis* are attributed to their high metabolite-secreting abilities. Among these, bacteriocins can be used as food preservatives or additives in cheese manufacture. The objective was to evaluate the technological characteristics and antibacterial activity of 10 *Lactococcus lactis* isolates from traditional fermented dairy products, including acidifying ability, proteolytic ability, diacetyl production and autolytic activity. *L. lactis* subsp. *cremoris* Y14 and Y15 showed significantly ($P < 0.05$) higher ΔpH than other strains. *L. lactis* subsp. *cremoris* Y17 exhibited the highest proteolytic, *L. lactis* subsp. *cremoris* Y15 and *L. lactis* subsp. *cremoris* Y18 were next in terms of proteolytic activity. All strains possessed the ability of diacetyl production, with the highest production of 10.39 mg/g observed in *L. lactis* subsp. *cremoris* Y15. *L. lactis* subsp. *cremoris* Y14 had the highest ($P < 0.05$) autolytic ability (8.96%), followed by *L. lactis* subsp. *cremoris* Y15 (8.05%). *L. lactis* Y15 exhibited discernible antibacterial activity with the largest zone of inhibition diameters (25.56 mm). Based on the above results, *L. lactis* subsp. *cremoris* Y15 was selected as the starter to manufacture cheese. There were no significant differences in texture and sensory acceptability of Cheddar cheese manufactured by *L. lactis* subsp. *cremoris* Y15 with *L. lactis* subsp. *lactis* KLDS4.0325 or commercial starter. *L. lactis* subsp. *cremoris* Y15 can be used as a starter to produce Cheddar cheese.

Keywords: *Lactococcus lactis*; technological property; antibacterial; bacteriocin cheese.

Practical Application: The research results provide a starter with excellent performance for the production of cheddar cheese.

1 Introduction

As a worldwide agricultural and sideline product, cheese is widely distributed all over the world. Cheeses are generally nutrient-dense foods and are a valuable source of high-quality proteins, lipids, vitamins (e.g.vitamin A, B2 and B12) and minerals (particularly calcium and phosphorus). In ddition to macro and micronutrients, some matured cheeses contain bioactive components (e.g. bioactive peptides), which have health benefits, while beneficial bacteria present in the cheese matrix can potentially improve human gut health by producing short-chain fatty acids (Santiago-López et al., 2018). A study by Rafiq et al. (2018) found that a water-soluble peptide (WSP) extract produced by cheese during maturation can exhibit significant growth of human lung (H-1299) cancer cells by causing cell cycle arrest and extensive apoptosis induction inhibitory activity. Meanwhile,current research suggests that for cheese in particular, a matrix effect exists, whereby the other components present interact with the overall structure, leading to health benefits (Feeney et al., 2021).

In the last few decades, food safety and stability are fields of continuous expansion, mainly involved in two aspects: the creation of a hostile environment for pathogenic and spoilage microorganisms; the chemical additive, such as thickener, sweeteners, emulgator and preservative frother et.al. With further research, a large body of evidence suggests that food additives have a negative impact on human health. Benzoic acid ($C_7H_6O_2$) and its derivatives commonly used as antibacterial and antifungal preservatives and/or flavoring agent in foods (Saravanan et al., 2013) has some toxic and negative effects on people (Downard et al., 1995; Olmo et al., 2017), not only exacerbation of orofacial granulomatosis (Campbell et al., 2011) but also irritation of the digestive mucous membrane (Iammarino et al., 2011). In addition, consumers increased their demand for more health and natural, low content of sugars, salts and fats foods. So, seeking for a safety and reliable alternative becomes deeply urgent.

Lactic acid bacteria (LAB) are a microbial group commonly isolated from fermented products have received increased attention as a potential food preservatives by reason of their antibacterial activity and as well as offer one or more organoleptic, technological, nutritional, or health advantages (Leroy & Vuyst, 2004). The majority of health promoting LAB belong to the *Lactobacillus* and *Bifidobacteria* genera, but other genera such as the *Lactococcus*, *Streptococcus* and *Enterococcus* are also used as probiotics (Ribeiro et al., 2016). Some members of LAB produce bacteriocins and bacteriocins-like substances defined as antimicrobial peptides or peptide complexes that

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[1] *Northeast Agricultural University, Food College, Harbin, China*
[2] *Heilongjiang Beidahuang Green Health Food Co., Ltd, Jiamusi, China*
[3] *Northeast Agricultural University, College of Food Science, Key Laboratory of Dairy Science, Harbin, China*
*Corresponding author: gzwname@163.com; 15846092362@163.com
[#]These authors contributed equally to this work.

present antagonism mainly inhibit growth of Gram-positive bacteria including spoilage and pathogenic microorganisms (Cotter et al., 2005; Gillor et al., 2008). It is well known that Nisin which belongs to the lantibiotic class of bacteriocins (Class I) (Moraanin et al., 2012) is the most important antimicrobial peptide and realize industrial mass production (Settanni & Corsetti, 2008). What's more bacteriocins and bacteriocins-like substances are harmless due to proteolytic degradation in the gastrointestinal tract (Cintas et al., 1995). In consequence, bacteriocins or the bacteriocin-producing LAB have been widely used as starter cultures and food preservative in over 50 countries (Campion et al., 2013; Leite et al., 2016; Hwanhlem et al., 2017).

Lactococcus lactis is one of the best known and characterized species of LAB. *Lactococcus lactis* plays a pivotal role in the cheese ripening and development of aroma process (Rasovic et al., 2017). It's widely found in various fresh cheeses such as Italian Mozzarella, cottage and cheddar cheese et.al, particularly in cheddar cheese, due to its characteristic: rapidly produce lactic acid, which promotes the coagulation and formation of curd; possess of a proteolytic enzyme system; produce other aromatic compounds such as diacetyl and acetoin through the metabolism of citrate or lipids (Parente & Cogan, 2004). Moreover, in a previous works *Lactococcus lactis* (L3A21M1) isolated from an artisanal Azorean cheese that produced a bacteriocin with some antimicrobial effects (Ribeiro et al., 2014). And, bacteriocinogenic cultures as lacticin (Rodríguez et al., 2001; McAuliffe et al., 1999) and pediocin (Rodríguez et al., 2005) producing strains of *Lactococcus lactis*, have also shown antimicrobial activity in cheese.

According to the complexity and production mode of starter culture, the cheese starter is divided into natural starter (NS), mixed strain starter (MSS) and defined strain starter (DSS). Researches have revealed that scientific literatures have a high degree of consistency in indicating a significant advantage in the use of defined and natural starter cultures as compared to adventitious microbiota in terms of acidification, sensory traits and acceptability of final ripened products, as well as in the control of undesired microorganisms (Bassi et al., 2015). Cheddar cheese originated in the United Kingdom but now has been produced in many countries, it is the most popular cheese all over the world. The potential of bacteriocin-producing lactic acid bacteria (LAB) to control undesirable microorganisms in cheese has been demonstrated. Recent efforts have focused on screening an valuable new lactococcal strains isolated from wild ecological niches, good quality raw milk and traditional cheeses. The objective of this study was to screen *Lactococcus lactis* with good technological properties and high antimicrobial activity. The screened strain was further used as the starter for cheese production to determine its texture profiles.

2 Materials and methods

2.1 Bacterial strains and culture conditions

Lactococcus lactis subsp. *cremoris* Y10, Y11, Y12, Y13, Y14, Y15, Y16, Y17, Y18, and Y19 were isolated from traditional cheese in Inner Mongolia, China and were anaerobically incubated in M17 broth at 30 °C for 24 h and were sub-cultured twice prior to the experiment. *Lactococcus lactis* subsp. *lactis* KLDS4.0325 were *preserved* at the Key Laboratory of Dairy Science, Ministry of Education of the Northeast Agricultural University, China. *Listeria monocytogenes* ATCC 19115 was provided by Heilongjiang entry-exit Inspection and Quarantine Bureau, China and used as the indicator strain for antibacterial activity assays. It was incubated in brain heart infusion broth (BHI) in aerobic condition at 37 °C.

2.2 Technological properties

Acid production

10 mL of reconstituted skim milk (RSM, 10% w/v) was inoculated (1%, v/v) with reactivated bacteria and stored at 37 °C. The pH was measured after 24 h of incubation and values were recorded as ΔpH.

Proteolytic activity

To evaluate the proteolytic activity of the strain, the method of o-phthalaldehyde (OPA) colorimetry was used by an assay modified from Nielsen et al. (2001). In short, 2.50 g of sterilized RSM and 1mL double-distilled water was mixed evenly. After that, 5 mL of 16% trichloroacetic acid was added and incubated at room temperature, let stand for 10 min. After preincubation, 150 μL supernatants that were collected by centrifugation at 3500 × g for 10 min were reacted with 3 mL OPA at room temperature for 2 min. Absorbance at 340 nm was measured with a spectrophotometer.

Diacetyl production

Diacetyl production was assessed by o-phenylenediamine colorimetric method according to Ribeiro et al. (2014). Briefly, fermented and solidified milk samples that ripened for 24 h at 4 °C was mixed evenly with 16% trichloroacetic acid solution. Supernatant was harvested by centrifugation at 4500 × g for 10 min, and filter-sterilized with a 0.22 μm filter membrane. Absorbance readings were recorded at 335 nm by UV-visible spectrophotometer.

Autolytic activity

The rate of autolysis was determined according to Yokoia et al. (2004) with some modifications. Briefly, the strains were harvested by centrifugation at 5000 × g for 15 min after culturing at 37 °C until stable phase. The cell pellets were then rinsed three times with 0.01 M PBS and re-suspended in the same buffer. Finally the cell suspension was adjusted to an absorbance value (A650) of approx. 0.8–1.0. The optical density at 650 nm of simples was measured using Spectrophotometer at different points in time. The extent of autolysis of strains (%) calculated with the equation given below (Equation 1):

$$EAS\% = \left(1 - \frac{A_t}{A_0}\right) \times 100 \quad (1)$$

where A_0 was the optical density of initial cell suspension; A_t was the optical density of the samples with different culture times.

2.3 Determination of the effect of strains on antibacterial activity

Cell-free culture supernatants (CFCS) of the 10 strains were harvested by centrifugation at 10000 × g for 15 min at 4 °C after incubation at 37 °C for 24 h. For detection of antagonistic activity of CFCS, agar well diffusion assay as described by Ribeiro et al. (2014) was used. Firstly, 10 mL of agar solution (1.2% agar, w/v) were mixed and poured into sterile petri dishes, and stood for an overnight in the clean bench. *Listeria monocytogenes* ATCC 19115 was inoculated into 10 mL cooled to 50 °C BHI soft agar (0.7% agar, w/v) with 2%, and poured into dry agar plates (1.2% agar). After that, wells (5 mm) were punched in the plate, 50 µL CFCS or sterilized distilled water (negative control) were placed in wells with stood for 3 h. Plates were then incubated at 37 °C for 24 h, inhibition zone diameters were observed and recorded.

In order to determine the effect of pH-value, temperature, H_2O_2, and catalase and proteolytic enzymes on the CFS Antagonistic Activity. CFCS aliquots were adjusted in a pH range from 2 to 12 using either sterile HCl or NaOH, respectively. CFCS aliquots were exposed at 60, 80, and 100 ◦C for 30 min, or at 121 °C for 15 min, respectively. The samples were then allowed to cool to room temperature before being tested. The sensitivity of the antagonistic substance to enzymatic degradation by catalase and proteolytic enzymes was evaluated using catalase, trypsin, and proteinase, and pepsin.

2.4 Texture analysis of cheese manufacture

Laboratory-scale cheese manufacture

Raw milk was obtained from a local dairy farm and pasteurised at 63 °C for 30 min, and cooled to 31 °C before cheese-making. Cheddar cheese was manufactured in triplicate in vats using the selected *L. lactis* subsp. *cremoris* strain with *L. lactis* subsp. *lactis* KLDS4.0325 and frozen DVS preparation of a mixed starter culture R604Y as control group. The experiment and control group were mixed with pasteurized milk at the rate of 2% and 0.02% (v/v) respectively. Starter culture was added to cheese milk at the rate of 0.012% (w/v), the curd was cooked to 31 °C, pitched at pH 6.15, and milled at pH 5.35, 0.02% (v/v) $CaCl_2$ was added. Subsequently cheeses were salted by addition of salt at the level of 2.5% (w/w) and then wiped dry, vacuum packed and ripened at 8 °C for 60 days. Grated cheese samples (10 g) were taken at 0, 14, 28 and 56 days of ripening to assess starter.

Texture Profile Analysis (TPA)

Textural properties of cheese were evaluated using a texture analyzer. Cheese samples were cut into 10 mm^3 cubes, let stand for 2 h at ambient temperature before testing. In the following, texture profile analysis (TPA) was used to assess texture changes of cheese (Kilcawley et al., 2012). Alling speed, measuring speed and returning speed of probe were 2.0 mm/s, 5.0 mm/s and 2.0 mm/s respectively. The lower deformation was 5 mm, trigger force was 5.0 g, probe types was p/0.5. Hardness, cohesiveness, springiness, gumminess, and chewiness were performed three times.

2.5 Sensory evaluation

The cheeses in both the control and experimental group had been ripened for 60 days at the time of evaluation. The evaluation method refers to the study of Ruvalcaba-Gómez et al. (2020). Visual impression, tactile texture, aroma, taste, buccal texture and overall impression of cheese were evaluated using a nonstructured 9-cm hedonic scale (0 = dislike extremely, 9 = like extremely). The evaluation was conducted in four rounds (1 randomised sample per round) with 20 untrained panellists including both students and staff members from our institution (men and women, age range of 20-34 years). All panellists were preselected as regular (at least weakly) Cheddar cheese consumers. Each round, the panellist received a wedge-shaped cheese portion for visual evaluation and four cubic-shaped portions (1.5 cm^3) for tactile texture, aroma, taste and buccal texture evaluation; cheese samples were tempered at 20 °C for 1 h prior to evaluation in individual booths using white fluorescent light. Unsalted crackers and water were provided for mouth rinsing between samples.

2.6 Statistical analysis

Statistical analyses were performed using the SPSS 19.0 software for Windows (SPSS Chicago, IL, USA). The analysis of variance (Duncan Test and T-test) was applied to determine difference in means. All the values are the mean values ± Standard Deviation (SD) obtained from three independent assays for each trait.

3 Results and discussion

3.1 Technological performance of Lactococcus lactis **strains**

The output of Cheddar cheese is more than all other cheese in the world and re-cheeses in the market are mostly made of cheddar cheese as raw material. So, traditional technologies and small workshops can not meet the needs of a large demand of consumer, now almost the Cheddar cheese are commonly produced in large factories. The uniformity of the product becomes particularly important. It's necessary to screen stability and nontoxic cheese starter (Soda, 2014).

Acidifying activity

Screening of cheese starter strains, acidification and rapid production of lactic acid are the most important criteria (Rasovic et al., 2017). Acid-producing ability of strain, fast acid production and strong acid production capacity of the cheese can not only increase the rate of cheese acidification, but also can reduce the fermentation process, and prevent of bacterial contamination from microbial contamination. The pH stability also may contribute to applications in acid and non-acid foods. The results of the acidification activity recorded after 24 h of bacterial growth in skim milk are shown in Figure 1. After 24 h, the strains presented values of ΔpH ranging from 1.12 to 1.93 pH units. Among all strains, *L. lactis* subsp. *cremoris* Y14 and Y15 showed significantly higher ΔpH than other strains ($P < 0.05$). The results supported the values recorded for *L. lactis* strains isolated from herbs, fruits and vegetables by Ho et al. (2018).

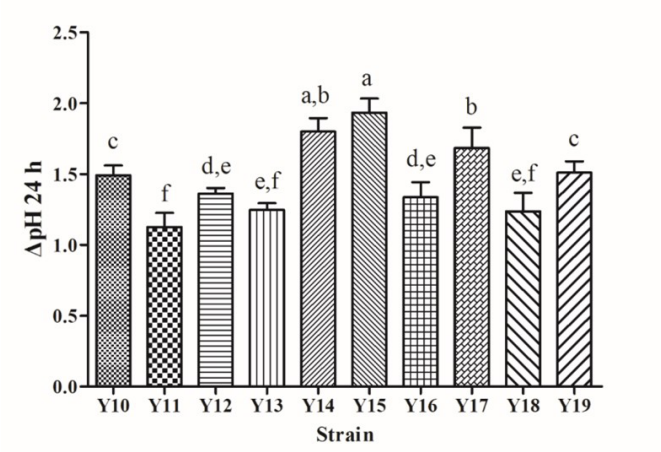

Figure 1. Changes of pH in Reconstituted Skim Milk (RSM) medium after inoculation of *Lactococcus lactis* strains for 24 h. Values are mean ± SD. Significant differences ($P < 0.05$) among the strains are indicated with different letters above the graphical bars.

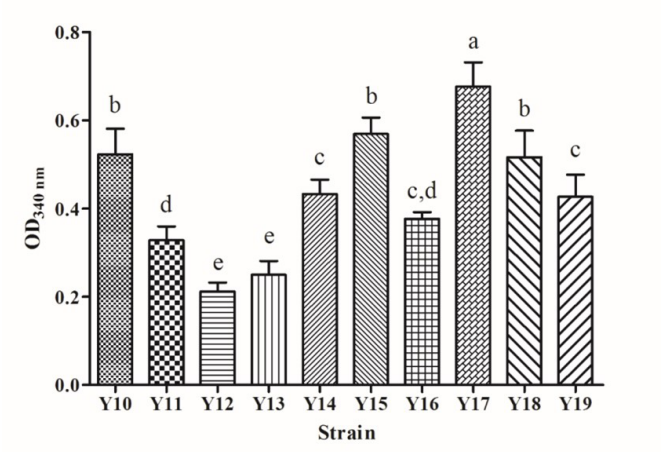

Figure 2. Proteolysis degree (OPA assay) of *Lactococcus lactis* strains. Values are mean ± SD. Significant differences ($P < 0.05$) among the strains are indicated with different letters above the graphical bars.

Proteolytic ability

The selection of starters for proteinases, peptidases, and aminopeptidases activity is necessary for growth of LAB in milk and for casein hydrolysis during cheese ripening (Ammor & Mayo, 2007). Researches have revealed that proteolytic system of *Lactococcus lactis* plays a key role in the process of cheese flavor formation. In this study, the proteolytic activity was determined by the method of o-phthalaldehyde colorimetry. OPA spectrophotometry is a quick, easy-to-use and accurate means of quantitatively assessing proteolysis in milk and milk proteins. The OPA reagent is easy to prepare, and one solution serves for stopping hydrolysis and color development. Serine was used as a standard, since this reaction resembled the average response of other amino acids (Nezhad et al., 2020). The results showed that *L. lactis* subsp. *cremoris* Y17 exhibited the highest proteolytic, *L. lactis* subsp. *cremoris* Y15 and *L. lactis* subsp. *cremoris* Y18 were next in terms of proteolytic activity (Figure 2). These findings were similar to those produced by several LAB strains isolated from traditional cheeses (Hemme & Foucaud-Scheunemann, 2004; Moreno et al., 2006; Domingos-Lopes et al., 2017). However, not all the LAB is able to hydrolyzed protein. It has been reported that the two selected *Lactococci* strains KM746 and KM721 did not show proteolytic activity (Allam et al., 2017). To the best of the author's knowledge, conflicting literature data concerning casein proteolysis in *Lactococcus* indicate a marked strain-to-strain variation of this phenotypic trait (Favaro et al., 2014).

Diacetyl production

Diacetyl is responsible for the distinct aroma properties and quality of fermented dairy products (Marilley & Casey, 2004). Moreover, most of the strains provided good sensory characteristics of the fermented product producing diacetyl (Terzić-Vidojević et al., 2015; Terzic-Vidojevic et al., 2013; Passerini et al., 2013). In the present study, as shown in Figure 3, all strains possessed the ability of diacetyl production, with the highest production of 10.39 mg/g observed in *L. lactis* subsp.

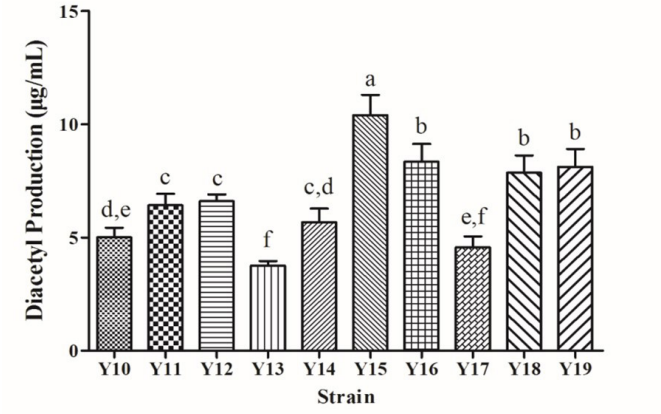

Figure 3. The diacetyl contents of *Lactococcus lactis* strains. Values are mean ± SD. Significant differences ($P < 0.05$) among the strains are indicated with different letters above the graphical bars.

cremoris Y15. Nevertheless, the ability to produce diacetyl from citrate was found to be species and strain dependent, as 60% of *Leuconostoc*, 33% of *Lactococci*, 82% of *Lactobacilli* and 92% of *Enterococci* produced diacetyl (Domingos-Lopes et al., 2017). In *L. lactis* subsp. *lactis*, aroma production is associated with the capacity to metabolize citrate, and diacetyl production is proportional to citrate consumption in aerobiosis (Laroute et al., 2017; Aymes et al., 1999).

Autolytic activity

The rapid release of intracellular enzymes due to autolysis of lactic acid bacteria in the cheese matrix post-manufacture not only accelerates the ripening of cheese, but also plays a decisive role in the flavor and sensory formation of cheese (Lortal & Chapot-Chartier, 2005; Law, 2001; Guinee et al., 2000). The rapid autolysis of the starter that promotes cell the early release of

intracellular peptidase and maintains the activity in the cheese, then bitter peptides were further decomposed for all amino acid, thus reduce the bitter taste of low salt cheese. Figure 4 presents the statistically significant differences in the Autolytic activity for the 10 *Lactococcus lactis* strains. Autolytic activity vary from 3.61% to 8.96%. *L. lactis* subsp. *cremoris* Y14 had the highest ($P < 0.05$) autolytic ability (8.96%), followed by *L. lactis* subsp. *cremoris* Y15 (8.05%). Hannon et al. (2003) added a highly autolytic strain of *Lactobacillus helveticus* (DPC4571) as an adjunct cultures or starter to make cheddar cheese, these two ways of adding can be accelerated cheese ripening and flavour development.

3.2 Antibacterial activity

Lactococcus lactis in milk is associated with the rapid production of lactic acid, which prevents the growth of pathogenic and spoilage bacteria and creates optimal biochemical conditions for ripening (Song et al., 2017). As well as, bacteriocin-producing LAB to control undesirable microorganisms in cheese has been demonstrated (Rodríguez et al., 2005). *Lactococcus lactis* has a long history of using in milk fermentation, from small-scale traditional operations to well-controlled industrial applications (Cretenet et al., 2011). Over the last decade, a variety of bacteriocins produced by species of *Lactococcus* have been reported. The antibacterial activity of CFCS of all tested strains against the indicator bacteria of *Listeria monocytogenes* ATCC 19115 was determined by the diameter of zones of inhibition on the agar well plates. As shown in Figure 5, CFCS of *L. lactis* Y15 exhibited discernible antibacterial activity with the largest zone of inhibition diameters (25.56 mm), *L. lactis* subsp. *cremoris* Y11 had the second-strongest antibacterial potential with an inhibition zone of 20.24 mm. *L. lactis* subsp. *cremoris* Y12 had the least antibacterial activity (25.56 mm) against *Listeria monocytogenes* ATCC 19115 in this study. Thus, *L. lactis* subsp. *cremoris* Y15 was selected for further study to physicochemically characterized of antagonistic substances. *L. lactis* subsp. *cremoris* L3A21M1 isolated from an artisanal Azorean cheese (Pico cheese) that produced a bacteriocin with anti-listerial activity (Ribeiro et al., 2016). The antimicrobial activity of two pediocin-producing transformants obtained from wild strains of *Lactococcus lactis* on the survival of *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* O157:H7 during cheese ripening was investigated (Rodríguez et al., 2005). The bacteriocin-producing strain exhibited a broad activity spectrum, inhibiting not only pathogenic strains of *L. monocytogenes*, but also all the other LAB strains (Ribeiro et al., 2016). *L. lactis* subsp. *lactis* produces nisin with an antimicrobial activity against closely related Gram-positive bacterial strains, food spoilage and foodborne pathogens such as *Bacillus cereus*, *B. thermosphacta*, *Clostridium botulinum*, *S. aureus*, *Listeria innocua* and *Listeria monocytogenes* (Hwanhlem et al., 2013). But, not all *Lactococcus* exhibit antimicrobial activity. The two selected *Lactococcus* strains KM746 and KM721 did not show neither proteolytic nor antimicrobial activity (Allam et al., 2017).

The effect of temperature, pH, catalase and proteolytic enzymes on the antibacterial activity of CFCS was determined. As shown in Table 1, the antibacterial activity of CFCS against *Listeria monocytogenes* ATCC 19115 was maintained within the pH range tested (2.0-12.0) and stable by heat treatment after 30 min at 60, 80, 100, or 121 ◦C. The antibacterial activity was observed after catalase treatment, but no bacteriostatic activities were detected, with CFCS treated with proteinase K, papain and trypsin. Therefore, it is possible to determine that the antibacterial substances of CFCS is protein or polypeptide as bacteriocin. With the increase of temperature, the diameter of the inhibitory ring hardly changed. In other words, bacteriocin has better thermal stability. This finding was consistent with a number of bacteriocins produced by *Lactococcus* spp (Ribeiro et al., 2016; Kelly et al., 1998). The heat tolerant of the bacteriocin produced by the strain tested could be a useful characteristic for application as a food preservative, since many food-processing procedures involve exposure to

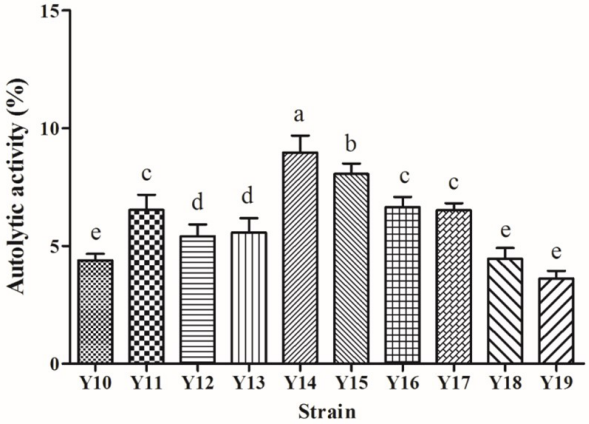

Figure 4. Autolytic activity of *Lactococcus lactis* strains. Values are mean ± SD. Significant differences ($P < 0.05$) among the strains are indicated with different letters above the graphical bars.

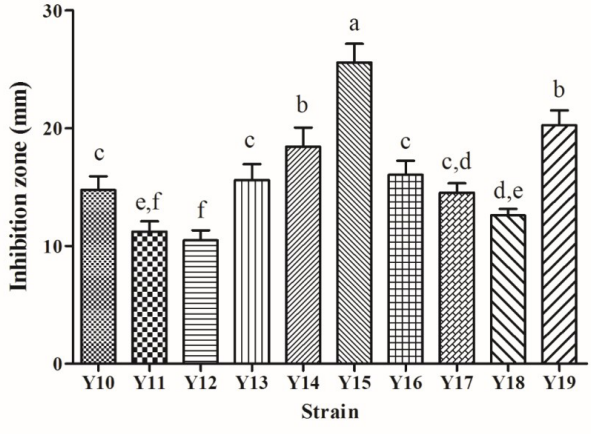

Figure 5. Zone of inhibition diameters induced by *Lactococcus lactis* strains against *Listeria monocytogenes* ATCC 19115. Values are mean ± SD. Significant differences ($P < 0.05$) among the strains are indicated with different letters above the graphical bars.

high temperature. LAB bacteriocins, such as nisin, exert a stronger effect at acidic pH values, rather than neutral pH. The pH stability also may contribute to applications in acid and non-acid foods (Ribeiro et al., 2016).

3.3 Texture analysis of cheese manufacture

The starter plays critical role in the texture of cheese. Based on the above results, *L. lactis* subsp. *cremoris* Y15 was selected as the starter to manufacture cheese. When the cheese is deformed by external pressure, the main component that controls its structure is the protein matrix, which is crucial to the degree of protein concentration, hydrolysis and hydration. As shown in Table 2, the results showed that the hardness of cheese and cohesion in the mature period gradually decreases, and the springiness, cohesiveness and chewiness were gradually increased. The general trend toward reduction in all TPA metrics during ripening may be due to the proteolytic breakdown of casein by remaining chymosin and the enzymes of starter, nonstarter, and adjunct LAB (Sousa et al., 2001; O'Mahony et al., 2005; Sheehan et al., 2007).

Table 1. Effect of temperature, pH, H_2O_2 and proteolytic enzymes on the antibacterial activity against *Listeria monocytogenes* ATCC 19115.

Treatments	Inhibitory activity of CFCS
Temperature	
60 ◦C, 30 min	++
80 ◦C, 30 min	++
100 ◦C, 30 min	++
121 ◦C, 15 min	++
pH	
2	++
4	++
6	++
8	++
10	++
12	+
Enzymes	
Catalase	++
Proteinase K	–
Papain	–
Trypsin	–

Note: + = presence of inhibition zone with growth of sparse colonies; ++ = presence of clear inhibition zone; – = absence of inhibition zone.

Cheese springiness is the extent to which the sample is reinstated after the first compression. Fresh Cheddar cheese inside is honeycomb structure, protein chain is unbroken and elastic. However, along with the extending of ripening time, continuous decomposition of protein, the honeycomb structures collapse, casein network blends with each other, and the return and elasticity of the cheese are constantly declining. The elasticity of the cheese increases first and then decreases in maturation (Awad, 2006), but this study concluded that the elastic gradually increase during ripening. This may be associated with shorter maturity.

Cheese hardness is the main parameter of cheese especially hard cheese texture analysis. During cheese ripening period, with the increase of the degree of hydrolysis, original network of cheese was destroyed, and the hardness was reduced. In general, the degradation of α_{s1}- casein is an important reason for the change of the state, texture of the cheese. In addition, with the increase of pH, the dissolution of calcium in the casein micelle can further lead to the destruction of casein network structure, and the cheese structure is more loose (Aminifar et al., 2010, 2013).

In the study, a decrease in cohesiveness in the control and experiment groups was observed during ripening; this is due to the decrease in moisture content and the proteolysis during ripening. Cheese cohesiveness was shown to decrease as cheese moisture content decreases (Pastorino et al., 2003). Cheese cohesiveness is inversely related to cheese proteolysis, with a trend of decreasing with increasing proteolysis (Lane et al., 1997). The internal bonding of cheese decreased with time, which was due to the change of protein interaction during ripening, chemical bonds of cheese to break down (Pastorino et al., 2003). As shown in Table 2, however, no significant differences were observed between the control and experiment groups in texture parameters at any time during ripening. This indicated that *L. lactis* Y15 can be used as a starter of cheeses manufacture.

3.4 Sensory analysis

Acceptability test results are shown in Table 3. There were no significant differences in appearance, tactile impression, aroma, taste, texture and overall impression between the control and experimental cheddar cheeses.

In a word, when compared to the frozen DVS preparation of a mixed starter culture R604Y, under the premise of not affecting the texture and sensory of the cheese, our selected *L. lactis* subsp.

Table 2. Texture parameters of the control and experiment groups.

Groups	Ripening time (w)	Springiness	Cohesiveness	Adhesion (g)	Chewiness (g)	Hardness (g)
Control	0	0.678 ± 0.012	0.811 ± 0.031	184.579 ± 25.85	185.366 ± 41.21	600.659 ± 40.26
	4	0.725 ± 0.021	0.789 ± 0.022	200.375 ± 47.26	231.502 ± 43.19	594.564 ± 38.92
	8	0.856 ± 0.037	0.736 ± 0.012	225.197 ± 32.88	273.266 ± 43.19	514.511 ± 50.63
	12	0.886 ± 0.035	0.718 ± 0.061	237.306 ± 96.45	343.199 ± 40.54	426.563 ± 30.81
Experiment	0	0.699 ± 0.015	0.834 ± 0.032	189.238 ± 41.27	172.389 ± 49.02	619.555 ± 38.98
	4	0.736 ± 0.053	0.795 ± 0.010	213.124 ± 45.46	219.611 ± 59.62	587.563 ± 48.22
	8	0.831 ± 0.090	0.752 ± 0.032	229.488 ± 68.93	262.395 ± 42.52	503.633 ± 56.36
	12	0.879 ± 0.031	0.728 ± 0.018	249.926 ± 47.31	345.537 ± 50.48	421.366 ± 40.35

Table 3. Sensory acceptability of the control and experiment groups.

Groups	Appearance	Tactile impression	Aroma	Taste	Texture	Overall impression
Control	8.23 ± 1.26	7.81 ± 0.31	8.45 ± 0.85	7.93 ± 1.44	7.73 ± 1.26	8.14 ± 1.71
Experiment	8.18 ± 1.07	7.78 ± 0.18	8.28 ± 1.31	8.02 ± 0.98	7.49 ± 2.01	8.03 ± 2.10

cremoris Y15 with *L. lactis* subsp. *lactis* KLDS4.0325 exhibited discernible antibacterial activity. However, their stability will be further studied to achieve commercial application.

4 Conclusion

Lactococcus lactis strains isolated from traditional dairy products showed a variety of technological characteristics. *L. lactis* subsp. *cremoris* Y15 showed good technological property (acidifying activity, proteolytic ability, and diacetyl production, autolytic activity) and high antibacterial activity. There were no significant differences in texture and sensory acceptability of Cheddar cheese manufactured by *L. lactis* Y15 or commercial starter. *L. lactis* Y15 can be used as a starter to produce Cheddar cheese.

Conflict of interest

The authors declare no conflicts of interest.

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