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Research article

Identification and characterization of *Lactococcus* starter strains in milk-based traditional fermented products in the region of Iran

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Abstract: The aim of the present research was identification and investigation of technological attributes of *Lactococcus* starter strains from traditional dairy products collected from the countryside of Boroujerd in Iran. 33 samples were cultured on selective media M17 and typical colonies surveyed for morphological properties. Totally, 37 strains were isolated based on the diversity in cell morphology and identified using API galleries and carbohydrate fermentation includes 17 strains of *Lactococcus lactis* (45.96%), 12 strains of *Lactococcus garvieae* (32.43%) and 8 strains of *Lactococcus plantarum* (21.62%). Strains were appraised for hydrolysis of L-arginine, casein and starch. Furthermore, strains were evaluated for the ability to grow at temperature 10 °C, 45 °C and presence of 4% and 6.5% NaCl, antibiotic sensitivity, acidification ability, proteolytic and lipolytic activities. Generally, 3 strains of *Lc. garvieae* (GYLC1, BWLC1, DCLC1) and 7 strains of *Lc. lactis* (GCLC4, GWLC2, GWLC3, SWLC1, SWLC3, BCLC5, DYLC1) exposed the highest levels of technological properties in order to use as starter cultures.

Keywords: identification; *Lc. garvieae*; *Lc. lactis*; *Lactococcus plantarum*; starter cultures

1. Introduction

From the far past, people knew that milk and its fermented products have a major impact on the health, therefore, even in traditional medicine they used these products. At that time, the existence of microorganisms in these products was not proven as long as the Russian scientist, Metchnikoff, demonstrated the secret of long life the Bulgarians due to valuable bacteria that live in dairy products. These products are the unique sources of energy, sugars, proteins, fats, vitamins and vital elements

including calcium and phosphor. These elements and macromolecules are essential for human growth, teeth health, bone stability and decreasing cardiovascular disease [1]. Starter cultures that contain chosen microorganisms that meticulously add to milk or dairy products under controlled and certain conditions to create favorable changes. Starter cultures microorganisms used in fermentations possessed by the family of bacteria known as lactic acid bacteria (LAB). These bacteria play a significant role as starter cultures in the dairy industry. Due to its pH lowering effect and production of antimicrobial metabolites, they often used in the food industry as an additive enhancing the organoleptic features, product quality and lifetime. Since milk provides the conditions for microbial growth, such as pH close to the neutral, water activity and reaches of nutrients is an appropriate place for many microorganisms. The process of all fermented dairy products relied on LAB due to their ability to ferment lactose in milk to make mainly lactic acid, which produces a distinguished fresh flavor to the product [2]. *Lactococcus* strains are the best known and specified species of the LAB which are mainly isolated from both dairy products and plant material. *Lactococcus* species have an active role in the ripening of cheese texture owing to its ability to produce intracellular peptidase enzymes. With increasing population growth and richer culture of dairy consumption related factories produce diverse dairy products which relied on import starter cultures from abroad. Contrary to what was said, not much research has not been done yet in Iran about dairy. Studies conducted in the field of the LAB are more focused on the dairy industry to optimize biochemical attributes, quality of dairy products and identification.

The aim of the present study was isolation, identification, and study of technological attributes of *Lactococcus* starter strains from traditional dairy products. By this research not only, we are able to preserve these valuable strains for extensive commercial exploitation and prevent the annual withdrawal of millions of dollars from the country, but also produce dairy products according to the taste of people in this area.

2. Material and methods

2.1. Sampling, enrichment and isolation

Iranian samples used in this study include and yogurt drinks (Doogh), whey and cheese obtained from different villages of Khoramabad and Boroujerd. All samples were collected during one month and immediately transported to the laboratory under the refrigerated and sterile condition in 20 mL Falcon tubes and maintained at 4 °C until analyses. Moreover, the primary pH of samples aseptically was measured. In order to enhance the solubility of the hard texture of some samples, 5 g of each sample were mixed with 45 mL sodium citrate 2% (w/v) under sterilized conditions by mortar for 1 min at 45 °C [3]. After 30 min, 10 mL of each suspension was cultivated separately in M17 (Quelab, Canada) for *Lactococcus* species at 30 °C for 24 h anaerobically, pour plate method. Due to the growth of *Lactococcus* strains in the oxygen-free plates, anaerobic conditions were achieved through the jar and Gas-pack technique (BBL Gas-pack anaerobic system S, VWR international haasrode, Belgium). Samples were incubated for 24 h, diluted by the serial dilution method and 1 mL of each dilution by pouring plate combined separately with 15 mL of M17. Afterward, plates were incubated anaerobically for 48–72 h. In order to distinguish acid producing colonies from others, was used Bromocresol purple indicator in M17. From the plates with countable colonies of *Lactococcus*, colonies with distinctive morphological features were isolated. The purity

of the isolates was checked twice by repeated streaking them into the suitable agar plates [4]. The bacteria colonies were stocked in 50% glycerol at −40 °C until the complementary test. All isolates were inspected by Gram staining, motility, catalase, hydrolysis of L-arginine, casein, citrate and starch, ability to grow at 10 °C, 45 °C, and presence of 4% and 6.5% NaCl.

2.2. Carbohydrate fermentation

In order to investigate the fermentation ability of eight various carbohydrates, include fructose, galactose, glucose, lactose, maltose, melibiose, raffinose and Sucrose by strains, used a basic medium contains 0.5 g/L phenol red, 0.5 g/L acid ascorbic, 5 g/L lactose, 0.25 g/L magnesium sulfate, 2.5 g/L meat peptone, 19 g/L sodium glycerophosphate, 5 g/L soya peptone, 2.5 g/L tryptone and 2.5 g/L yeast by pH 7.0 ± 0.2. The 0.5% stock solution of each strain was prepared and sterilized by a membrane filter 0.2 μm. Subsequently, 0.5 mL of each strain was mixed with 4.5 mL of basic medium was contained every eight carbohydrates and incubated for 24 h at 30 °C. The results were interpreted based on the color changes of phenol red to yellow when the environment inside the tube becomes acidic [5]. Moreover, the ability to making CO_2 from glucose fermentation was inquired by inoculation strains in Durham tubes containing glucose and incubating for 24 h at 30 °C.

2.3. Hydrolysis of L-arginine, starch and casein

Ability to hydrolyze L-arginine by strains was checked in arginine dihydrolase broth, which contains 0.5 g peptone, 2.5 g NaCl, 5 g L-Arginine HCl and 0.15 g dipotassium phosphate in 0.5 L distilled water. A significant ingredient of the medium is an appropriate amount of glucose, necessary for the process to proceed and pH indicator used in medium (0.5 g/L Bromcresol purple) turns yellow at pH < 5.2. The inoculated tubes were incubated at 35 °C for 24 h and the preliminary results determined. Starch agar is a medium that assesses the potential of a microorganism to release particular Exo-enzymes, include α-amylase, oligo-1 and 6-glucosidase which hydrolyze starch. In order to prepare this medium, was used from 3 g beef extract, 5 g gelatin, 10 g potato starch, 5 g gelatin, 15 g agar and 1 L distilled water [6]. Strains were cultured on starch and incubated for 24 h at 30 °C. Afterward, strains were checked after adding few drops of Iodine, which changes color from a brown to dark brown-black in the presence of starch. The casein test was conducted on milk agar which used from 50 g dry milk, 12.5 g agar, 2.5 g yeast extract, 5 g casein, 1 g glucose and 1 L distilled water. Strains were cultivated on milk agar and incubated at 30 °C for 24–48 h. Existence a clear zone around the bacteria owing to Exo-enzyme released by strains are a clue that strains are able to hydrolyze casein.

2.4. Antibiotic susceptibility of strains

Antibiotic susceptibility was interpreted by diffusion disks method and results compared with MIC for strains, which have determined earlier toward different antimicrobial agents. M17 agar plates were overlaid with 8 mL of M17 soft agar (0.8%) containing 50 μl of freshly grown culture and the plates were incubated at 4 °C for the soft agar to set. Antibiotic disks (Mast Co, UK) were placed on solid medium and incubated at 30 °C for 24 h, anaerobically and then inhibition zone

measured. Eight antibiotics were used in the following concentration: 10 µg ampicillin, penicillin, gentamicin, 30 µg chloramphenicol, novobiocin, erythromycin, nalidixic acid and 130 µg bacitracin. Generally, strains were considered as resistance if the inhibition zone diameter was ≤ 10 mm for penicillin, ampicillin and gentamicin, ≤ 15 mm for erythromycin, chloramphenicol, novobiocin and nalidixic acid, ≤ 18 mm for bacitracin. The results were considered according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for streptococci, available in CA-SFM [7].

2.5. Assessment of acid production

This test relies on incubating overnight of strains in the skim milk medium. Strains were initially grown in M17 broth and then in sterile reconstituted skim milk supplemented with 0.3% of yeast extract and 0.2% of glucose for three sequential subcultures [8]. The 1% of overnight activated strains were inoculated with 100 mL of sterile reconstituted skim milk 10% and incubated for 24 h at 30 °C. pH shifts were determined using a pH meter (Teika Co, Japan). Strains considered as fast, medium or slow acidifying when pH obtained to below the isoelectric point and milk started to curdle at various intervals of time 6, 12, 24 h, respectively.

2.6. Assessment of proteolytic and lipolytic activity

Proteolytic activity of strains was evaluated by casein hydrolysis on M17 agar and supplemented with 10% of skim milk as poured and solidified. After preparing the mediums, 1% of fresh strains with concentration 0.5 MacFarlane was combined with basic mediums which pointed already. The plates were incubated anaerobically at 30 °C within 24 h. Strains with the protease enzyme which grew on the medium were distinguished due to the production of the clear zone after precipitation with 1 M HCl solution against an opaque background of unhydrolyzed protein.

Lipolysis can be considered as an enzyme-catalyzed hydrolytic cleavage of milk lipids, which resulting production of partial glycerides and FFA [9]. Lipase broadly applies in dairy products in order to cheese ripening, hydrolysis of milk fat and its ability to produce fragrance and taste. In this test, the lipolytic activity of strains was evaluated based on qualitative methods by Nile blue indicator. The strains were overnight incubated in the same condition of the proteolytic test, cultivated on the medium by combining 5% of the butter, 1.5% of agar, 0.5% (w/v) each extract of meat, yeast, peptone and glucose in Tween-80 1%, 3%, 5% [10]. After one-week incubation at 30 °C, colonies with blue or green-blue fat cells were considered as lipolysis. At the end**,** clear zone diameter in the proteolytic and lipolytic zone was measured.

3. Results and discussion

In this study, thirty-seven *Lactococcu*s strains were isolated from thirty-three dairy samples of villages around Boroujerd and Khoramabad using phenotypic methods and identified based on API galleries 20 strep (BioMérieux, France) and the ability to metabolize different carbohydrates. Generally, strains were cocci that occur singly, in pairs, or in chains, gram-positive, non-spore forming catalase negative, not motile considered as *Lactococcu*s genus. Since *Lactococcu*s *lactis* strains were grown poorly or not at 45 °C and also a large number of *Lactococcus garvieae* and

Enterococci usually grow very well at 45 °C, that is a proper test to differentiate between *Lc. lactis* and other gram-positive cocci. Results of carbohydrate fermentation were compared with Bergey's manual [11,12], thus *Lc. garvieae* strains were distinguished from other strains. *Lc. garvieae* strains were able to ferment lactose and mannitol but did not produce acid from raffinose. Also *Lc. lactis* could not hydrolyze mannitol and raffinose but were able to hydrolyze lactose, however, some strain of *Lc. lactis* were not able to produce acid from all three carbohydrates. *Lactococcus plantarum* strains were able to hydrolyze only mannitol. Furthermore, *Lc. plantarum* and some *Lc. lactis strains* (probably *L*c. *lactis* ssp. *cremoris*) were not able to hydrolyze arginine. The results of biochemical tests in table 1 and carbohydrates fermentation are summarized in table 2, briefly.

3.1. Antibiotic susceptibility of strains

Lactococcus species might be as a source of antibiotic resistance gene that transfers to pathogenic bacteria through the gastrointestinal tract or the food chain [13]. It is absolutely essential before using starter strains become confident these bacterial strains do not include any antibiotic resistance genes [14]. Both *Lc. lactis* and *L. plantarum* are generally recognized as safe status microorganisms. However, *L. garvieae* can be considered as an opportunistic potential zoonotic pathogen involved in several clinical human infections. In order to evaluate the use of these *L. garvieae* isolates as starters, further studies regarding the study of other virulence genes could be carried on. Indeed, high antibiotic resistance is not a proper characteristic of strains in order to use them as dairy starter cultures. Susceptibility results of strains toward eight antibiotics are shown in Table 3. Totally, three strains of *Lc. garvieae* (GYLC1, BWLC1, DCLC1) and seven strains of *Lc. lactis* (GCLC4, GWLC2, GWLC3, SWLC1, SWLC3, BCLC5, DYLC1) had no any resistance to different antibiotics thus, considered as suitable strains. These results are in agreement with Sharma's finding who declares *Lactococcus* species isolated from probiotics have an intrinsic trait of low resistance to gentamicin, ampicillin, chloramphenicol and penicillin [15].

3.2. Acidification activity evaluation

Lactic acid is responsible to prepare an acidic fresh flavor in fermented dairy products as plays an active role in forming the curd. *Lactococcus* species lower pH by way of the acid production from carbohydrate fermentation leading to, development of favorable sensory characteristic and preventing the growth of pathogenic microbes to ensure the final product is highly safe and stable [16]. In this study only six strains (GCLC4, GWLC2, GWLC3, SWLC3, BCLC5, DYLC1) which all belong to *Lc. lactis* lower pH below the Isoelectric point within 6 h (pH = 4.6). Generally, three strains during 12 h and fourteen strains during 24 h were able to reach pH below 4.6. Furthermore, fourteen strains were unable to make the curd. Meanwhile, pH shifts of all strains were evaluated by pH meter (Figure 1). The most acidification activity was, respectively, related to *Lc. lactis, Lc. garvieae, Lc. plantarum* within 24 h (Figure 2). These results confirm findings of Mangia et al. [17] that argued *Lc. lactis* strains isolated from Fiore Sardo cheese and raw milk were able to produce acid within 24 h.

Table 1. Biochemical characteristics of strains.

Strain	Motility	Spore	Hydrolysis of			Growth at temperatures		Growth with NaCl	
			Arginine	Casein	Starch	10 °C	45 °C	4%	6.5%
GYLC1	−	−	+	+	+	−	(+)	+	−
GYLC2	−	−	+	+	+	−	+	+	−
GYLC3	−	−	+	+	+	+	−	−	−
GCLC1	−	−	−	+	+	−	−	−	−
GCLC2	−	−	+	+	+	+	(+)	+	−
GCLC3	−	−	−	+	+	−	−	−	−
GCLC4	−	−	+	+	nd	−	−	−	+
GCLC5	−	−	+	−	+	−	(+)	+	+
GWLC1	−	−	+	+	+	−	−	−	−
GWLC2	−	nd	−	+	+	+	−	−	−
GWLC3	−	−	+	+	+	−	−	−	+
GWLC4	−	−	−	+	nd	+	−	−	−
SYLC1	−	−	−	+	+	−	−	+	−
SYLC2	−	−	−	+	+	−	−	+	+
SYLC3	−	−	+	+	+	−	+	+	+
SYLC4	−	−	+	+	+	+	−	−	−
SCLC1	−	−	−	+	+	−	−	+	+
SCLC2	−	−	+	+	+	−	−	−	+
SCLC3	−	−	+	−	+	−	(+)	+	+
SWLC1	−	−	+	+	+	−	−	−	−
SWLC2	−	−	+	+	nd	−	(+)	+	−
SWLC3	−	−	+	+	nd	−	+	+	−
SWLC4	−	−	+	+	+	−	+	+	+
BYLC1	−	−	+	−	+	−	(+)	+	+
BYLC2	−	−	+	+	+	+	+	+	−
BYLC3	−	−	+	+	+	−	+	+	−
BCLC1	−	−	+	+	+	+	+	+	−
BCLC2	−	−	+	+	+	−	(+)	+	−
BCLC3	−	−	+	+	+	−	−	−	+
BCLC4	−	−	−	+	+	−	−	+	−
BCLC5	−	−	+	+	+	+	−	−	−
BWLC1	−	−	+	+	+	−	(+)	+	−
BWLC2	−	−	+	+	+	−	+	+	−
DYLC1	−	−	+	−	+	−	+	+	−
DYLC2	−	−	+	+	+	−	(+)	+	−
DCLC1	−	nd	+	+	nd	+	(+)	+	−
DCLC2	−	−	−	+	+	−	−	+	+

+: week, (+): strong, −: negative, nd: no data. After 24 h incubation at optimal temperature.

Table 2. Carbohydrates fermentation test.

Strain	Fermentation of									Identification
	Fru	Gal	Glu	Lac	Man	Mel	Raf	Suc	Xyl	
GYLC1	−	−	+	+	(+)	−	−	(+)	−	*Lc. garvieae*
GYLC2	−	−	+	(+)	−	−	−	+	−	*Lc. lactis*
GYLC3	+	−	+	−	+	−	−	+	−	*Lc. plantarum*
GCLC1	+	−	+	−	+	−	−	+	−	*Lc. plantarum*
GCLC2	−	−	+	+	(+)	−	−	+	−	*Lc. garvieae*
GCLC3	−	−	+	+	−	−	−	+	−	*Lc. lactis*
GCLC4	−	−	+	(+)	−	−	−	(+)	−	*Lc. lactis*
GCLC5	−	−	+	+	(+)	−	−	+	−	*Lc. garvieae*
GWLC1	−	−	+	+	−	−	−	+	−	*Lc. lactis*
GWLC2	−	−	+	(+)	−	−	−	+	−	*Lc. lactis*
GWLC3	−	−	+	(+)	−	−	−	(+)	−	*Lc. lactis*
GWLC4	−	−	+	+	−	−	−	+	−	*Lc. lactis*
SYLC1	−	−	+	−	+	−	−	+	−	*Lc. plantarum*
SYLC2	+	−	+	−	+	−	−	+	−	*Lc. plantarum*
SYLC3	−	−	+	(+)	−	−	−	+	−	*Lc. lactis*
SYLC4	−	−	+	+	−	−	−	+	−	*Lc. lactis*
SCLC1	+	−	+	−	+	−	−	+	−	*Lc. plantarum*
SCLC2	−	−	+	+	−	−	−	+	−	*Lc. lactis*
SCLC3	−	−	+	+	(+)	−	−	+	−	*Lc. garvieae*
SWLC1	−	−	+	(+)	−	−	−	(+)	−	*Lc. lactis*
SWLC2	−	−	+	+	(+)	−	−	+	−	*Lc. garvieae*
SWLC3	−	−	+	(+)	−	−	−	+	−	*Lc. lactis*
SWLC4	−	−	+	(+)	(+)	−	−	(+)	−	*Lc. garvieae*
BYLC1	−	−	+	+	(+)	−	−	+	−	*Lc. garvieae*
BYLC2	−	−	+	+	−	−	−	+	−	*Lc. plantarum*
BYLC3	−	−	+	(+)	−	−	−	(+)	−	*Lc. lactis*
BCLC1	−	−	+	(+)	(+)	−	−	(+)	−	*Lc. garvieae*
BCLC2	−	−	+	+	−	−	−	+	−	*Lc. garvieae*
BCLC3	−	−	+	+	−	−	−	+	−	*Lc. lactis*
BCLC4	−	−	+	−	+	−	−	+	−	*Lc. plantarum*
BCLC5	−	−	+	(+)	−	−	−	+	−	*Lc. lactis*
BWLC1	−	−	+	(+)	(+)	−	−	(+)	−	*Lc. garvieae*
BWLC2	−	−	+	+	−	−	−	+	−	*Lc. lactis*
DYLC1	−	−	+	+	−	−	−	+	−	*Lc. lactis*
DYLC2	−	−	+	(+)	(+)	−	−	(+)	−	*Lc. garvieae*
DCLC1	−	−	+	+	(+)	−	−	+	−	*Lc. garvieae*
DCLC2	−	−	+	−	+	−	−	+	−	*Lc. plantarum*

+: weak; (+): strong; −: negative; Fru: fructose; Gal: galactose; Glu: glucose; Lac: lactose; Mal: maltose; Mel: melibiose; Raf: raffinose; Suc: sucrose; Xyl: xylose.

Table 3. Resistance and sensitivity toward different antibiotics.

Strain	Antibiotics							
	Amp	Bac	Cam	Erm	Gen	Pcn	Nvb	Nx
GYLC1	(S)	(S)	S	S	(S)	(S)	S	(S)
GYLC2	(S)	S	R	S	S	S	(S)	S
GYLC3	R	S	S	S	(S)	(S)	R	S
GCLC1	R	(S)	S	S	S	(S)	S	S
GCLC2	S	S	S	S	S	(S)	S	S
GCLC3	S	(S)	R	S	S	S	S	(S)
GCLC4	(S)	S	S	S	(S)	(S)	S	S
GCLC5	(S)	S	R	S	S	S	S	S
GWLC1	S	S	S	S	R	(S)	S	S
GWLC2	S	S	S	(S)	(S)	(S)	S	S
GWLC3	(S)	(S)	S	S	(S)	(S)	S	S
GWLC4	S	S	S	S	S	(S)	R	R
SYLC1	R	S	S	(S)	S	S	S	R
SYLC2	S	S	S	(S)	S	S	S	S
SYLC3	(S)	S	(S)	S	S	S	S	S
SYLC4	R	S	(S)	(S)	S	S	S	S
SCLC1	S	S	R	S	S	R	S	(S)
SCLC2	S	S	S	S	S	R	(S)	S
SCLC3	S	S	R	S	S	R	(S)	S
SWLC1	(S)	S	S	S	(S)	(S)	S	S
SWLC2	S	R	(S)	S	S	(S)	R	S
SWLC3	(S)	S	S	S	(S)	(S)	S	S
SWLC4	R	R	(S)	S	S	S	S	S
BYLC1	S	S	(S)	S	S	R	R	S
BYLC2	S	S	R	S	S	(S)	S	S
BYLC3	R	S	S	S	S	(S)	S	R
BCLC1	(S)	S	S	S	S	(S)	S	R
BCLC2	S	S	R	S	(S)	S	S	(S)
BCLC3	(S)	S	S	S	S	R	S	S
BCLC4	S	S	S	S	S	S	S	S
BCLC5	(S)	S	S	S	S	(S)	(S)	S
BWLC1	(S)	S	(S)	S	S	(S)	S	S
BWLC2	S	(S)	S	S	R	S	S	S
DYLC1	(S)	S	S	S	(S)	(S)	S	S
DYLC2	S	S	S	S	(S)	R	S	S
DCLC1	(S)	(S)	(S)	S	S	(S)	S	S
DCLC2	R	S	S	S	(S)	S	S	S

R: resistant; S: low sensitive; (S): high sensitive; Amp: ampicillin; Bac: bacitracin; Pcn: penicillin; Cam: chloramphenicol; Erm: erythromycin; Gen: gentamicin; Nvb: novobiocin; Nx: nalidixic acid.

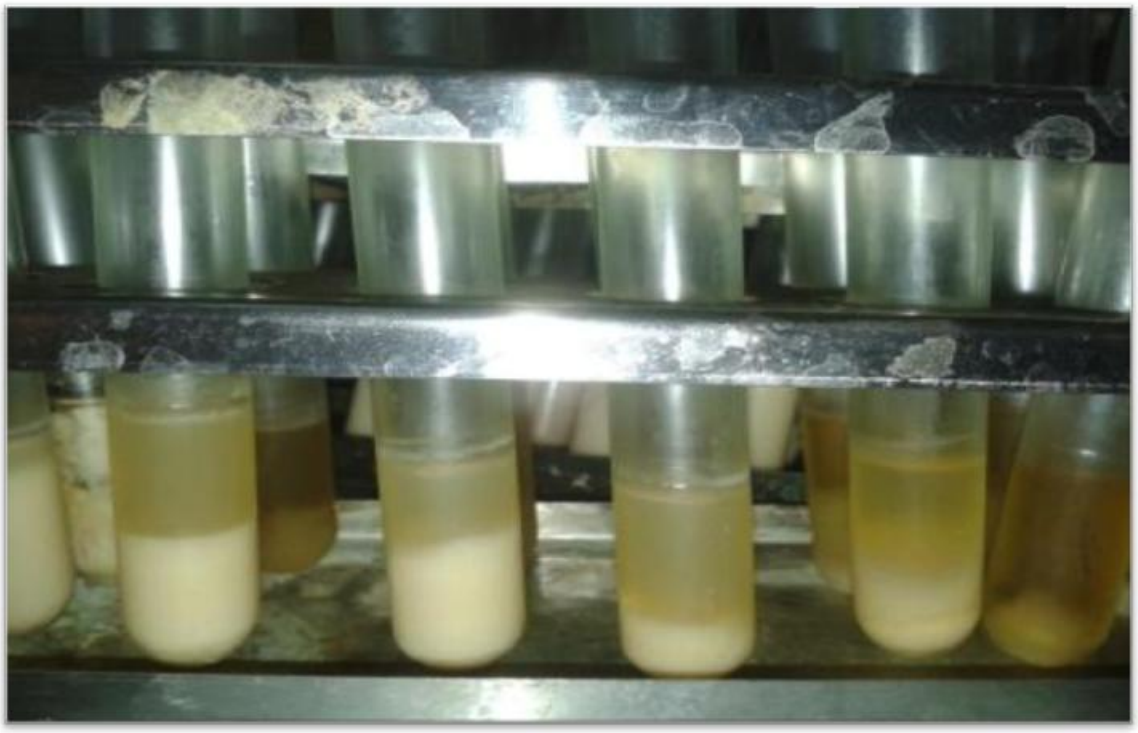

Figure 1. Acidification test of strains in skim milk medium.

3.3. Investigating proteolytic and lipolytic activities

Achieving of high-quality fermented dairy products relied on the starter bacterium's proteolytic ability [18]. Owing to the active role of proteolysis for improvement of flavor, the proteolytic attribute of starter cultures is considered as a key phenotypic factor as the production of peptides and amino acids are a result of protease activity. Free amino acids and complex peptides influence the flavor and aroma producing composition by way of secondary catabolic reactions. While the proteolysis diameter is between 15 and 21 mm, the strain is called proteolytic, Vuillemard et al. [19] argued. Considering table 4, strains exposed different protease activities as nine strains of *Lc. lactis* (GCLC4, GWLC2, GWLC3, SWLC1, SCLC2, SWLC3, BCLC5, BCLC3, DYLC1) and three strains of *Lc. garvieae* (GYLC1, BWLC1, DCLC1) exhibited relevant activity and had a diameter of between 15 and 20. This result is in agreement with research of Ma et al. [20] which argue *Lactococcus* species isolated from dairy products show various proteolytic activities and strains with the highest proteolytic activity significantly, are capable to lower pH.

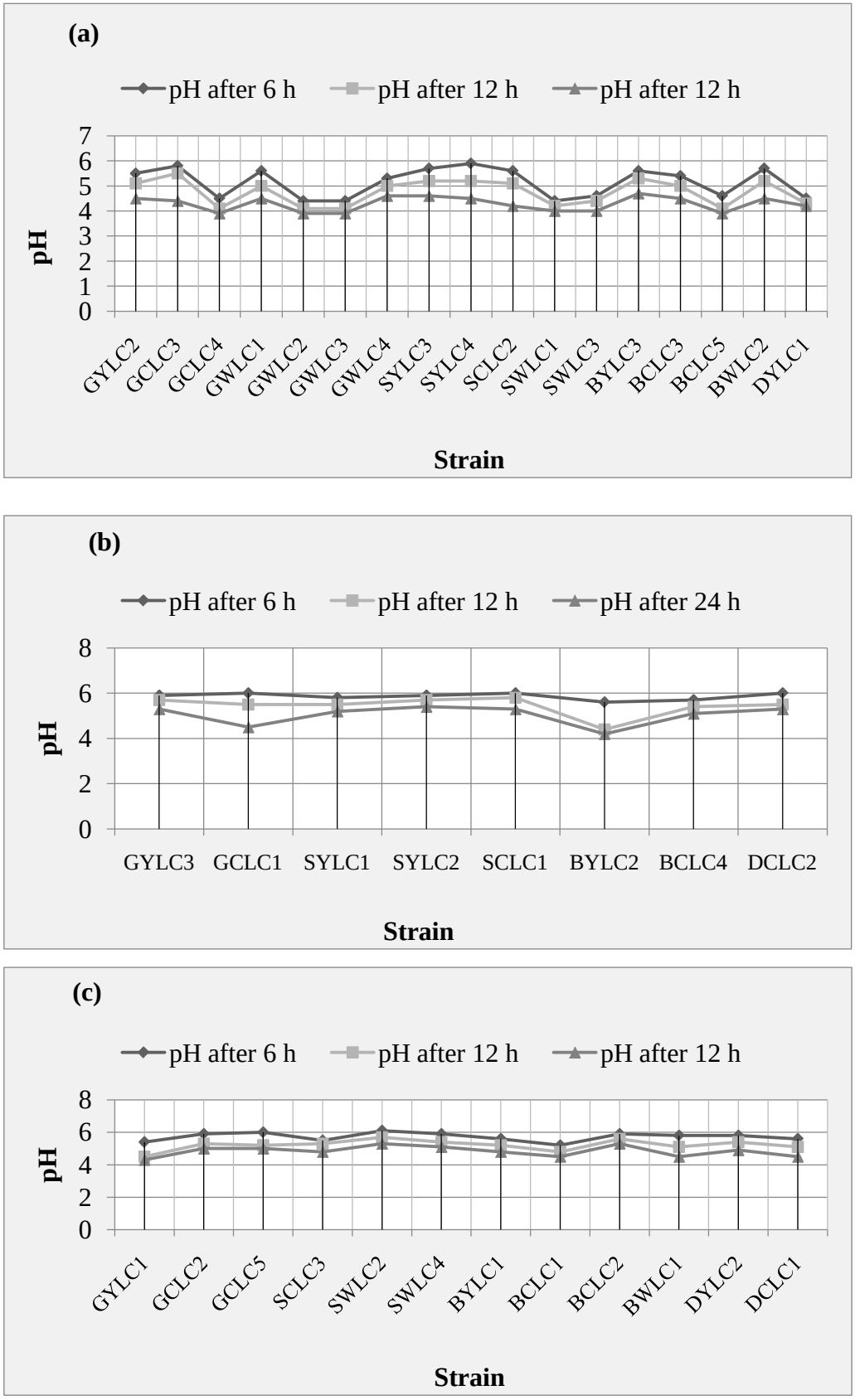

Figure 2. The pH changes in acidification test (a): *Lc. lactis*; (b): *Lc. plantarum*; (c): *Lc. garvieae*.

Rancidity as short-chain fatty acids like butyric is a result of lipolysis of milk fat by bacteria. In order to reduce bacterial lipase, usually, apply ultra high-temperature treatment in the dairy industry. As far as fat milk gets captured by globule's membrane, they are protected from lipase enzyme therefore, this test has a top priority in the dairy industry. Although the lipolytic activity of LAB is weak, nevertheless, they have a significant role in cheeses ripping [21]. The results of proteolytic and lipolytic tests were summarized in table 4. Fourteen out of thirty-seven strains include eleven strains *Lc. lactis* and four strains of *Lc. garvieae* and one strain of *Lc. plantarum* were able to hydrolyze the milk fat. This finding demonstrates that *Lactococcus* species specially *Lc. plantarum* has a weak lipolytic activity and are not in agreement with the study were conducted by Oterholm et al. [22], which extract two esterase enzymes from bacteria and expressed *Lc. plantarum* had the maximum esterase activity among LAB.

4. Conclusion

The similar studies have carried out earlier is in agreement with this research. Kacem et al. [23] identified 50 strains of *Lc. lactis* subsp. *lactis*, 34 strains of *Lc. lactis* subsp. *biovar* and 35 strains of *Lc. lactis* subsp. *cremoris* from milk. Furthermore, the research conducted by Edalati et al. [24] demonstrated the presence of 25.26% *Lc. Lactis* subsp. *lactis* from Lighvan cheese.

In sum, based on to this study, thirty-seven strains from thirty-three samples were isolated according to morphological differences in cell and identified relied on API galleries 20 strep galleries and carbohydrate fermentation pattern which, consequently led to identification 17 strains of *Lc. lactis* (45.96%), 12 strains of *Lc. garvieae* (32.43%) and 8 strains of *Lc. plantarum* (21.62%). Strains were evaluated for hydrolysis of L- arginine, casein, starch. Furthermore, strains were evaluated for the ability to grow at temperature 10 °C, 45 °C and presence of 4% and 6.5% NaCl, antibiogram test with various antibiotics, acidification, proteolytic and lipolytic activities. Totally, 3 strains of *Lc. garvieae* GYLC1 (isolated from yogurt), BWLC1 (isolated from whey), DCLC1 (isolated from cheese) and 7 strains of *Lc. lactis* BCLC5, GCLC4 (isolated from cheese), GWLC2, GWLC3, SWLC1, SWLC3 (isolated from whey), DYLC1 (isolated from yogurt) exposed the best performance of technological characteristics to apply them as starter cultures in dairy industries These strains had the lowest resistance to antibiotics of penicillin, ampicillin, nalidixic acid, bacitracin, chloramphenicol, gentamicin, novobiocin, erythromycin and their protease and lipase enzymes were highly active. Meanwhile, they were able to hydrolyze milk protein, casein, and had high acidifying activity in less time in order to make the curd. Generally, these strains as domestic starter cultures prepare conditions for product procurement on an industrial scale, but it absolutely requires more studies like the molecular identity of strains which demonstrate the compatibility of them. Chosen strains in this research indicated that significant intrinsic properties may supply imminent gene pool to develop genetically modified strains with unique features.

Table 4. Proteolytic and lipolytic activities of strains.

Strain	Proteolytic zone diameter (mm)	Lipolytic zone diameter (mm)		
		1% Tween-80	3% Tween-80	5% Tween-80
GYLC1	20	14	14	16
GYLC2	14	n	n	n
GYLC3	n	n	n	n
GCLC1	14	9	9	12
GCLC2	12	n	n	n
GCLC3	11	n	n	n
GCLC4	19	15	15	15
GCLC5	13	n	n	n
GWLC1	n	n	n	n
GWLC2	19	12	15	18
GWLC3	20	17	20	20
GWLC4	12	n	n	n
SYLC1	n	n	n	n
SYLC2	14	8	10	10
SYLC3	14	n	n	n
SYLC4	12	n	n	n
SCLC1	n	n	n	n
SCLC2	15	n	n	n
SCLC3	13	n	n	n
SWLC1	19	18	20	20
SWLC2	13	n	n	n
SWLC3	20	18	15	15
SWLC4	12	n	n	n
BYLC1	14	10	10	12
BYLC2	14	12	12	10
BYLC3	n	n	n	n
BCLC1	11	n	n	n
BCLC2	14	n	n	n
BCLC3	15	n	n	n
BCLC4	14	n	n	n
BCLC5	18	17	15	20
BWLC1	18	17	18	18
BWLC2	n	n	n	n
DYLC1	18	17	17	17
DYLC2	14	n	n	n
DCLC1	19	15	17	17
DCLC2	n	n	n	n

n: no activity.

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Conflict of interest

The author declares there are no conflicts of interest in this paper.

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