

DETERMINATION OF STARTER CULTURE PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM CHEESE

İlkay Turhan^{1*}, Zübeyde Öner²

¹T.C. İstanbul Arel University, School of Health Sciences, Nutrition and Dietetic Department, İstanbul

²Süleyman Demirel University, Faculty of Engineering, Food Engineering Department, Isparta

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Abstract

In this study, starter properties of 83 lactic acid bacteria (LAB) strains which were isolated from 13 cheese samples that were produced from raw milk and were characterized by using phenotypic, API and FTIR spectroscopy methods were determined. Proteolytic activity, acidification and decarboxylase activity were analyzed as starter culture properties for 22 *Lactococcus* sp., 36 *Enterococcus* sp. and 25 *Lactobacillus* sp. of 83 LAB. 18 isolates could decrease pH to less than six in 6 hours, 38 isolates indicated lower than 20 µg tyrosin/ml proteolytic activity and also 42 isolates indicated no decarboxylase activity. These isolates are thought to be the appropriate starter culture for cheese industry.

Keywords: Starter culture, proteolytic activity, acidification and decarboxylase activity

PEYNİRDEN İZOLE EDİLEN LAKTİK ASİT BAKTERİLERİNİN STARTER KÜLTÜR ÖZELLİKLERİNİN BELİRLENMESİ

Özet

Bu çalışmada çeşitli işletmelerden alınan çiğ süttten üretilmiş 13 adet peynir örneğinden izole edilen ve biyokimyasal, API test kitleri, FTIR spektroskopisi yöntemleri ile tanıları gerçekleştirilen 83 adet laktik asit bakterisi (LAB) suşunun başlangıç kültür olma özellikleri belirlenmiştir. 22'si *Lactococcus* sp., 36'sı *Enterococcus* sp. ve 25 tanesi *Lactobacillus* sp. olarak belirlenmiş izolatların başlangıç kültür olma özelliklerinden olan asit oluşturma yeteneği, proteolitik aktivite ve dekarboksilaz aktiviteleri incelenmiştir. 18 izolat, 6 saatte pH'yı 6'nın altına düşürmesi; 38 izolat, 20 µg tirozin/ml miktarının altında bir proteolitik aktivite göstermesi ve 42 izolat, hiçbir dekarboksilaz aktivitesi göstermemesi açısından peynir endüstrisine uygun başlangıç kültür olabileceği düşünülmüştür.

Anahtar kelimeler: Starter kültür, asit oluşturma, proteolitik aktivite, dekarboksilaz aktivitesi

*Corresponding author/ Yazışmalardan sorumlu yazar

✉ ilkayturhan@arel.edu.tr,

☎ (+90) 212 867 2500 / 1175,

☎ (+90) 212 860 04 815

INTRODUCTION

The most important desired properties for cheese are its texture, flavor, taste, aroma, smell and appearance. The raw milk quality, total dry matter, protein content of raw matter, acidity, properties of starter cultures and additives used are factors that affect these properties during production of cheese. Kashar cheese is one of the most important cheeses in Turkey (1, 2).

Lactic acid bacteria, especially in milk and milk products, are widely available in nature. LAB are widely used as starter cultures for fermentation in the dairy, meat and other food industries. Their properties have been used to manufacture products like cheese, yoghurts, etc. The most important feature of these microorganisms increases acidity of milk by producing lactic acid from lactose. The increasing of acidity affects the stability of casein as well as and promotes the activity of rennin. After acidification, milk proteins begin coagulation. In addition, due to the lipolytic and proteolytic activities during cheese ripening, lactic acid bacteria have a significant impact on the formation of characteristic taste, aroma and texture (3-5).

Acid production, proteinase and peptidase activity of LAB and many other features like these are important for their using as a starter culture. Proteolytic systems of LAB include their cell membrane proteins, amino acid and peptide transport systems and many of the intracellular peptides and proteases (6). Proteolysis is important for the formation of the specific taste of cheese (6, 7) and different LAB species have different proteolytic activity (6, 8, 9).

In this study, ability of producing acid, proteolytic activity and decarboxylase activities of lactic acid bacteria, which were isolated from Kashar cheese and diagnosed by biochemical tests and FTIR spectroscopy method, were determined.

MATERIALS AND METHODS

Material

A total of 12 Kashar cheese and 1 White cheese samples which were produced in laboratory conditions and were supplied from various

factories were used as isolation materials. For the characterization of LAB, biochemical tests and FTIR Spectroscopy (Perkin Elmer, Spectrum 100) method was applied. In order to identify isolates according to using of carbohydrates, BD BBL Crystal™ Gram-Positive ID Kits were used for isolates that was determined as coccus and bioMerieux API 50 CHL (Ref. 50410 to 10 x 10 ml) test kits were used for isolates that was determined as bacillus (10).

Total 157 isolates were obtained from all samples as a result of isolation procedures. The physiological and biochemical analyzes were continued with 83 isolates in consequence of morphological characteristics, catalase activities, and gram reactions. Biochemical tests were carried out according to the Sherman classification. Cocci that could grow under such conditions 10 °C, 15 °C, 45 °C, 6.5 % NaCl and pH 9.6 were identified as enterococci and the rest of the cocci strains were evaluated as lactococci. Taking into account these criteria, 36 of 83 isolates was approved as *Enterococcus* sp. and 22 of them was approved as *Lactococcus* sp. according to their phenotypic characteristics. All of the 25 isolates which were morphologically identified as bacilli was evaluated as *Lactobacillus* sp. because of being able to grow at 15 °C (10).

After these cultures were isolated and identified, acidification, proteolytic and decarboxylase activity of bacteria were determined in order to determine starter culture properties of isolated bacteria (10).

METHODS

Determination of acidification activity

1% of the active cultures were inoculated to the tubes which contain 5 ml Skim Milk medium. After *Lactococcus* sp. at 28 °C, *Lactobacillus* sp. at 30 °C and *Enterococcus* sp. at 37 °C were incubated for 18 hour, 1% of the cultures were inoculated into other tubes which contain 5 ml Skim Milk medium. The cultures were incubated for 6 and 24 hours at the appropriate temperatures and their pH was measured. Δ pH values were determined taking into account the initial and final pH values (11, 12).

Determination of Proteolytic Activity

Isolated strains were grown in Skim Milk medium and proteolytic activity was measured by a spectrophotometer. Sterilized Skim Milk medium was used as a control in order to eliminate the proteolysis products which would come from the medium. Proteolytic activity was expressed as tyrosine equivalent (12, 13).

Determination of Decarboxylase Activity

Isolated cultures were inoculated into the base medium which was enriched with a variety of 2 % amino acids (histidine, tyrosine, lysine, ornithine, phenylalanine, and tryptophan) and incubated for 7 days at anaerobic conditions (14).

After *Lactococcus* and *Enterococcus* strains were activated in M17 medium and *Lactobacillus* strains were activated in MRS medium, these strains were inoculated on M17 and MRS slope agar medium and *Lactococcus* sp. and *Lactobacillus* sp. were incubated at 30 °C for 24 hours, *Enterococcus* sp. was incubated in the 37 °C for 24 hours. At the end of this period, bacteria whose Mac Farland adjustment was done were inoculated in media which contain amino acids and amino acid-free media which used as a control and then were incubated in the anaerobic conditions at 30 °C for 7 days (14).

RESULTS and DISCUSSION

Acid Production Activities of Isolates

Δ pH changes at 6 h and 24 h is given in Table 1. for isolates were evaluated as *Lactococcus* sp., *Enterococcus* sp. and *Lactobacillus* sp..

When it was considered Karakus (15)'s evaluation in the study with white cheese, it was determined all isolates that was evaluated as *Lactococcus* sp. were produced low acid level (Δ pH <1.00) in our study. It was pointed out that *Lactococcus* strains which have good acid production should reduce pH under 5.3 at the end of 6 hours and at 30 °C (16). In our study, only two strains of *Lactococcus* sp. isolates could reduce pH to 5.85 and 5.81. Similarly, none of isolated *L. lactis* strains from Durlu-Özkaya et al. (17)'s study with white cheese produced by using goat's milk could not reduce pH to 5.0 ± 0.2 at 6 hours.

17 of 27 *L. lactis* which were isolated from Orinotyri cheese produced by using goat milk strains showed slow acid production (Δ pH 0.00 to 0.18), while the rest of 10 isolates showed the middle level acid production (Δ pH 0.20 to 0.54) in the first 6 hours in Prodromou et al. (18)'s study. In the same study, only one of isolates each *Enterococcus* sp. and *Lactobacillus* sp. could reduce pH under 6 within 6 hours. In Elçioğlu (19)'s study, it has been pointed out that only 2 of *Lb. plantarum*, 2 of *E. durans* and one of *E. faecium*'s, from 96 of isolates from Kargı Tulum cheese, can be used for preparation of starter culture because of their rapid acidification. In Dağdemir (20)'s study with white cheese, Δ pH changes for both *Enterococcus* sp. and *Lactobacillus* sp. varied low levels in 6 hour. It is observed that both enterococci and lactobacilli were capable of producing acid more slowly than lactococci in Tunail et al. (12)'s study. In our study, two isolates evaluated as *Enterococcus* sp. reduced the pH level to 4.08 in 24 hours, may be preferred as an alternative acid producing culture in the cheese production as their acidity is close to the level of acidity of *Lactococcus* sp. isolates which show highest possible levels of acidity. However, as the isolates evaluated as *Lactobacillus* sp. could reduce the pH level to maximum 4.48 within 24 hours and therefore, lactobacilli were not considered to be at the level sufficient to be selected as an acid producing culture.

Proteolytic Activity of Isolates

Proteolytic activity values (PAV) which were expressed as tyrosine equivalent of *Lactococcus* sp., *Enterococcus* sp. and *Lactobacillus* sp. cultures were given as μ g tyrosine / ml in Table 1. Tyrosine curve value (R^2) was 0.9966.

It is known that proteolytic activity is an important property for selection of starter culture. Proteolytic activity of cultures which will be selected is required to be low or high according to the type of cheese. While cultures which produced low proteolytic activity are preferred for fresh cheeses, cultures with high proteolytic activity are required for matured hard cheeses (12, 21). Proteolytic activity values (PAV) were assessed as weak level (PAV < 10.00), mid-level (10.00 < PAV < 20.00) and strong level (PAV > 20.00) at Karakus (15)'s study with white cheese.

Table 1. Acidification values of *Lactococcus* sp. *Lactobacillus* sp. and *Enterococcus* sp. strains at 6 h and 24 h and proteolytic activities

Species	Acidification		Proteolytic activities
	Δ pH (6h)	Δ pH (24 h)	μ g tyrosin / ml
<i>E. faecalis</i> (n=27)			
Minimum	0.42	0.72	-
Maximum	0.75	2.62	238.1
Mean	0.58 \pm 0.09	1.94 \pm 0.58	68.51 \pm 89.46
<i>Enterococcus</i> sp. (n=9)			
Minimum	0.08	0,26	1,6
Maximum	0.7	2,44	210,5
Mean	0.45 \pm 0.19	1,67 \pm 0.67	36,27 \pm 65.55
<i>L. lactis</i> (n=17)			
Minimum	0.19	0.65	12.1
Maximum	0.79	2.58	49.2
Mean	0.49 \pm 0.15	2.06 \pm 0.60	22.67 \pm 10.38
<i>L. cremoris</i> (n=3)			
Minimum	0.03	0.90	13.6
Maximum	0.2	1.72	21
Mean	0.12 \pm 0.09	1.34 \pm 0.41	18.33 \pm 4.11
<i>L. diacetylactis</i> (n=2)			
Minimum	0.27	1.37	17.3
Maximum	0.4	2.23	20.5
Mean	0.34 \pm 0.09	1.8 \pm 0.60	18.9 \pm 2.26
<i>Lactobacillus</i> sp. (n=13)			
Minimum	0.08	0.31	11.3
Maximum	0.65	2.12	29.2
Mean	0.37 \pm 0.21	1.16 \pm 0.68	17.35 \pm 6.03
<i>L. plantarum</i> (n=12)			
Minimum	0.04	1.68	7.4
Maximum	0.4	2.06	78.6
Mean	0.28 \pm 0.1	1.94 \pm 0.12	35.38 \pm 22.52

According to the evaluation of the study, it was determined that 58.82 % of the isolates had mid level proteolytic activity and 41.18 % of them had strong level proteolytic activity in our study. PAV average (25.95 mg tyrosine / ml) of *Lactobacillus* sp. isolates and PAV average (21.8 mg tyrosine / ml) of *Lactococcus* sp. isolates was found very close. It is also found that PAV average (60.3 mg tyrosine / ml) of *Enterococcus* sp. isolates were quite high and PAV average of 25 % (9 isolates) of them is higher than PAV average (60.3 mg tyrosine / ml) of all the enterococci strains.

While PAV values of lactococci and lactobacilli isolates were close to each other, values of enterococci isolates changed between wide limits (from 29.3 to 121.1 μ g of tyrosine / ml) and 36 % of them was found to have higher proteolytic activity than 60 μ g tyrosine / ml at Durlu-Özkaya *et al.* (17)'s study with cheese produced by using raw goat milk. Similar results were found at Tunail *et al.* (12)'s study and Dağdemir (20)'s study, in general *Enterococcus* sp. especially *E. faecium* strains had high proteolytic activity.

Prodromou *et al.* (18) isolated 27 *L. lactis* strains from Orinotyri cheese produced by using raw goat milk and determined that 9 of them had low proteolytic activity (<50 μ g glycine / ml), 7 of them had medium proteolytic activity (50 to 150 μ g glycine / ml), and 3 of them had high proteolytic activity (> 150 μ g glycine / ml).

Determination Decarboxylase Activity of Isolates

Microorganisms which have decarboxylase activity can form biogenic amine by enzymatic decarboxylation of amino acids in foods. Histamine poisoning results from consumption of foods typically certain types of fish and cheeses that contain unusually high levels of histamine. Therefore, decarboxylase activity is an important problem at the selection of culture. In this study Joosten and Northolt (14)'s method was used to determine decarboxylase activity. Biogenic amine forming capabilities of the cultures by using histidine, tyrosine, lysine, ornithine, phenylalanine, tryptophan, amino acids were determined as shown in the Table 2.

Table 2. Decarboxylase activities

Species	Orn	His	Tyr	Trp	Phe	Lys
<i>E. faecalis</i> (n=27)	0	0	25	0	0	0
<i>Enterococcus</i> sp. (n=9)	0	0	7	0	0	0
<i>L. cremoris</i> (n=3)	0	0	0	0	0	0
<i>L. diacetyllactis</i> (n=2)	0	0	1	0	0	0
<i>L. lactis</i> (n=17)	0	0	2	0	0	0
<i>Lactobacillus</i> sp. (n= 13)	0	0	0	0	0	0
<i>Lb. plantarum</i> (n=12)	0	0	3*	2*	0	0

Orn: Ornithine, His: Histidine, Tyr: Tyrosine, Trp: Tyryptophane, Phe: Phenilalanine, Lys: Lysine

*: Weak reaction.

Table 3. Acidification and proteolytic activities of selected isolates

Species	Acidification		Proteolytic activities
	Δ pH (6h)	Δ pH (24 h)	μ g tyrosin / ml
İLc12 (<i>L. lactis</i>)	0.75	2.4	17.3
İLc13 (<i>L. lactis</i>)	0.79	2.52	16.7
İLb74 (<i>Lb. fermentum</i>)	0.7	1.18	21.4
İE33 (<i>Enterococcus</i> sp.)	0.7	2.44	18.2

It was determined that white cheeses include a high proportion of Tyramine (22, 23). Enterococci, lactobacilli and lactococci isolated from cheeses produce the most of tyramine (24). Durlu-Özkaya *et al.* (17) reported that lactococci and lactobacilli strains isolated from white cheese didn't reduce none of histidine, tyrosine, lysine, ornithine, phenylalanine and threonine amino acids, on the contrary all of the enterococci except two of them decarboxylated tyrosine. Similar results were also obtained from Tunail *et al.* (12)'s study. It was reported that tyramine is the only biogenic amine produced by *Enterococcus* sp. in milk (25-27). Tuncer (28) determined that all 36 of 39 enterococci isolates except 3 of them isolated from Tulum cheese, formed tyramine from the tyrosine amino acid. It was determined that determination of decarboxylase activity of strains by using medium does not show very healthy results (12, 24, 29, 30). Kucerová *et al.* (31) assigned that 20 of 33 *Enterococcus* strains isolated from fresh cheese and semi-hard cheese produced by using raw cow's milk, had tyrosine decarboxylase activity. Joosten and Northolt (14) assigned that only 5 *Lb. buchneri* of lactobacilli strains isolated from cheese produced by using raw milk, had histidine decarboxylase activity and one *Lb. brevis* of them had tyrosine decarboxylase activity.

CONCLUSION

As a result, when acid producing capabilities, proteolytic activities and decarboxylase activities of isolates were evaluated, it was determined that İLc12 (*L.lactis*) ve İLc13 (*L. cremoris*) isolates among lactococci, İLb74 (*Lb. fermentum*) isolate among lactobacilli and İE33 (*Enterococcus* sp.) isolate among enterococci showed the best starter characteristics (Table 3.). All of these isolates could reduce < 6 pH in 6 hours, have had moderate proteolytic activity (<20 mg tyrosine / ml) and were decarboxylase negative isolates. To research possibilities of these isolates to be used as a starter culture, in terms of antibiotic resistance, phage susceptibility and aroma substances formation should be assessed.

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