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Amino Acid Decarboxylase Activity of Some Lactic Acid Bacteria

Pelin ERTÜRKMEN^{1*}, İlkyay TURHAN², Zübeyde ÖNER³

¹ Mehmet Akif Ersoy University - Burdur Vocational School of Higher Education, Burdur

² İstanbul Arel University - School of Health Sciences, İstanbul

³ Süleyman Demirel University - Faculty of Engineering, Isparta

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✉ Sorumlu Yazar (Corresponding author)*: perturkmen@mehmetakif.edu.tr

☎ +90 248 2132278 📠 +90 248 2132288

ABSTRACT

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 lactic acid bacteria as a starter culture in fermented products. In this study, decarboxylase activities of 161 lactic acid bacteria (LAB) strains isolated from 20 cheese samples were determined. As a result, 37 of the 59 *Enterococcus* spp. (62.71%), 4 of the 47 *Lactococcus* spp. (8.51%), 4 of the 55 *Lactobacillus* spp. (7.27%) produced tyramine from tyrosine and also 3 of the 55 *Lactobacillus* spp. (5.45%) produced tryptamine from tryptophan. None of the 108 strains (70.19%) decarboxylated ornithine, histidine, tyrosine, tyrtophane, phenylalanine, or lysine. These isolates are thought to be the appropriate starter culture for cheese industry.

Keywords: Decarboxylase activity, lactic acid bacteria, cheese

Bazı Laktik Asit Bakterilerinin Amino Asit Dekarboksilaz Aktivitesi

ÖZ

Dekarboksilaz aktivitesine sahip olan mikroorganizmalar gıdalarda bulunan amino asitleri enzimatik olarak dekarboksile ederek biyogen amin oluştururlar. Gıdalardaki tipik histamin zehirlenmelerinde yüksek düzeyi en çok peynir ve balık içermektedir. Bu nedenle fermente ürünlerde kültür seçiminde dekarboksilaz aktivitesi önemli bir problemdir. Bu çalışmada, 20 adet peynir örneğinden izole edilen 161 laktik asit bakterisinin dekarboksilaz aktivitesi belirlenmiştir. Çalışmanın sonucunda, 59 *Enterococcus* spp.'nin 37'si (62.71%), 47 *Lactococcus* spp.'nin 4'ü (8.51%), 55 *Lactobacillus* spp.'nin 4'ü (7.27%) tirozinden tiramin ve 55 *Lactobacillus* spp.'nin 3 'ü (5.45%) triptofandan triptamin üretmiştir. 108 suşun (70.19%) hiçbirinde ornitin, histidin, tirozin, triptofan, fenilalanin, ya da lizin dekarboksile edememiştir. Bu izolatların peynir endüstrisi için uygun başlatıcı kültür olduğu düşünülmektedir.

Anahtar Kelimeler: Dekarboksilaz aktivitesi, laktik asit bakterileri, peynir

INTRODUCTION

Biogenic amines are low-molecular, toxic and nitrogenous compounds that are formed by decarboxylation of amino acids or amination or transamination of aldehydes and ketones (Turantaş and Özsüz, 1998; Gingerich et al., 1999). The separation of carbon dioxide from the amino acids is called decarboxylation and the formation process of biogenic amines is catalyzed by decarboxylase enzymes. Decarboxylases can be formed by animal tissues, plant tissues and microorganisms. The most important biogenic amines formed in food are histamine, tyramine, putrescine, cadaverine, β -phenylethylamine, tryptamine, spermidine and spermine (Shalaby, 1996; Santos, 1996; Paulsen et al., 1997; Kalač et al., 1999). The production of these compounds proceeds through several steps, starting from proteins via peptides to amino acids, the decarboxylation of which leads to biogenic amine formation (Halász et al., 1994; Buňková et al., 2011). The formation of biogenic amines in food depends on the quantities of free amino acids and the presence of microorganisms with decarboxylase activities (Landete et al., 2008).

The importance of observing biogenic amines content lies in potential toxicity to humans, mainly if the concentration is >100 mg/kg (or >100 mg/L). Thus, the presence of biogenic amines significantly influences the food quality and safety (Christensen et al., 1999; Halász et al., 1994; Santos, 1996; Smit et al., 2005; Buňková et al., 2010). Fermented dairy products and especially cheeses belong to the most common sources of biogenic amines, mainly histamine, tyramine, putrescine and cadaverine (Shalaby, 1996; Valsamaki et al., 2000; Buňková et al., 2010). Histamine and tyramine have been the most studied biogenic amines due to the toxicological effects derived from their vasoactive and psycho-active properties (Karmas, 1981; Vidal-Carou et al., 1990; Bover-Cid and Holzapfel, 1999). Tyramine can lead food-induced migraines and hypertensive crisis in patients under antidepressive treatment with mono-amine oxidase inhibitor (MAOI) drugs (Karmas, 1981; Vidal-Carou et al., 1990; Bover-Cid and Holzapfel, 1999). The excessive intake of histamine could cause e.g. dilatation of peripheral blood vessels, hypotension, urticaria, flushing and headache (Shalaby, 1996; Valsamaki et al., 2000; Buňková et al., 2010).

Microbial strains with high proteolytic enzyme activity also potentially increase the risk of biogenic amines formation in food and food products (Landete et al., 2008). Various bacteria strains have different kind of amino acids decarboxylation activity. Some species have substrate specific decarboxylase activity and

effect only a single amino acids, while some of them decarboxylated several amino acids (Sinell, 1978; Yerlikaya and Gökoğlu, 2002). Microorganisms demonstrating decarboxylase activity can get into foods spontaneously or they might be contained in starter cultures which are commonly used for technological purposes added intentionally to food (Ladero et al., 2012; Santos, 1996; Buňková et al., 2013). The amino acid-decarboxylating activity was reported for some LAB strains like *Bacillus*, *Citrobacter*, *Pseudomonas*, *Klebsiella*, *Clostridium*, *Escherichia*, *Proteus*, *Enterobacter*, *Salmonella*, *Shigella*, *Staphylococcus*, *Photobacterium*, *Streptococcus*, *Lactobacillus*, *Enterococcus*, *Lactococcus* and *Leuconostoc* (Ten Brink et al., 1990). Tyramine biosynthesis capability has been detected in a variety of LAB, including several strains of enterococci and lactobacilli that are present during the manufacture of most cheeses (Joosten and Northolt, 1989; Rea et al., 2004; Komprda et al., 2008; Ladero et al., 2010). These can be present in the raw milk or in the starter culture, and develop as secondary microbiota during the fermentation process (Novella-Rodríguez et al., 2002; Ladero et al., 2010). Therefore, it is recommended to test starter cultures for decarboxylase activity before they are used in dairy industry (Buňková et al., 2011).

The aim of the study is to determine decarboxylase activity of lactic acid bacteria which will be used as a starter culture in dairy industry.

MATERIALS AND METHODS

Materials

The microorganisms used in this study which were produced in laboratory conditions and were supplied from various factories were used as isolation materials. A total of 161 bacterial strains including 59 *Enterococcus* spp., 47 *Lactococcus* spp. and 55 *Lactobacillus* spp. isolated from different cheese were tested.

Methods

Joosten and Northolt's (1989) method was used to determine decarboxylase activity in this study. *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* spp. and *Lactobacillus* spp. were incubated at 30 °C, *Enterococcus* spp. was incubated in the 37 °C. At the end of this period, cell concentration of isolated cultures were measured with MacFarland equipment and the 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 were incubated in the

anaerobic conditions at 30 °C for 7 days (Joosten and Northolt, 1989).

RESULTS AND DISCUSSION

Determination Decarboxylase Activity of Lactic Acid Bacteria Isolates

Biogenic amine forming capabilities of the cultures by using histidine, tyrosine, lysine, ornithine, phenylalanine, tryptophan. Amino acids were determined as shown in the Table 1.

Table 1. Decarboxylase activities result of lactic acid bacteria isolates

Species	Orn	His	Tyr	Trp	Phe	Lys
Enterococcus						
<i>E. faecalis</i> (n=31)	0	0	28	0	0	0
<i>E. durans</i> (n=2)	0	0	1	0	0	0
<i>E. avium</i> (n=2)	0	0	0	0	0	0
<i>E. faecium</i> (n=2)	0	0	0	0	0	0
<i>E. raffinosus</i> (n=1)	0	0	0	0	0	0
<i>E. solitarius</i> (n=1)	0	0	0	0	0	0
<i>P. pentosaceus</i> (n=4)	0	0	0	0	0	0
Lactococcus						
<i>Lc. cremoris</i> (n=7)	0	0	0	0	0	0
<i>Lc. diacetylactis</i> (n=2)	0	0	1	0	0	0
<i>Lc. lactis</i> (n=36)	0	0	3	0	0	0
<i>Lactococcus</i> spp. (n=2)	0	0	0	0	0	0
Lactobacillus						
<i>L. jensenii</i> (n=10)	0	0	0	0	0	0
<i>L. plantarum</i> (n=19)	0	0	3*	3*	0	0
<i>L. curvatus</i> (n=7)	0	0	1*	0	0	0
<i>Lactobacillus</i> spp. (n= 19)	0	0	0	0	0	0

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

*: Weak reaction (red-brown zone).

Enterococci, lactobacilli and lactococci isolated from cheeses mostly produce tyramine (Durlu-özkyaya et al., 1999). In this study, Enterococci and lactococci isolates produced tyramine whereas lactobacilli isolates produced tyramine and tryptophan, mostly (Table 1). 37 of the 59 *Enterococcus* spp. (62.71%), 4 of the 47 *Lactococcus* spp. (8.51%), 4 of the 55 *Lactobacillus* spp. (7.27%) produced tyramine from tyrosine and also 4 of the 55 *Lactobacillus* spp. (7.27%) produced tyramine from tryptophan. Colony growing with yel-

low zone in the medium was evaluated as negative reaction and colony growing with purple zone or transparent zone (only for tyrosine) in the medium was evaluated as positive reaction (Figure 1). Fourth and third of lactobacilli isolates produced red-brown zone from tyrosine and tryptophan, respectively (Figure 2). They were evaluated as weak reaction. None of the 108 strains (70.19%) decarboxylated ornithine, histidine, tyrosine, tyryptophane, phenylalanine or lysine.

Durlu-Özkaya et al. (1999) 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.

The previous studies showed that tyramine was only biogenic amine formed by enterococcal strains (Giraffa et al., 1995; Bover-Cid and Holzapfel, 1999; Sarantinopoulos et al., 2001; Yousif et al., 2005; Tuncer, 2009; Kalhotka et al., 2012), as confirmed in this study. Bover-Cid and Holzapfel (1999) analysed *E. durans* (1), *E. faecalis* (15) and *E. faecium* (10) strains decarboxylase activity and they found that all of the enterococci strains decarboxylated tyrosine. In the same study, It was reported that none of the strains decarboxylated the other amino acids (histidine, lysine and ornithine). In another similar study, a total of 129 food, animal and human origin enterococci strains (*E. faecium*, *E. faecalis* and *E. durans*) were studied and while none of the strains decarboxylated none of histidine, lysine and ornithine amino acids, the majority of the strains decarboxylated tyrosine amino acids (Sarantinopoulos et al., 2001). Kalkotk et al. (2012), researched biogenic amine production properties of enterococcus strains isolated from goat milk and goat cheese on decarboxylase medium containing six different amino acid (arginine, phenylalanine, histidine, lysine, tyrosine and tryptophan) and they reported 8 of 9 enterococci isolates decarboxylated tyrosine. Similar results were also obtained from Tunail et al. (2001)'s study. It was reported that tyramine is the only biogenic amine produced by *Enterococcus* spp. in milk (Celano et al., 1992; Schirone et al., 2012). Tuncer (2009) 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 (Tunail et al., 2001; Durlu-ozkaya et al., 1999; Sumner and Taylor, 1989; Ayhan et al., 2000). Kucerová et al. (2009) 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.

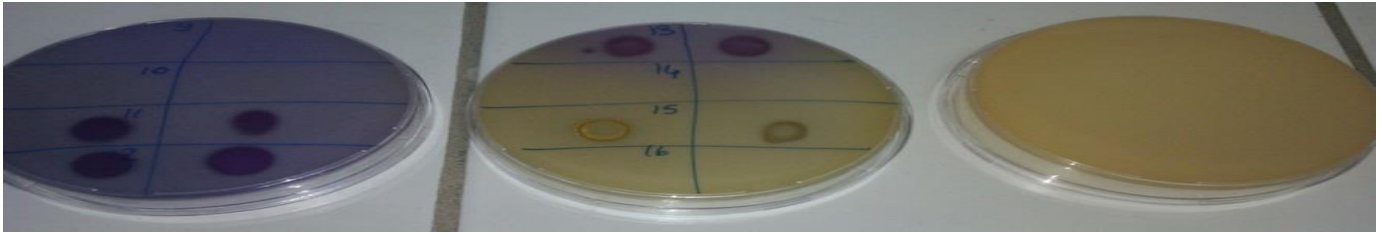


Figure 1. Tyrosin positive medium with purple zone, tyrosin positive medium with transparent zone and tyrosin medium without bacteria strains, respectively.

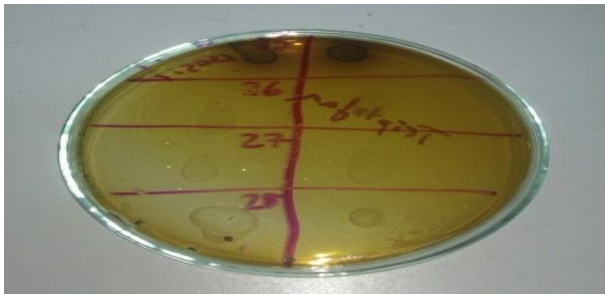


Figure 2. Sample of tyrosine and tryptophan positive-weak reaction with red-brown zone

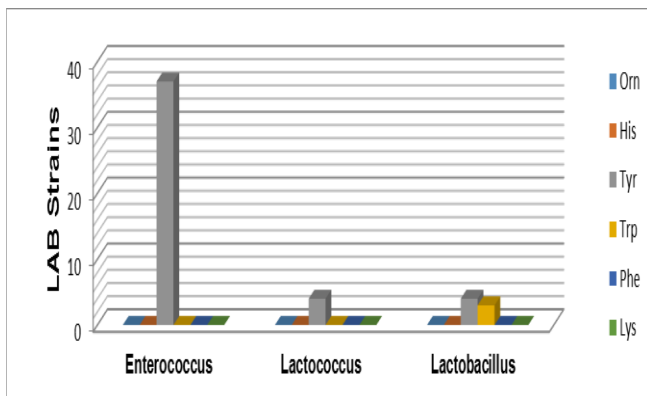


Figure 3. Distribution of decarboxylase activities of LAB strains in cheeses.

These studies indicate that *Enterococcus* strains have a high proportion of tyrosine decarboxylation property. Some authors relate high enterococci counts in milk to the later presence of large quantities of tyramine in cheese (Joosten and Northolt, 1989). In fact, the capacity to decarboxylate tyrosine to tyramine is a general characteristic of the strains belonging to the species *Enterococcus faecalis* (Marcobal et al., 2004; Ladero et al., 2010). In addition, several isolates of *Lactobacillus brevis* and *Lactobacillus curvatus*, both considered to be non starter LAB, have been identified as tyramine producers in fermented products (Roig-Sagués et al., 1999; Komprda et al., 2008; Ladero et al., 2010). Joosten and Northolt (1989) assigned that only 5 *L. buchneri* of lactobacilli strains isolated from cheese produced by using raw milk, had histidine decarboxylase activity

and one *L. brevis* of them had tyrosine decarboxylase activity.

In recent years, molecular methods for detection and identification of biogenic amine producer bacteria are becoming more widely accepted as an alternative to conventional culture methods (Marcobal et al., 2006).

CONCLUSIONS

Lactococci and lactobacilli strains generally showed negative decarboxylase activity with the exception some of lactobacilli strains showed weak tyrosine and tryptophan activities. Almost all enterococci strains showed tyrosine positive reaction and the strains formed purple or transparent zone in the medium. It is determined that enterococci strains generally decarboxylated tyrosine and have potential forming biogenic amine. Therefore enterococci strains have potential risk forming biogenic amine in cheeses. This study indicates that it is important to be considered biogenic amine production features as well as virulence factors of the strains on selection of starter culture during cheese production. The use of enterococcus strains as starter cultures requires a careful evaluation for food safety on fermented products. This will be possible by increasing the number of studies.

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