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Histidine and tyrosine decarboxylase activities of lactic acid bacteria in sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*)

Yağmur Nil DOĞAN[1,a,✉], Şebnem PAMUK[2,b], Zeki GÜRLER[2,c]

[1]Gaziantep University, İslahiye Vocational School, Department of Veterinary, Gaziantep; [2]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Afyonkarahisar, Turkey
[a]ORCID: 0000-0002-1309-0936; [b]ORCID: 0000-0001-7227-3364; [c]ORCID: 0000-0002-9037-2945

✉Corresponding author: yagmurnildemirel@hotmail.com
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Abstract: Biogenic amines (BAs) are formed by decarboxylation of amino acids, amination and transamination of aldehyde and ketone groups. The excess of BAs is harmful to human health. BAs play a significant role in determining the shelf life and quality of foods. Released type and amount of BAs depend on factors such as the quality of the raw material, the diversity of natural microbiota, processing and storage conditions. In fish, the release of BAs is affected primarily from microbial growth as well as other reasons and may cause poisoning. It was aimed to determine the possibility of histidine decarboxylase activity gene (*hdc*) and tyrosine decarbocylase activity gene (*tyrdc*) in lactic acid bacteria (LAB) which were isolated from sea bream and sea bass. A total of 18 Gram positive-catalase negative LAB was isolated from 84 fish samples from 14 different fish markets. It was found that 12 out of 18 LAB (67%) isolates showed negative histidine and tyrosine decarboxylase activities. While 2 out of 6 (11%) LAB isolates were determined positive only tyrosine decarboxylase and 4 of them (22%) were positive for histidine and tyrosine decarboxylase. As a result of the Polymerase Chain Reaction (PCR), 9 out of 12 LAB isolates (75%) were found to have histidine decarboxylase activity gene. As a result, the prevalence of histidine decarboxylase activity gene in the LAB has detected more extensive than tyrosine decarboxylase activity gene. Increasing the studies examining the presence of aminobiogenic microorganisms in fish is important for the protection of public health.

Keywords: Fish, histidine decarboxylase, lactic acid bacteria, public health, tyrosine decarboxylase.

Çipura (*Sparus aurata*) ile levrek (*Dicentrarchus labrax*) balıklarındaki laktik asit bakterilerinin histidin ve tirozin dekarboksilaz aktiviteleri

Özet: Biyojen aminler (BA), aminoasitlerin dekarboksilasyonu, aldehit ve keton grupların aminasyonu ve transaminasyonu sonucu oluşan fazlası insan sağlığına zararlı olan maddelerdir. Gıdaların raf ömrü ve kalitesinin belirlenmesinde biyojen aminlerin rolü büyüktür. Açığa çıkan BA çeşit ve miktarı hammaddenin kalitesi, doğal mikrobiota çeşitliliği, işleme ve depolama şartları gibi faktörlere bağlı olarak değişiklik göstermektedir. Balıklarda başta mikrobiyel üremeye bağlı olmak üzere birçok faktöre bağlı BA oluşabilmekte ve zehirlenmelere sebep olabilmektedir. Bu çalışmada, çipura ve levrek balıklarından izole edilen laktik asit bakterilerinin (LAB) muhtemel histidin dekarboksilaz (*hdc*) ve tirozin dekarbokilaz gen (*tyrdc*) aktivitelerini belirlemek amaçlanmıştır. Toplamda 14 farklı balık marketlerinden toplanan 84 örnekten 18 Gram pozitif katalaz negatif LAB izole edilmiştir. İzole edilen 18 laktik asit bakterisinden 12'si (%67) histidin ve tirozin dekarboksilaz negatiftir. Geriye kalan 6 LAB izolatının 2'si (%11) sadece tirozin dekarboksilaz pozitif, 4'ü ise (%22) histidin ve tirozin dekarboksilaz pozitif belirlenmiştir. Elde edilen toplam 18 LAB izolatının yapılan Polimeraz Zincir Reaksiyonu (PZR) sonucunda 12 laktik asit bakterisinin 9'unun (%75) histidin dekarboksilaz genine sahip olduğu tespit edilmiştir. Sonuç olarak, LAB izolatlarında histidin dekarboksilaz gen prevalansı tirozin dekarboksilaz geninden fazla bulunmuştur. Aminobiyojenik mikroorganizmaların balıklardaki varlığını inceleyen çalışmaların arttırılması halk sağlığının korunması açısından önem arz etmektedir.

Anahtar sözcükler: Balık, halk sağlığı, histidin dekarboksilaz, laktik asit bakterileri, tirozin dekarboksilaz.

Introduction

Biogenic amines (BAs) can be synthesized by plant and animal metabolism or by the production of microbial carboxylation from free amino acids. They are known as potential indicator substances for determining the shelf life and the quality of foods (13, 31). In humans, BAs are used for body activities such as brain activity, the regulation of body temperature and stomach pH, gastric acid secretion, the immune response, cell growth, and differentiation. However, the consumption of foods with high concentrations of BAs can be quite toxic to health (16). Histamine, putrescine, cadaverine, tyramine, β-

phenylethylamine, spermine, and spermidine are the most common BAs in foods (13, 31).

Some of BAs, such as histamine and tyramine, are characterized by the control of the nervous system and blood pressure. While histamine causes a decrease in blood pressure, increased capillary permeability, increased gastric acid secretion, and allergic reactions, tyramine causes hypertension (2, 21, 35). It is reported that histamine is determined for the quality and microbial degradation index in fish and fish products (32, 39, 40). Histamine related poisoning cause gastrointestinal and neurological disorders (17). The maximum limit is recommended as 100-800 mg/kg for tyramine. Normally, human monoamine oxidase (MAO) detoxification system can inhibit small amounts of BAs. However, some people have a genetically or pharmacologically impaired MAO detoxification system. For this reason, the low level of BAs concentration may cause adverse effects such as migraine and, hypertension risk (35, 38).

Fish-borne intoxications are mostly caused by microbial activities or some substances such as BAs. There are considerably the lowest level biogenic amines in the fresh fish. Many *Enterobacteriaceae*, *Clostridium*, *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Streptococcus, Micrococcus,* and *Pseudomonas* are capable for the formation of BAs in food. Therefore, contamination with these microorganisms can take place at each stage during the transport of fish (33). In addition, these microorganisms can be present in microbiota or introduced through contamination before, during or after the processing of products (18). The aminobiogenic microorganisms can cause decarboxylating of free amino acids and lead to the formation of biogenic amines damaging human health (3, 6, 8, 9, 11, 12, 13). Recently, the genes of diverse pathways producing BA were identified in LAB (18). It was defended that the pathways were dependent to strain specific (5). There are some conditions for BA accumulation in foods such as presence of aminobiogenic microorganisms and favourable conditions for their growth (1). Under these conditions, BA production by LAB could be controlled at during fermentation (36). Several authors (3, 15) have reported the presence of tyrosine and histamine decarboxylase activity in LAB. This study aimed to determine whether lactic acid bacteria isolated from sea bream and sea bass have the tyrosine and histidine decarboxylase genes and a possible risk of histamine and tyramine.

Material and Methods

A total of 84 fish samples (42 sea bass and 42 sea bream) were collected from fourteen different fish markets between at October-December 2018 and January 2019. The samples were brought to the laboratory under the cold chain and fish bones and skins were removed. All samples were diluted 10-fold with a sterile 0.85% NaCl solution. Then, 0.1 ml of each dilution was spread onto De Man Ragosa Sharpe (MRS) agar (LAB223) and it was incubated at 37ºC/24-48 hour under anaerobic conditions. After the inoculation, the isolates which were Gram positive and catalase negative isolates were stocked at -18ºC in MRS broths (LAB094) with glycerine until the next step. *Lactobacillus* 30a ATCC 33222 and *Lactobacillus brevis* ATCC 367 were used as positive control strains in each stage of isolation and identification.

***Determination of decarboxylase activities*:** Medium was prepared by the modified Maijala's (23) method (5 g/L tryptone; 4 g/L yeast extract; 8 g/L meat extract; 0.5 g/L Tween 80; 0.2 g/L $MgSO_4$; 0.05 g/L $MnSO_4$; 0.04 g/L $FeSO_4$; 0.1 g/L $CaCO_3$; 20 g/L amino acid; 0.06 g/L bromocresol purple; 20 g/L agar), and pH was adjusted to 5.3. The medium separately was added with tyrosine and histidine as precursors. Besides, the medium was prepared without amino acids to control positive reactions. The isolates were incubated at 37ºC for 24 hours under aerobic conditions. The change of color's medium from yellow to purple showed the presence of amino acid decarboxylase activity (24).

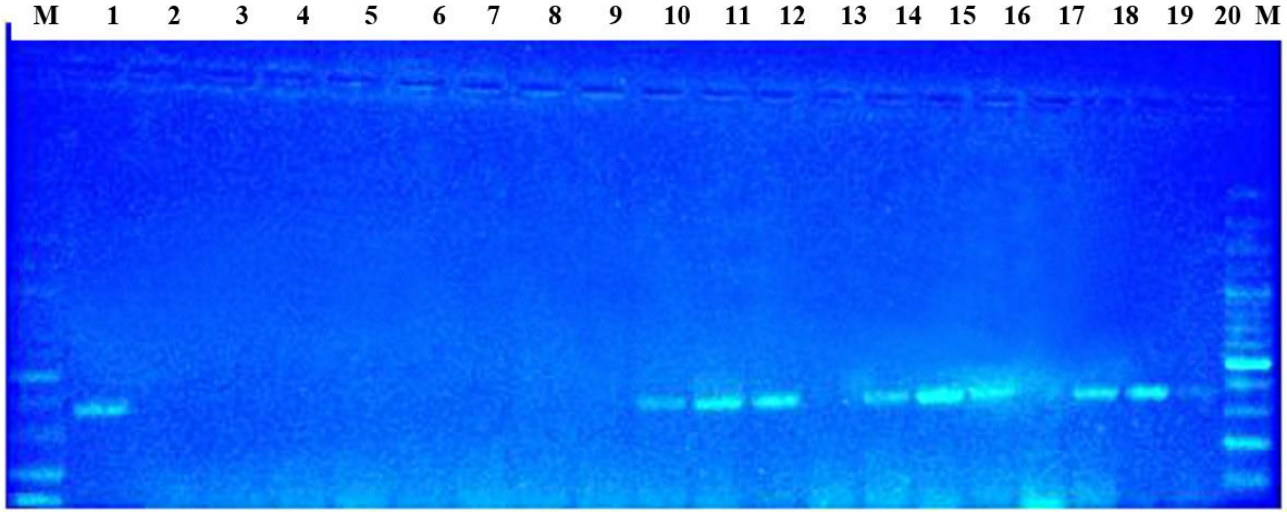
***Verification of lactic acid bacteria by polymerase chain reaction*:** A DNA extraction protocol was performed according to the manufacturer's introduction (Thermo Scientific, K0721). Polymerase Chain Reaction (PCR) amplification of DNA samples was performed (25).The PCR was performed in a 25 µl amplification reaction mixture containing 20 mM Tris-HCl, pH8.0, 50 mM KCl; 2.5 mM $MgCl_2$; each 200 µM dNTP; 1 µM primers, 1U Taq polymerase and 12.5 ng target DNA (25).

Modified methods were used for amplification (4, 25). The reactions were performed in a Thermal Cycler using the following cycling parameters: 5 min for first denaturation at 94°C, 35 cycles of 45 s at 94°C, 45 s at 48°C, 1min at 72 °C, and a final extension step of 7 min at 72°C. The primer pairs used in the amplification process are shown in Table 1. Amplified products were examined on a 1,8% agarose gel with stained ethidium bromide.

Table 1. Oligonucleotide primer pairs used in the PCR method for lactic acid bacteria

Target Gene[a]	Primer Sequence(5'-3')	Amplicon size	Reference
hdc	F: AGATGGTATTGTTTCTTATG	367 bp	Marcobal et al. (25)
	R: AGACCATACACCATAACCTT		
tyrdc	F: GCATACCAGAGTCCCTCAAG	906 bp	Lucas et al. (22)
	R: CGGATACGGACGCACAATTG		

[a]*hdc*, histidine decarboxylase; *tyrdc*, tyrosine decarboxylase



M: 100 bp DNA ladder; 1: *Lactobacillus brevis* ATCC 367; 2-9: negative LAB isolates from sea bass samples; 10-20: LAB isolates from sea bream samples; 10-12; 14-16; 18-20; positive isolates

Figure 1. The tyrosine decarboxylase activity gene PCR gel image of the isolates.

Results

A total of 18 LAB which was Gram positive-catalase negative was isolated from 84 fish samples from different fish markets. Eleven isolates which were obtained from sea bream were evaluated as negative for a histidine and tyrosine decarboxylase activity and one of sea bass sample was negative, too. Two (11%) of the positive isolates showed only a tyramine decarboxylase activity, four (22%) isolates had both histamine and tyramine decarboxylase activities.

According to the PCR study, 18 isolates did not have a *tyrdc* gene (data not shown). *Hdc* gene was determined in 9 of 12 LAB which were shown negative reaction in cultural method for both amino acid. The tyrosine decarboxylase activity gene PCR gel image of the isolates is shown in Figure 1.

Discussion and Conclusion

Histamine formation is the critical control point during the production of fish products (37). It has been found that 7 of 27 LAB isolates showed a decarboxylase activity, especially tyrosine (34). On the contrary, it has not been found any *hdc* gene in 74 LAB isolates. It was identified a *tyrdc* gene in 34 isolates which belonged to *Enterococcus* spp (29). The amino acid decarboxylase capacity depends on the species of microorganisms and environmental factors (8, 9, 26, 28, 34). It is reported that the detection of histamine producing microorganisms is not reliable with classical culture techniques (37). It was widely used in Niven et al.'s (30) modified medium for classical cultural methods (20, 27, 41). However, several researchers (14, 19) reported that cultural methods using Niven et al. (30) medium had a false positive or negative result. It has been concluded that 13 (17%) of lactic acid bacteria isolated from 77 fermented fish products were able to the decarboxylation of amino acids (7). Our findings have a negative relation with Dapkevicius et al. (7)'s study because of the differences between preparation of material and fish product. If LAB does not have a *tyrdc* gene activity, it does not mean that it cannot present tyramine in fish (29). Even though histamine forming microorganisms lose viability in the frozen-thawed fish, histamine can be detected in fish (10). BAs cannot be removed by technological methods such as freezing or heat treatment. Therefore, the absence of aminobiogenic microorganisms in fish does not indicate that the product can be reliable.

According to our results, it is determined that classical culture methods could give false positive or false negative results for the determination of BAs decarboxylase. It is recommended that molecular methods should be used to eliminate this complexity and to take faster results. In sea breams, LAB with histidine decarboxylase is more than sea basses. For this reason, the public should be made aware of the possibility of BA in fish especially during transport to non-coastal areas. It is mainly focused on Gram negative microorganisms related to the formation of biogenic amines in fish. However, it should be kept in mind that LAB may cause unwanted conditions such as the formation of BAs as well as desired properties such as fermentation or bacteriocin production in foods. Therefore, it is important for public health to know the aminobiogenic LAB isolated from fish. To prevent BA formation and to ensure food safety, it is required that hygienic quality during hunting, processing, distribution, storage conditions, and marketing should be

taken into consideration. Especially, during transport to non-coastal areas, there is a need that fish and fish products must be brought under controlled conditions.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors have no conflicts of interest relevant to this article.

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