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Genetic screening of biogenic amines production capacity from some lactic acid bacteria strains

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ABSTRACT

There is an increasing interest for using lactic acid bacteria (**LAB**) as a starter and adjunct cultures for producing novel foods with particular functional traits. The ability of the starter to produce biogenic amines (**BA**) should be taken into account wherein protective starters should be selected to avoid hygienic and toxicological risks. This work aimed to study the possibilities of forming BA (histamine, putrescine, agmatine and tyramine) from thirty two LAB strains belonging to species of the genera *Lactobacillus* and *Streptococcus* that used in food products as well as strains isolated from healthy breast-fed infants. The analytical protocol involved using polymerase chain reaction (PCR) and thin layer chromatography (TLC) techniques to determine the ability of LAB strains to form BA. The presence of key genes involved in the biosynthetic pathways of the BA was also assessed by PCR. Six LAB strains gave positive results for putrescine production wherein the maximum level was 14.6 mg/kg. Six strains gave positive results for histamine production (maximum level was 31.7 mg/kg) and were positive for the presence of histidine decarboxylase (HDC) gene. Seven strains exhibited positive results for tyramines production (maximum level was 2.85 mg/kg) and were positive for the presence of tyrosine decarboxylase (TDC) gene. Eight strains gave positive results for agmatine production (maximum level was 174.5 mg/kg) and were positive for the presence of dihydrolase (deiminase) gene that responsible for agmatine formation. It could be concluded that the microorganisms used in food and dairy production should be screened carefully by PCR for their ability to produce BA.

1. Introduction

Biopreservation refers to the processes in which the extension offood shelf life and safety improvement are obtained using microorganisms (Papagianni, 2012; Tabanelli et al., 2014). Interest has been posed on protective strains, which are selected food-grade bacteria, due to their antagonistic properties rather than for their influence on organoleptic or nutritional values (Rodgers, 2001). Lactic acid bacteria (**LAB**) are often used as biopreservers because they can produce antimicrobial metabolites, such as organic acids, antifungal peptides and bacteriocins without safety implications (Ghanbari, Jami, Domig, & Kneifel, 2013; Tabanelli et al., 2014). LAB major function is the rapid production of lactic acid from lactose, resulting in low pH (Ladero et al., 2015). Safe history of applying LAB in food products has resulted in the assignment of Qualified Presumption of Safety (QPS) status [awarded by the European Food Safety Authority (EFSA)] to the majority of LAB strains. However, some enzymatic activities can induce toxic compounds such as biogenic amines (**BA**), wherein the presence of BA should be avoided in food (Linares et al., 2012).

BA are naturally occurring low molecular weight compounds involved in different biological activities in living organisms. BA-contained food, however, can trigger human health problems leading to hypertension, headaches and flushing (Elsanhoty, Mahrous, & Gohnamy, 2009; Lonvaud-Funel & Joyeux, 1994). Studies supported the view that BA are formed in winemaking mainly by LAB due to the decarboxylation of free amino acids (Costantini, Cersosimo, Del Prete, & Garcia-Moruno, 2006; Lucas, Claisse, & Lonvaud-Funel, 2008; Landete, de las Rivas, Marcobal, &

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Muñoz, 2008). In the case of fermented foods, some LAB are able to convert amino acid precursors into BA *via* decarboxylase or deiminase activities during or following fermentation. LAB can produce BA in fermented foods and beverages including meat (Aymerich et al., 2006; Bover-Cid, Hugas, Izquierdo-Pulido, & Vidal-Carou, 2001; Ruiz-Capillas & Jimenez-Colmenero, 2004; Suzzi & Gardini, 2003), cheese (Burdychova & Komprda, 2007; Fernández, Linares, Rodríguez, & Alvarez, 2007**),** wine (Izquierdo Canas et al., 2009; Moreno-Arribas & Polo, 2008; Moreno-Arribas, Polo, Jorganes, & Munoz, 2003) and cider (Del Campo, Lavado, Duenas, & Irastorza, 2000; Garai, Duenas, Irastorza, Martin-Alvarez, & Moreno-Arribas, 2006, 2007).

In a survey of 118 wines from different wine-producing areas of Southwest France, Coton, Rollan, Bertrand, and Lonvaud-Funel (1998) found almost half of the tested wines possessed bacteria carrying the histidine decarboxylase (**HDC**) gene. Among LAB, many strains are endowed with high decarboxylating potential. For example, Straub, Kicherer, Schilcher, and Hammes (1995) reported that some *Lactobacillus buchneri* strains may form putrescine and cadaverine. Gonzalez de Llano, Cuesta, and Rodriguez (1998) described two strains of *Leuconostoc* showing tyrosine decarboxylase (**TDC**) activity. *Enterococci* are known as the most efficient tyramine producers in fermented foods (Ladero et al., 2012; Marcobal, De Las Rivas, Landete, Tabera, & Muñoz, 2012; Tabanelli et al., 2014). In addition, the presence of efficient histaminogenic strains of *Streptococcus thermophilus* has been reported (Calles-Enríquez et al., 2010; Tabanelli, Torriani, Rossi, Rizzotti, & Gardini, 2012; Trip, Mulder, Rattray, & Lolkema, 2011).

The main BA produced by LAB species are histamine *via* **HDC** (Coton et al., 1998; Lonvaud-Funel & Joyeux, 1994), tyramine *via* tyrosine decarboxylase (**TDC**) (Lucas, Landete, Coton, Coton, & Lonvaud-Funel, 2003), putrescine *via* ornithine decarboxylase (**ODC**) (Arena & Manca de Nadra, 2001) and agmatine *via* agmatine deiminase (**AgDI**) pathway (Arena & Manca de Nadra, 2001; Lucas et al., 2007). Many factors may affect BA production, including food physicochemical traits (i.e., pH and temperature), raw material quality, manufacturing processes, presence of decarboxylase-positive microorganisms, and availability of free amino acids (Linares et al., 2012).

After food consumption, BA are commonly metabolized in the human gut to physiologically less active forms through the activity of the amine oxidizing enzymes, monoamine and diamine oxidases. Therefore, consumption of BA-contained food or beverages could have toxic impact such as hypertension, cardiac palpitations, nausea, diarrhea, flushing, and localized inflammation (Suzzi & Torriani, 2015). Therefore, assessing the potential risk of BA accumulation in food at an early stage of production will assist in managing the fermentation process in order to reduce the spoilage.

The selection of strains with no BA-producing capacity would be a good starting point for reducing BA formation in food products. Qualitative and quantitative tests to determine BA have been reported, whereas most of tests involved the measurement of amino acid decarboxylase-positive single strain which isolated from food (Maijala & Eerola, 1993). Some methods tested the capacity of LAB to produce BA, including the use of differential media and pH indicators (Bover-Cid & Holzapfel, 1999). However, the strong acidification of the medium occasioned by LAB can affect the result. Moreover, these methods target the presence of amino acid decarboxylases and do not test the presence of deimination routes involved in the production of BA such as putrescine (Linares et al., 2012). The method used in detecting microorganisms that have amino acids decarboxylases and agmatine deiminase cannot determine the final BA levels, but the risk of BA spoilage is linked to the presence of the genes in the bacteria population (Lucas et al., 2008). Marcobal, de las Rivas, and Muñoz (2006) developed detecting methods of tyramine-producing microorganisms. These culture-independent methods were specific, sensitive, rapid, and were subject to less variability than phenotypic characterization. Ladero, Martínez, Martín, Fernández, and Alvarez (2010) described the real time PCR assay that allows the quantification of tyramine-producing microorganisms in cheese. Culture-independent methods based on PCR techniques, aimed to detect the genetic determinants involved in the synthesis of BA, are now regarded as the most suitable for screening collections of isolates. Agreement between the results obtained by analytical and molecular methods strengthens the case for the use of the latter (Ladero et al., 2012; 2015).

This work aimed to detect specific LAB strains that have coding genes for the enzymes involved in BA production. LAB strains were screened for BA production using both polymerase chain reaction (PCR) and thin layer chromatography (TLC) methods.

2. Materials and methods

2.1. Materials

2.1.1. Strains and media

Strains including *Lactobacillus acidophilus* p2, 4, 5, 6, 7, 8, 9, 112, 106; *Lactobacillus plantarum* p1, *Lactobacillus brevis* p102, *Lactobacillus pentosus* p160, *Enterococcus faecium* p187, and BL *bifidobacterium longum* were identify by Khalil et al. (2007). Strains were isolated from healthy breast-fed infants (15–30 days old) and used after the selection had been done according to Bergey's Manual of Determinative Bacteriology (Holt, Krieg, Sneath, Staley, & Williams, 1994) with identification by SDS-PAGE technique and API System. Table 1 presents the origin and incubation conditions of various strains used in the study. The strains were tested for their probiotic characteristic such as gastric acid resistance, bile salt tolerance, antibacterial activity, adhesion to human mucus. *Lactobacillus* strains were cultivated in MRS (de Man Rogosa Sharpe) broth (Lab M, IDG, UK) and incubated at 37 °C in BBL anaerobic jar (Becton Dickinson Microbiology Systems, Sparks, MD) provided with disposable BBL gas generating pack (CO_2 system envelopes, Oxoid Ltd., West Heidelberg, Victoria, Canada). All LAB strains under investigation were grown at 30 °C in MRS broth supplemented with or without 2.5 g/l L-tyrosine disodium salt (Sigma–Aldrich, USA), 2.5 g/L L-histidine mono hydrochloride, 2.5 g/L L-ornithine mono-hydrochloride, 2.5 g/L L-lysine mono hydrochloride and/or 1 g/L agmatine sulfate salt (Sigma, USA). Cultures were incubated without agitation for 24 h to 8 days according to the species.

2.2. Chemicals and reagents

Amines (histamine, putrescine and tyramine) as their crystalline hydrochlorides, dansyl chloride (5-dimethylaminonaphtalene-1-sulphonyl) and TLC plates (20 × 20 cm aluminum sheets coated with 0.20 mm silica gel G-60) were obtained from Merck (Merck, Darmstadt, Germany).

2.3. Detection of BA-producing strains

2.3.1. DNA extraction

DNA was extracted from pure cultures cells wherein 2 mL of culture were harvested by centrifugation at 13.000 × g for 15 min. The pellet was suspended in 600 μL of TE buffer (Tris–HCl 10 mM, EDTA 1 mM) containing lysozyme (10 mg/mL) and incubated at 37 °C for 30 min. The extraction was continued according to De et al. (2010). The final pellet was dissolved in 50 μL Tris–EDTA (10:1, pH 8) and stored frozen at −20 °C till further analysis.

Table 1
Origin and incubation conditions of bacterial strains under study.

Strains	Origin	Growth temperature (°C) and O_2 need
Lactobacillus acidophilus ATCC 20552	1	37 °C Anaerobic
Lactobacillus paracasei TISTR 453	2	30 °C Aerobic
Lactobacillus rhamnosus TISTR 541	2	37 °C Aerobic
Lactobacillus salivarius TISTR 390	2	37 °C Aerobic
Lactobacillus acidophilus TISTR 450	2	37 °C Anaerobic
Lactobacillus johnsonii ATCC 33200	3	30 °C Aerobic
Lactobacillus casei DSMZ 20011	4	30 °C Aerobic
Lactobacillus acidophilus DSM 9126	4	37 °C Aerobic
Lactobacillus acidophilus DSM20079	4	37 °C Aerobic
Lactobacillus acidophilus DSM20242	4	37 °C Aerobic
Lactobacillus sanfrasiscensis DSM20451	4	37 °C Aerobic
Lactobacillus plantrum	3	30 °C Aerobic
Bifidobacterium infantis DSMZ 20088	4	37 °C Anaerobic
Bifidobacterium angulatum DSMZ 20098	4	37 °C Anaerobic
Lb. delbrueckii subsp *bulgaricus*	3	37 °C Aerobic
Lactobacillus acidophilus p 2	5	37 °C Anaerobic
Lactobacillus acidophilus p4	5	37 °C Anaerobic
Lactobacillus acidophilus p5	5	37 °C Anaerobic
Lactobacillus acidophilus p 6	5	37 °C Anaerobic
Lactobacillus acidophilus p7	5	37 °C Anaerobic
Lactobacillus acidophilus p 8	5	37 °C Anaerobic
Lactobacillus acidophilus p 9	5	37 °C Anaerobic
Lactobacillus acidophilus p112	5	37 °C Anaerobic
Lactobacillus acidophilus p106	5	37 °C Aerobic
Lactobacillus plantarum p1	5	37 °C Aerobic
Lactobacillus brevis p102	5	30 °C Aerobic
Lactobacillus pentosus p160	5	30 °C Aerobic
Enterococcus faecium p187	5	42 °C Aerobic
Bifidobacterium longuium	5	37 °C Anaerobic
Lactobacillus brevis	5	30 °C Aerobic
Lactobacillus buchneri	5	37 °C Aerobic
Streptococcus thermophilus	5	37 °C Aerobic

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2. Thailand Institute of Scientific and Technological Research, Bangkok (Thailand).
3. American Type Culture Collection, Manassas, VA (USA).
4. Deutsche Sammlung von Mikroorganismen und Zellkulturen Gmbh (DSMZ, Germany).
5. Institute of Genetic Engineering and Biotechnology, Industrial Biotechnology Department, Food and Dairy Biotechnology Branch, Sadat City University (Egypt).

2.3.2. Quantification of genomic DNA

Genomic DNA concentration was determined by recording the absorbance at 260 nm (A_{260}) using a Nanodrop spectrophotometer (Wilninggto, DE, USA) according to producer's instructions. DNA purity was measured using the appropriate ratio of OD260:OD280 (1.65–1.85). Concentrations (μg/L) and A_{260}/A_{280} readings were recorded for each sample (Sambrook, Fritsch, & Maniatis, 1989). The quality of the isolated DNA was also evaluated by gel electrophoresis (0.9% agarose) using 2 μL of isolated DNA. The type of band pattern indicates the quality of the DNA isolated. A known amount of bacteriophage lambda DNA was used to compare the intensity and approximate size of the isolated DNA. The extracted DNA concentration was measured and adjusted by dilution to 20–25 μg/L prior to PCR using bi-distilled, deionized and sterile water (Fluka, Germany).

2.3.3. Oligonucleotide primers

The primers used in the study together with their target specific part of the investigated DNA are listed in Table 2. All primers were synthesized by Biosynthesis (USA) and obtained in a lyophilized state. All primers were dissolved before use to obtain a final concentration of 20 pmol/L for each.

2.3.4. DNA amplification and PCR condition

PCR was carried out on thermo cycler (Biometra, T1) using a prepared master mix. Amplification by PCR is performed in 25 μL reaction mixture containing 12.5 ng of template DNA, 20 mM Tris–HCl, pH 8, 50 mM KCl, 2.5 mM $MgCl_2$, 200 μM of dNTP, 1 mM of each primer, and 1 U of DNA polymerase wherein water was added to reach 25 μL. Oligonucleotide primer sequences for the amplification of internal fragments of the genes coding histidine, tyrosine, ornithine decarboxylase, and agmatine deiminase by PCR have been designed by research groups. The reactions were performed according to the cycling parameters given in Table 3. The PCR products are analyzed by electrophoresis in a 1.5% agarose gel and revealed under UV after staining with ethidium bromide.

2.4. Detection of specific BA-producing strains

2.4.1. Detection of histamine-producing strains

The methods described by Coton and Coton (2005) with the specific primers HDC3/HDC4 was used to detect the ability of strains to produce histamine. The hdcA gene was amplified as codes to detect HDC.

2.4.2. Detection of tyramine-producing strains

To detect the tyrosine decarboxylase (TDC) gene in the strains under investigation, the TDC gene was used according to Marcobal, Las Rivas, Moreno-Arribas, and Muñoz (2004).

2.4.3. Detection of putrescine-producing strains (via ornithine decarboxylase)

ODC gene that used as code for conversion of ornithine to putrescine was detected in the stains under investigation according to Marcobal et al. (2004).

Table 2
Oligonucleotide primer pairs sequence and their target elements.

Primer	Sequence	Fragment length	Target element	References
hdcAf/hdcAr	5′-ATGAAGCCAGGACAAGTTGG 3′ 5′-AATTGAGCCACCTGGAATTG 3′	84 bp	Histidine decarboxylase gene	Coton and Coton (2005)
tdcf/tdcr	5′-CAAATGGAAGAAGAAGTTGG 3′ 5′-GAACCATCAGCA ACAATGTG 3′	213 bp	Tyrosine decarboxylase (tdc) gene	Lucas et al. (2008).
HDC3/HDC4	5′-GATGGTATTGTTTCKTATGA 3′ 5′-CCAAACACCAGCATCTTC 3′	435 bp	Histidine decarboxylase (hdc) gene	Coton and Coton (2005)
Odcf/Odcf	5′-TGCA CTTCCATATCCTCCAG-3′ 5′-GAATTTCTGGAGCAAATC CA3′	127 bp	Ornithine decarboxylase gene	Granchi et al. (2006) Nannelli et al. (2008)
AGD1f/AGDIR	5′-GAACGACTAGCAGCTAGTTAT-3′ 5′-CCAATAGCCGATACTACCTTG-3′	90 bp	Agmatine dihydrolase (deiminase) gene	Lucas et al. (2007)
ODF/ODR	5′ CATCAAGGTGGACAATATTTCCG 3′ 5′ CCGTTCAACAACTTGTTTGGCA 3′	500 bp	Putrescine gene	Granchi et al. (2006)

Table 3
Time/temperature profiles for qualitative PCR with DNA extracted from microorganisms using the primer pairs described in Table 2.

Primer pair	Initial denaturation	Denaturation	Annealing	Extension	Cycles	Reference
HDC3/HDC4	95 °C, 30 s	95 °C, 30 s	48 °C, 45 s	72 °C, 2 min	35	Coton and Coton (2005)
hdcAf/hdcAr	95 °C, 5 min	95 °C, 30 s	55 °C, 30 s	72 °C, 30 s	40	Coton and Coton (2005)
tdcf/tdcr	95 °C, 30 s	95 °C, 30 s	52 °C, 30s	72 °C, 2 min	30	Marcobal, de las Rivas, Moreno-Arribas, and Muñoz (2005)
p1rev/p0303	95 °C, 1 min	95 °C, 30 s	50 °C, 1 min	72 °C, 1min	35	Landete et al. (2007)
Agdif/agdif	95 °C, 5 min	95 °C, 30 s	55 °C, 30 s	72 °C, 2 min	35	Lucas et al. (2007)
ODCF/OCDR	95 °C, 5 min	95 °C, 30 s	55 °C, 30 s	72 °C, 30 s	40	Granchi et al. (2006) Nannelli et al. (2008)

2.4.4. Detection of agmatine dihydrolase (deiminase) in the strains

The primer pair AGD1f/AGDIR was used for detection of agmatine dihydrolase (deiminase) gene according to Lucas et al. (2007).

2.5. BA extraction and determination

2.5.1. BA extraction

To extracted and determined histamine, putrescine, agmatine and tyramine in all homogenized tested samples, the method described by Maijala and Eerola (1993) was applied. Briefly, samples (100 mL of microbial culture) were centrifuged and the supernatants were blended with 125 mL of trichloroacetic acid (TCA, 5%) for 3 min using a Warning blender. The homogenized sample was filtered using Whatman (No. 1). The filtrate (10 mL) was transferred into a glass tube with 4 g NaCl and 1 mL of NaOH (50%), then shacked and extracted with (3 × 5.0 mL) *n*-butanol:chloroform (1:1, v/v). The upper layers were transferred to separating funnel (100 mL) with 15 mL *n*-heptane and extracted with HCl (0.2 N, 3 × 1.0 mL). HCl layers were collected in a glass tube and evaporated to dryness at 95 °C with aid of a gentle current of air, submitted to dansylchloride derivatization (1 h at 55 °C) for formation of dansylamines. Saturated $NaHCO_3$ solution (0.5 mL) was added to the residue of both samples and working standards then carefully mixed. Dansyl chloride solution (1 mL) was added and mixed thoroughly using vortex mixer. The mixture was incubated at 55 °C for 45 min. Distilled water (10 mL) was added to the dansylated mixture and carefully mixed. The mixture was extracted with diethyl ether (3 × 5.0 mL). The ether layers were carefully evaporated at 35 °C with aid of current air. The dry film was kept under −20 °C for TLC analysis of BA.

2.5.2. Determination of dansylamines by TLC

One-dimensional silica gel G60 TLC plates (20 × 20 cm) (Merck, Darmstadt, Germany) were used for the chromatographic separation of the dansylamines. The dry film of standards and samples were dissolved with 500 μL methanol. The dissolved standards and samples (10 μL) were spotted. The plate was developed using chloroform: benzene: triethyl amine (6: 4.5: 1, v/v/v). The plate was dried at room temperature and visualized using UV lamp (365 nm). The resulting spots were marked and the marked areas were determined using CS-9000 Dual wavelength flying spot scanning densitometer (SHIMADZU) at 254 nm. Standard curve of each dansylamine was used to calculate the concentrations of BA in the tested samples.

3. Results and discussion

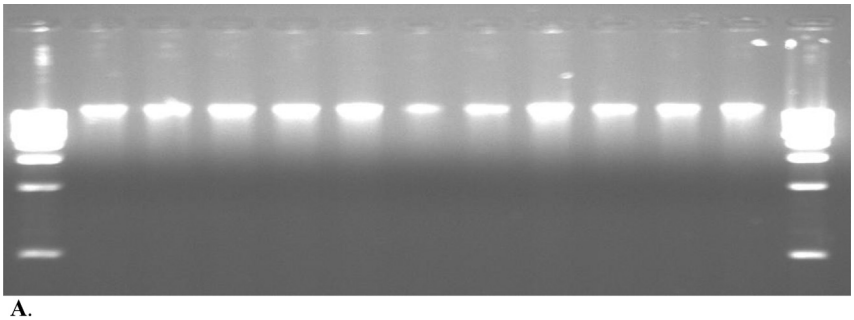
3.1. DNA isolation

Three steps were used to detect genes that gave microorganisms the ability for BA production. The first step involves DNA extraction and amplification of specific microorganisms sequence (16s r DNA) from DNA which is necessary to discriminate between negative and positive results due to inhibition in the amplification (Gezginc, Akyol, Kuley, & Zogul, 2013). The second step entails amplification of specific sequences, represented by HDC, TDC, ornithine decarboxylase (ODC), agmatine dihydrolase (deiminase) and putrescine genes. The quality of the extracted DNA from the strains using CTAB method was examined by gel electrophoresis. DNA bands of high intensity appeared in the lanes (Fig. 1A), showing high yield of DNA. The results presented in this study confirmed that the CTAB protocol can be used for DNA extraction and purification from the strains as a first step of detection of different decarboxylase genes. After gel electrophoresis, the DNA was present as a high molecular weight band from all strains (Fig. 1B).

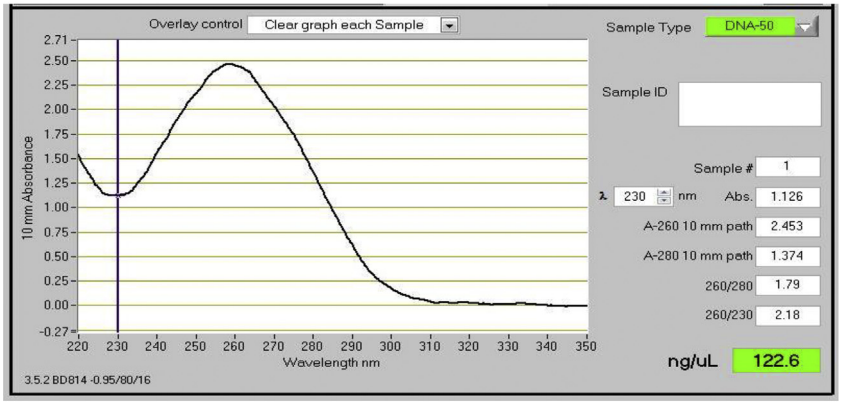
3.2. Detection of BA genes

3.2.1. Detection of HDC and TDC genes

The detection sensitivity of BA-producing bacteria in the first stages is important for preventing BA production and accumulation in food. The application of conventional culture technique for detecting BA-producing bacteria is often tedious and unreliable. On the other hand, it is more important to find a good correlation



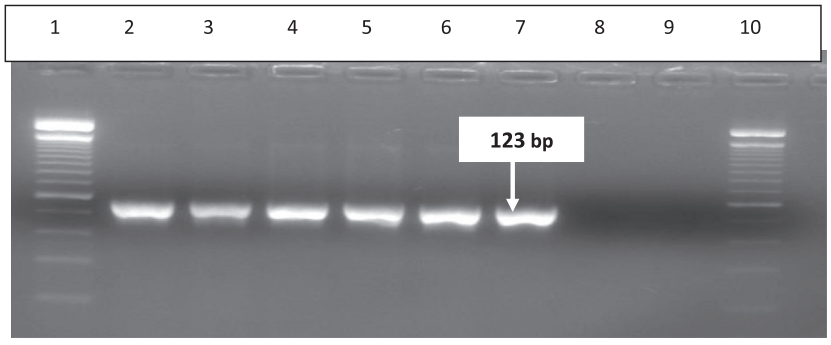
A.



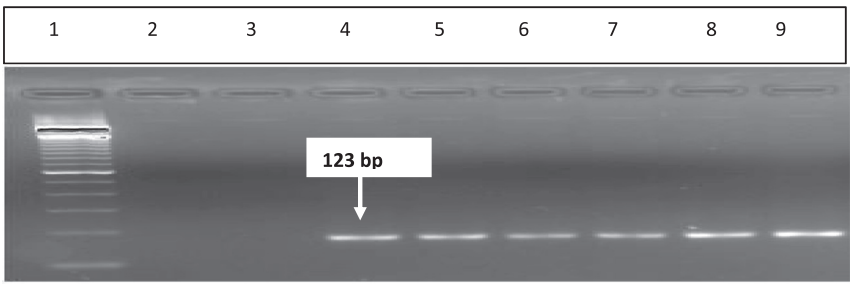
B.

Fig. 1. A. DNA electrophoresis on agarose gel for DNA extracted from different strains. B. Example of the results obtained by NANODROP 2000 spectrophotometer for DNA amount of extracted DNA from some strains using of CTAB method.

between histamine production and the presence of HDC gene. To detect HDC gene in the strains under investigation, the primer pair HDC3/HDC4 was used, wherein the positive samples gave PCR products at 435 bp (Coton & Coton, 2005). Fig. 2A presented the



A.



B.

Fig. 2. A. PCR results for the presence of genes involved in BA production. Results obtained by the primer pair HDC3/HDC4 for *S. thermophiles* (lane 2), *L. lactis* (lane 3), *L. brevis* (lane 4), *L. pentosus* p160 (lane 5), *Enterococcus faecium* (lane 6) and *L. rhamnosus* TISTR 541 (lane 7). B. Results of PCR tests for the presence of genes involved in BA production. Positive results obtained by primer pair hdcAf/hdcAr for DNA ladder 50 bp (lane 1), *S. thermophiles* (lane 4), *L. lactis* (lane 5), *L. brevis* (lane 6), *L. pentosus* p160 (lane 7), *Enterococcus faeciumand* (lane 8) and *L. rhamnosus* TISTR 541 (lane 9).

positive results obtained using the primer pair HDC3/HDC4 (lines 2, 3, 4, 5, 6, 7). To confirm the obtained results, the primer pair hdcAf/hdcAr was used because it is more sensitive wherein the PCR reveal at 89 bp as shown in Fig. 2B. From the results in the same figure, we could see that only six strains (*S. thermophilus, Lactobacillus lactis, L. brevis, L. pentosus* p160, *Enterococcus faeciumand* and *Lactobacillus rhamnosus* TISTR 541) gave positive results for the presence of HDC gene.

Data in Fig. 3A show the results obtained by the primer pair tdcf/tdcr to detect TDC gene. The PCR amplicon appear at 213 bp. The same strains that showed positive results for the presence HDC gave also positive results for the presence TDC gene. The obtained results were in agreement with the results of Gezginc et al. (2013) who reported that *S. thermophilus* isolates have the ability to form BA, especially histamine and tyramine. Similar results were obtained by Lorencová et al. (2012) who found that some strains (*Bifidobacterium* and *L. rhamnosus*) can produce BA. The three genes oriented polycistronic HDC cluster (HDCAPB) were identified in *S. thermophilus*. Moreover, Lucas et al. (2007) indicated that the presence of TDC gene was a strain-dependent trait in *L. brevis* strains isolated from wines.

The cluster began with the HDCA gene, followed by a transporter HDCP gene and ended with HDCB gene, which catalyzes maturation of the pyruvoyl-dependent hdcA (Calles-Enriquez et al., 2010). The gene order of HDCAPB operand is similar to *Staphylococcus capitis* and *Clostridium perfringens*, which, however, lack HDCB (De las Rivas, Rodriguez, Carrascosa, & Munoz, 2008). HDCB is a functional enzyme and substoichiometric amounts of HDCB were required to cleave HDCA (Trip et al., 2011). The HDC cluster is located either on the plasmid or on the chromosome. In *Lactobacillus hilgardii* and *Tetragenococcus halophilus* strains, HDC cluster are located on the plasmid (Satomi, Furushita, Oikawa, Yoshikawa-Takahashi, & Yano, 2008), while in *S. thermophilus* strain the cluster is located on the chromosome (Calles-Enriquez et al., 2010). The sequence of HDC cluster has homology with a phase resistance pER35 plasmid from *S. thermophilus*. In addition, TDCA gene was identified in *S. thermophilus* strains (La Gioia et al., 2011). These similarities point to horizontal transfer of HDC cluster and TDCA in *S. thermophilus* and the strains that gave positive results was similar with gene sequence showed similarity with TDCA and HDC genes. The HDCA enzyme was synthesized in milk even in the absence of histidine (Rossi et al., 2011) which means that enzyme activity was not significantly different in cell-free extract obtained from cultures grown with or without histamine.

3.2.2. Detection of ODC and agmatine dihydrolase genes

The primer Odcf/Odcf was used to detect ODC gene wherein PCR products were appear at 127 bp (Granchi, Paperi, Rosellini, & Vincenzini, 1998). In addition, the primer AGD1f/AGDIR was used to detect the agmatine dihydrolase gene wherein PCR products reveal at 90 bp (Lucas et al., 2007). Data in Fig. 4A showed the results obtained from tested stains (lanes 4 and 5 for *Lactobacillus paracasei*, lanes 6 and 7 for *Lactobacillus casei*, lanes 8 and 9 for *L. brevis*, and lanes 10 and 11 for *S. thermophilus*). The reaming stains did not give positive results. The results of our study show that only 4 strains have the gene for ODC. Similar results were observed by Landete, de Las Rivas, Marcobal, and Muñoz (2007) who found that some LAB species such as *L. casei, L. paracasei* and *Oenococcus oeni* have the genetic capability for BA production (Moreno-Arribas & Polo, 2008). The primer pair AGD1f/AGDIR was used to detect agmatine dihydrolase (deiminase) gene. Fig. 4B presented the positive results revealed from PCR. The same strains that showed positive results for the presence of ODC gene also gave positive results for the presence of deiminase gene. The obtained results were in agreement with Landete et al. (2007) who detected genes for BA production in *Lb. delbrueckii* subsp. *Bulgaricus*. On the other hand, many other *lactobacilli* can produce BA, including tyramine such as some strains of *L. brevis* or *L. paracasei* (Landete et al., 2008). Some studies have shown the existence of a strain-dependent ability to form BA such as *O. oeni* strains which described as being histamine- or putrescine-formers (Coton et al., 1998; Marcobal et al., 2006). *L. hilgardii* strain (IOEB 0006) was also described as being a histamine-former; in this case the amine producing pathway was harbored by an unstable plasmid (Lucas, Wolken, Claisse, Lolkema, & Lonvaud-Funel, 2005).

Data in Fig. 5 presented the obtained PCR results. From the date in Fig. 5 we could be concluded that the putrescine gene was detected in *L. paracasei*, *L. casei*, *L. brevis*, *S. thermophilus, E. faecium,* and *Lb. delbrueckii* subsp. *bulgaricus*. The obtained results were in agreement with Costantini, Pietroniro, Doria, Pessione, and Garcia-Moruno (2013) who found that the majority of *L. brevis* analyzed harbor both AgDI and TDC genes were tyramine and putrescine producers. Conversely, among the other tested LAB, only one *L. hilgardii* and one *Pediococcus pentosaceus* produced putrescine. The AgDI gene was also detected in two other LAB (*Lactobacillus mali* and *Pediococcus parvulus*), but no putrescine production was detected. The HDC gene and histamine production were found in the strains. The obtain results were in agreement with a previous report (Costantini et al., 2006), wherein the presence of HDC gene *in L. hilgardii* 5211 strain isolated from wine was confirmed. The HDC gene was also detected in *L. casei* 18 isolated from cider. These two strains were not putrescine producers.

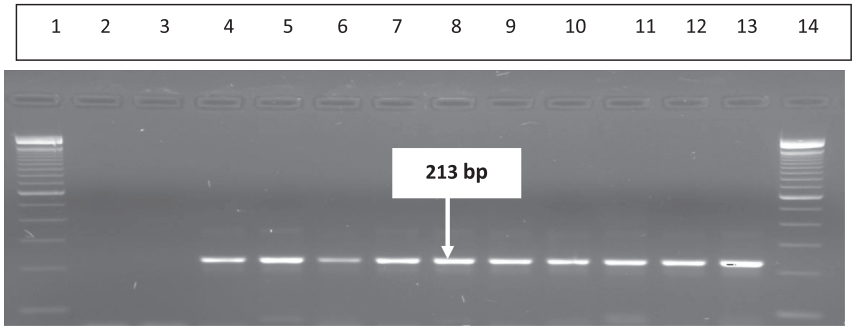


Fig. 3. Results of PCR tests for the presence of genes involved in BA production. Positive results obtained using the primer pair hdcAf/hdcAr for identification of TDC gene; lanes 1 and 14: DNA ladder 100 bp; lane 2 and 3 PCR control negative; lanes 4 and 5, PCR products by DNA from *S. thermophiles*; lanes 6 and 7, PCR products by DNA from *L. brevis* p102 and *L. brevis*; lanes 8 and 9, PCR products by DNA from *L. pentosus* p160 and *Enterococcus faecium;* lanes 10 and 11, PCR products by DNA from *L. rhamnosus* TISTR 541 and *lactobacillus casei* DSMZ 20011; lanes 11 and 12, PCR products by DNA from *L. Paracasei* TISTR 453 and *Lb. delbrueckii* subsp. *bulgaricus.*

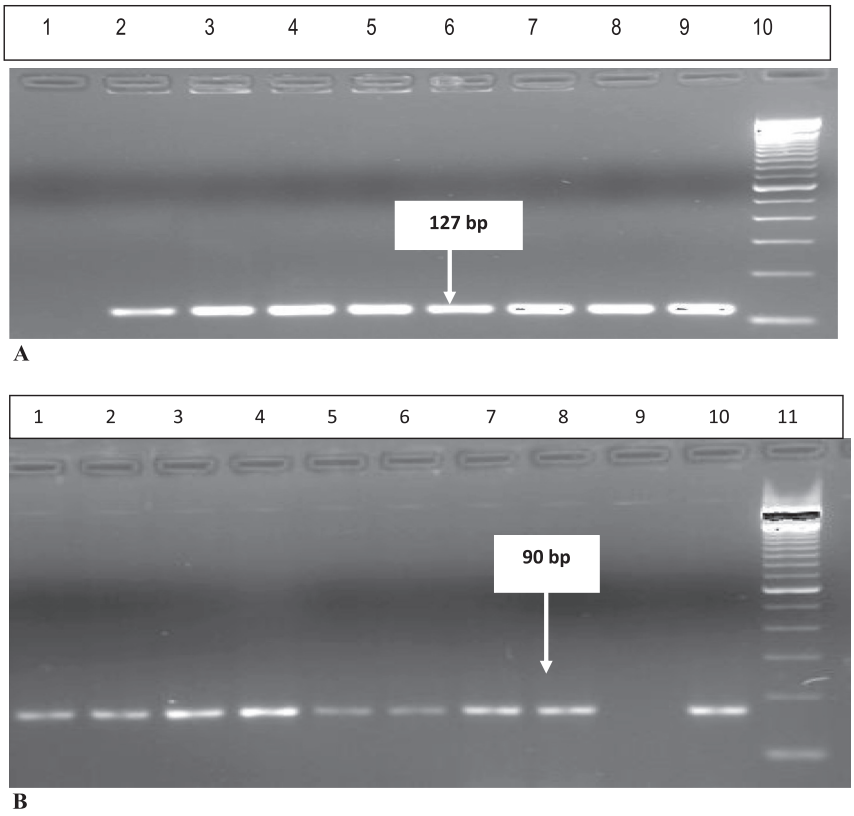


Fig. 4. A. Detection of ODC gene in some stains. PCR products obtained with the primer AGD1f/AGDIR; lane 1, PCR control; lanes 2 and 3, PCR products of DNA from *L. Paracasei*; lanes 4 and 5, PCR products of DNA from *L. Casei* DSMZ 20011; lanes 6 and 7, PCR products of DNA from *L. brevis*; lanes 8 and 9, PCR products of DNA from *S. thermophiles* and lane 10, DNA ladder 100 bp. B. Detection of deiminase gene in some stains. PCR products obtained with the primer AGD1f/AGDIR. Lane 1, PCR products by DNA from *L. Paracasei* TISTR 453; lane 2, PCR products by DNA from *L. Casei* DSMZ 20011; lane 3, PCR products by DNA from *L. brevis*; lane 4, PCR products by DNA from *S. thermophiles*; lane 5, PCR products by DNA from *L. brevis* p102; lane 6: PCR products by DNA from *L. buchneri*; lane 7: PCR products by DNA from *L. sanfrasiscensis*; lane 8, PCR products by DNA from *L. pentosus* p160; lane 9, PCR products by water; lane 10, PCR control positive and lane 11, DNA ladder 50 bp.

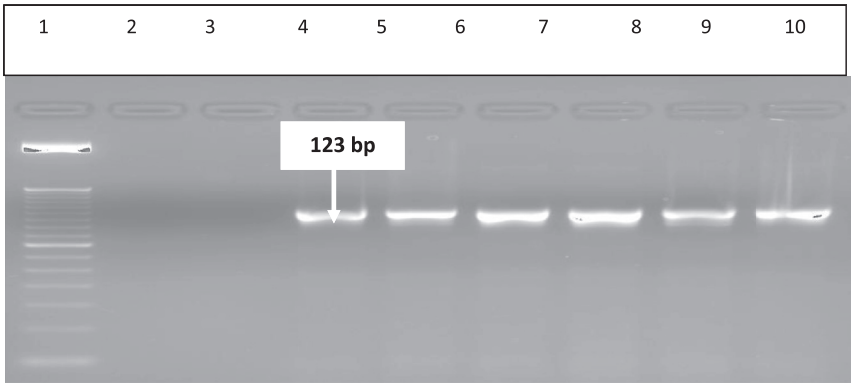


Fig. 5. Detection of putrescine gene in some stains. PCR products obtained with the primer ODF/ODR. Lane 1, DNA ladder 50 bp; lanes 2 and 3, PCR control without DNA; lane 4, PCR products by DNA from *L. Paracasei* TISTR 453; lane 5, PCR products by DNA from for *L. Casei* DSMZ 20011; lane 6, PCR products by DNA *Enterococcus faecium* p187; lane 7, PCR products by DNA from *L. brevis*; lane 8, PCR products by DNA from *S. thermophiles*; lane 9, PCR products by DNA from *L. delbrueckii* subsp *bulgaricus*.

3.3. Determination of BA by TLC

Data in Table 4 present the levels of BA in microorganisms under study as determined by TLC. Only six strains showed positive results for producing putrecine. Under our culture conditions, *L. casei* DSMZ 20011 gave the highest ability for putrecine production (17.38 mg/kg) then *L. paracasei* TISTR453 (14.65 mg/kg), *L. brevis* (13.59 mg/kg), *E. faecium* p187 (10.53 mg/kg) and *S. thermophilus* (3.53 mg/kg) wherein *Lb. delbrueckii* subsp *bulgaricus* gave the lowest ability for producing putrecine. In the same table, the ability of microorganisms to produce histamine was presented. *E. faecium* p187strain gave the highest ability for histamine production then *L. brevis* (12.88 mg/kg), *L. pentosus* p160 (4.46 mg/kg), *L. casei* DSMZ20011 (4.24 mg/kg), *L. rhamnosus* TISTR541 (2.75 mg/kg), while *S. thermophilus* produced the lowest level of histamine. The data in the same table also show the levels of tyramine production by different strains under investigation. The results showed that seven strains gave positive results for tyramine production. *L. casei*

Table 4
Concentration (mg/kg) of BA produced by LAB and bifidobacteria.

Strain	Putrescine	Histamine	Tyramine	Agmatine
Lactobacillus acidophilus ATCC 20552	ND	ND	ND	ND
Lactobacillus paracasei TISTR 453	14.65	ND	ND	174.50
Lactobacillus rhamnosus TISTR 541	ND	2.75	ND	ND
Lactobacillus salivarius TISTR 390	ND	ND	ND	ND
Lactobacillus acidophilus TISTR 450	ND	ND	ND	ND
Lactobacillus johnsonii ATCC 33200	ND	ND	ND	ND
Lactobacillus casei DSMZ 20011	17.28	4.24	ND	10.63
Lactobacillus acidophilus DSM 9126	ND	ND	ND	ND
Lactobacillus acidophilus DSM20079	ND	ND	ND	ND
Lactobacillus acidophilus DSM20242	ND	ND	ND	ND
Lactobacillus sanfrasiscensis DSM20451	ND	ND	ND	4.89
Lactobacillus plantrum	ND	ND	ND	ND
Bifidobacterium infantis DSMZ 20088	ND	ND	ND	ND
Bifidobacterium angulatum DSMZ 20098	ND	ND	ND	ND
Lb. delbrueckii subsp *bulgaricus*	1.96	ND	ND	ND
Lactobacillus acidophilus P 2	ND	ND	ND	ND
Lactobacillus acidophilus P4	ND	ND	ND	ND
Lactobacillus acidophilus P5	ND	ND	ND	ND
Lactobacillus acidophilus P 6	ND	ND	ND	ND
Lactobacillus acidophilus P7	ND	ND	ND	ND
Lactobacillus acidophilus P 8	ND	ND	ND	ND
Lactobacillus acidophilus P 9	ND	ND	ND	ND
Lactobacillus acidophilus P112	ND	ND	ND	ND
Lactobacillus acidophilus P106	ND	ND	ND	ND
Lactobacillus plantarum p1	ND	ND	ND	ND
Lactobacillus brevis p102	ND	ND	ND	3.49
Lactobacillus pentosus p160	ND	4.46	ND	6.34
Enterococcus faecium p187	10.53	31.77	ND	ND
bifidobacterium longuium	ND	ND	ND	ND
L. brevis	13.59	12.88	ND	42.40
L. buchneri	ND	ND	ND	11.76
S. thermophilus	3.53	1.74	ND	1.35

ND = not detected.

DSMZ20011 gave the highest ability for tyramine production (2.85 mg/kg) while *L. brevis* p102 gave the lowest ability for tyramine production (0.76 mg/kg). In addition, the ability of the strains under investigation to form agmatine was presented in Table 4. Eight strains showed the ability for agmatine production. *L. paracasei* TISTR453 showed the highest ability for agmatine production (74.5 mg/kg) while *S. thermophilus* showed the lowest ability for agmatine production (1.35 mg/kg). Similar results were obtained by Costantini et al. (2013) who found that the majority of *L. brevis, L. hilgardii* and *P. pentosaceus* can form putrescine. In addition, Al Bulushi, Susan, Hilton, and Gary (2009) found that most of *L. brevis* strains can produce tyramine, and putrescine. Tyramine and histamine are the most represented BA in wine and cider. Tyramine has toxicological properties and was presumed to play an active role in the development of migraine, hypertension, psychological depression, schizophrenia and parkinson disorder (Branchek & Blackburn, 2003). Histamine has been associated with headache, allergies and hypotension (Maintz & Novak, 2007). In the present investigation, most tyramine production observed as ascribable to *L. brevis*. Furthermore, the results indicated that two strains belonging to different species of *Lactobacillus* including *L. hilgardii* 5211 (isolated from wine) and *L. casei* 18 (isolated from cider) also biosynthesize histamine in line with their genetic pattern. Histamine production by *L. casei* has been previously demonstrated (Garai, Duenas, Irastorza, & Moreno-Arribas, 2007).

Our results are in agreement with the statement that the production of BA in bacteria seems to be strain-dependent rather than related to bacterial species or even genera. The study also revealed that with the elevated amount of the precursor (tyrosine), the production of the corresponding BA (tyramine) is increased in decarboxylation broth, which is in line with Fernández et al. (2007).

4. Conclusion

The work showed that some LAB strains under study can produce BA, highlighting the importance of carefully selecting indigenous strains for inclusion in starter and adjunct cultures. It could be noted that the ability to form BA are not a species-dependent trait in LAB and provides the molecular basis for strains to strain variation in BA-formation. The presence of putrescine producing LAB strains is noteworthy. The literature contains little on this, even though putrescine is one of the commonest BA. The obtained results will allow to study the BA formation trait in other LAB encountered in various fermented food products. As shown in this work, PCR testing affords many advantages to screen the capacity of LAB to produce BA. It is recommended to genetically screen the strains for amino acid decarboxylase activity before selecting LAB as a starter or probiotic strains in food and dairy production.

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