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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.