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SHORT COMMUNICATION

Effect of abiotic stress conditions on expression of the *Lactobacillus brevis* IOEB 9809 tyrosine decarboxylase and agmatine deiminase genes

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Abstract *Lactobacillus brevis* IOEB 9809, isolated from red wine, is able to produce both tyramine and putrescine. Using a real-time quantitative reverse transription PCR (qRT-PCR) we analyzed the relative expression of *L. brevis* IOEB 9809 *tdc* and *aguA1* genes, encoding, respectively, a tyrosine decarboxylase and an agmatine deiminase enzyme. Relative gene expression was monitored either during the different growth phases under optimal conditions or under abiotic stress commonly found in wine. The *tdc* and *aguA1* specific cDNA were amplified with specific primers. Our results indicate that *tdc* and *aguA1* genes are differently expressed during the different growth phases and transiently induced by ethanol (12% v/v) and acidic stresses (pH 3.2 and 5.0).

Keywords Tyrosine decarboxylase · Agmatine deiminase · *Lactobacillus brevis* · Biogenic amine · Stress

Introduction

Biogenic amines (BA) are naturally occurring low molecular weight compounds in humans that are involved in natural biological processes. Although BA are involved in important physiological functions, the consumption of foods containing large amounts of these amines can have toxicological consequences (Shalaby 1996). Foods likely to contain high levels of biogenic amines include fish, fish products and fermented foodstuffs (meat, dairy, vegetables) and beverages (wines, ciders and beers). BA poisoning problems are more severe in consumers with less efficient detoxification systems due to their genetic constitution or their medical treatments (Bodmer et al. 1999; McCabe-Sellers et al. 2006). Most common symptoms are headache, edema, vomiting, diarrhea, hypo- or hypertension (Silla Santos 1996). BA occurrence in food is mainly due to the microbial transformation of amino acids. Many lactic acid bacteria (LAB) are able to produce BA through amino acid decarboxylation via the activity of specific decarboxylases (Lonvaud-Funel 2001; Fiocco et al. 2007; Arena et al. 2007; Montel et al. 1999; Bover-Cid et al. 2001). By using catabolic pathways that convert amino acids into BA, LAB can produce metabolic energy and/or increase their acid resistance (Molenaar et al. 1993; Fernández and Zúñiga 2006; Griswold et al. 2006). Recently, the genes of diverse pathways producing biogenic amines were identified in LAB. Interestingly, the pathways seem to be rather strain-dependent than species-specific, suggesting that horizontal gene transfer may account for their dissemination in LAB (Lucas et al. 2005; Marcobal et al. 2006; Coton and Coton 2009). In addition, enzymes of pathways involved in biogenic amines production can be encoded by unstable plasmids (Lucas et al. 2005; Satomi et al. 2008), and only strains harboring BA-related plasmids are able to produce BA (Lucas et al. 2005).

Lactobacillus brevis IOEB 9809 harbors two biogenic amine-producing pathways in the same locus: the tyrosine decarboxylation operon and, immediately downstream, the

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agmatine deiminase pathway genes. The *tdc* gene, encoding a tyrosine decarboxylase (tyrDC), was preceded by a gene homologous to tyrosyltRNA synthetases (tyrRS) and followed by two genes coding for secondary transporters, a putative tyrosine transporter (tyrP) and a putative Na+/H+antiporter (nhaC) (Lucas et al. 2003; Wolken et al. 2006). The agmatine deiminase pathway includes the genes encoding: putrescine transcarbamylase (PTC), agmatine/putrescine exchanger (AgmP), agmatine deiminase (AgDI), carbamate kinase (CK), and transcription regulator (TR) (Lucas et al. 2007).

In order to increase our knowledges on the molecular and physiological bases of these specific amino acid decarboxylations in *L. brevis* IOEB 9809, we analyzed their relative gene expression in presence of abiotic stresses commonly found in wine. Our results suggest that the *tdc* and *aguA1* genes in *L. brevis* IOEB 9809 show a specific transcriptional behavior during growth phases and are differently inducible by low pH and high ethanol contents.

Materials and methods

Strain, medium and growth conditions

Lactobacillus brevis IOEB 9809, isolated from a red wine (Moreno-Arribas et al. 2000), was routinely grown in de Man–Rogosa–Sharpe (MRS) broth (pH 6.8) at 28°C without shaking. Growth of *L. brevis* IOEB 9809 under optimal conditions (pH 6.8, 28°C) was monitored by optical density (OD) at a wavelength of 600 nm (GeneQuant Pro RNA/DNA Calculator; Amersham Bioscience). Aliquots were removed during latent phase (Lag1, OD600 0.16; Lag2, OD600 0.20), exponential phase (Log1, OD600 0.34; Log2, OD600 0.45; Log3, OD600 0.64), and stationary phase (Sta1, OD600 1.13; Sta2, OD600 1.24), and total RNA was extracted and used for quantitative real-time PCR (qRT-PCR) analysis. Furthermore, the microorganism was subjected to different abiotic stress conditions, in particular presence of metabisulfite (15 g hl^{-1}), low pH values (pH 3.2 and 5.0), low temperature (18°C) and low ethanol concentrations (8% and 12% v/v). Stationary-phase *L. brevis* IOEB 9809 cells were diluted in fresh MRS medium and allowed to grow to mid-exponential phase (OD_{600}, 0.6). Potassium metabisulfite was added to the cultures to a final concentration of 15 g hl^{-1}. For acid stress (pH 3.2 and 5), culture was centrifuged at 2,000 rpm for 7 min and the pellet obtained was resuspended in MRS broth supplemented with malic acid (10 g l^{-1}). For low temperature condition, the cultures were transferred to water baths maintained at 18°C (with 5 min of pre-stress acclimatization). For ethanol stresses (8 and 12% v/v), culture was centrifuged at 2,000 rpm for 7 min and the pellet obtained was resuspended in MRS broth containing 8% and 12% v/v of ethanol, respectively. The stresses were imposed for periods of 3, 5 and 15 min.

Reverse transcription and real time PCR analysis

Total RNAs were extracted using the ultraclean microbial DNA isolation kit, (Mo-Bio Laboratories, Carlsbad CA USA) according to the manufacturer's instructions. The quality of the RNA samples was verified by electrophoresis on 1.2% agarose gels, and RNA concentrations were calculated both spectrophotometrically (GeneQuant Pro RNA/DNA Calculator; Amersham Bioscience) and using Quantity One software (Bio-Rad). cDNAs were synthesized using 0.3 μg of total RNA and the Quantitect Reverse Trascription (Qiagen) which includes a DNaseI treatment step and reverse transcription. Absence of chromosomal DNA contamination was confirmed by real-time PCR on DNaseI-treated, non-retrotranscribed RNAs. Primers for real-time PCR were designed in order to have a length around 20 bases, a GC content of approximately 50% and a Tm around 60°C. OligoPerfect Designer software (Invitrogen, Carlsbad, CA, USA) was used to select primers sequences. Secondary structures and dimer formation were predicted using Oligo Analyzer 3.0 software (Integrated DNA Technologies, Coralville, IA, USA). Primers were purchased from Sigma-Aldrich (St Louis, MO, USA). The *tdc*-specific cDNA was amplified with primers 5′ TGA GAA GGG TGC CGA TAT TC 3′ forward and 5′ GCA CCT TCC AAC TTC CCA TA 3′ reverse. The *aguA1* specific cDNA was amplified with primers 5′ TCT TGA AAA TGC GAC AGA CG 3′ forward and 5′ TCC AAC GTA GCC TGA GCT TT 3′ reverse. The amplicon lengths were between 141 bp and 240 bp for the *tdc* and the *aguA1* genes, respectively.

Real-time PCR was performed on Applied Biosystems 7300 Real-Time PCR System. After 20-fold dilution of cDNA, 5 μL were added to 15 μL of a real-time PCR mix containing Power SYBR Green PCR Master Mix (Applied Biosystems), according to the manufacturer's instructions, and 100 nM of each primer. In each run, a negative control was included. Thermal cycling conditions were designed as follows: initial denaturation at 95°C for 5 min followed by 35 cycles at 95°C for 10 s, and 60°C for 40 s. A melting curve analysis was performed in order to verify the specificity of real-time PCR. Data were measured in function of the amount of total RNA, according to Torriani et al. (2008).

Real-time PCRs were performed in triplicate for each sample of cDNA. For each measurement, a (Ct) was

determined. In this study, the threshold value was determined with a baseline settled automatically.

Results and discussion

Biogenic amines content in wine have been receiving increasing attention because (1) of the toxicity episodes which may be produced in sensible individuals when these amines are consumed at high concentrations and (2) of the precursor limits in the histamine contents in wines that some countries have established (García-Villar et al. 2009). In general, putrescine is the most abundant amine in wines although significant amounts of histamine, tyramine and phenylethylamine are sometimes identified (Saurina 2009; Ancin-Azpilicueta et al. 2008).

Initially, the expression of *tdc* and *aguA1* genes was analyzed during the different growth phases at 28°C (Fig. 1). This analysis revealed that early growing cells contain substantial amounts of *tdc* mRNAs, which rapidly decline at the entry into the stationary phase (Fig. 1a). In contrast, the *aguA1* was found induced at the end of the exponential growth phase. Then, the amount of transcript decreased during the stationary growth phase, although *aguA1* gene expression was still observed during the late stationary growth phase (Fig. 1b). The different expression pattern observed during growth at optimal temperatures may be a consequence of the different functions of the *tdc* and *aguA1* genes in *L. brevis*.

It is possible to suggest that the expression of *aguA1* gene, mainly observed in the late exponential and stationary phases, might be related to energy production and/or acidic response. Consistent with these findings, in *Lactobacillus buchneri* Molenaar et al. (1993) observed that histidine decarboxylase gene expression is highest in the exponential growth phase. In contrast, in *Lactobacillus hilgardii*, Landete et al. (2006) found a clear induction during the exponential phase, followed by repression in the stationary growth phase. To our surprise, the *tdc* gene showed a different behavior in gene expression during growth, with a pronounced induction in the latent phase, that should require further studies. Indeed, it is important to consider that, in *L. brevis* IOEB 9809, the TDC enzymatic activity was found to have increased during the rapid growth phase and decreased thereafter (Moreno-Arribas et al. 2000).

Furthermore, the expression of the *tdc* and *aguA1* genes was monitored in presence of abiotic stresses commonly encountered in wine such as metabisulfite stress (data not

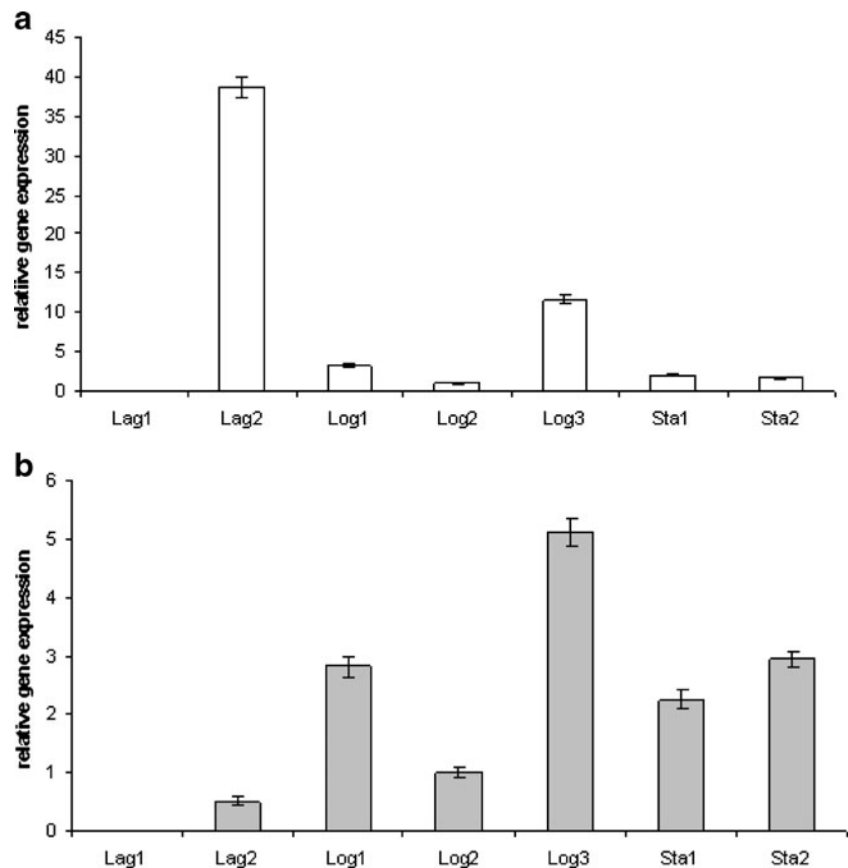

Fig. 1 Real-time RT-PCR analysis of *tdc* (**a**) and *aguA1* (**b**) relative gene expression in *Lactobacillus brevis* IOEB 9809 during the different growth phases under optimal conditions. The expression levels observed were normalized to Log2. Data shown are mean ± standard deviations from three independent experiments

shown), low temperature (data not shown), acid and ethanol stresses (Fig. 2). A significant increase of the expression of the genes *tdc* and *aguA1* (six- and three-fold, respectively) was observed at pH 3.2 after 3 min, while a reduction (about 0.1- and 0.2-fold, respectively) was found after 5 and 15 min (Fig. 2a). The expression patterns of *tdc* and *aguA1* genes were similar during less severe acid condition (pH 5.0), with an induction observed only after 3 min (twofold) (Fig. 2b).

Furthermore, the expression of *tdc* and *aguA1* genes was monitored in presence of different ethanol concentrations (Fig. 3). The *tdc* gene was apparently unaffected by ethanol 8% (v/v). In contrast, the *aguA1* gene was induced by ethanol 8% after 3 min (about twofold). A repression of the genes *tdc* and *aguA1* (0.4- and 0.5-fold, respectively) was found after 5 and 15 min of ethanol stress exposure (Fig. 3a). However, in the presence of ethanol 12% (v/v), the *tdc* and *agA1* genes were induced after 3 min (three- and fivefold, repectively), and repressed after 5 and 15 min (Fig. 3b). Relative *tdc* and *aguA1* gene expressions in presence of sulphite (potassium metabisulphite 15 g/hL) and during low temperatures stress (18°C)

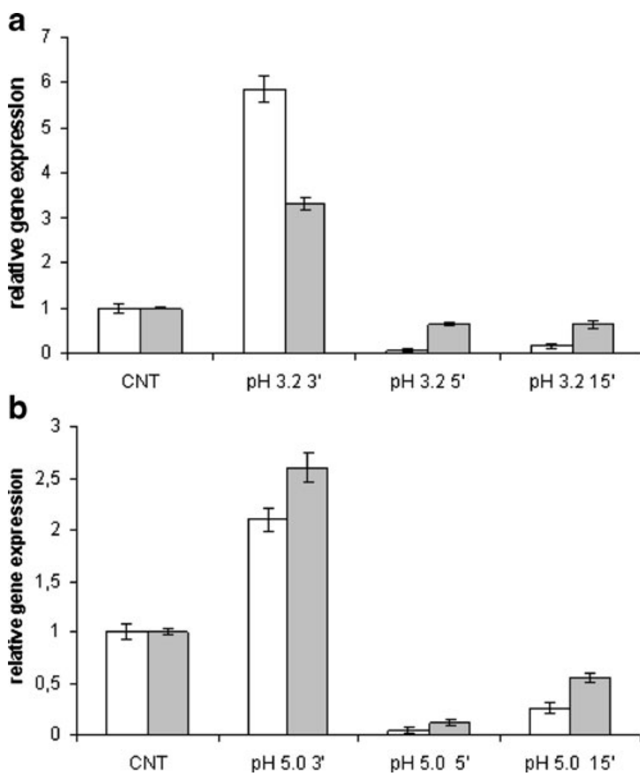

Fig. 2 Real-time RT-PCR analysis of *Lactobacillus brevis tdc* and *aguA1* relative gene expression (*tdc*, *white bar charts*; *aguA1*, *gray bar charts*) in response to 3, 5 and 15 min exposure to pH 3.2 (**a**) and pH 5.0 (**b**). Total RNA was extracted from exponentially growing *L. brevis* cultures before (control) and after stress. The expression levels observed in the stressed samples were normalized to those detected in the control unstressed sample. Data shown are mean ± standard deviations from three independent experiments

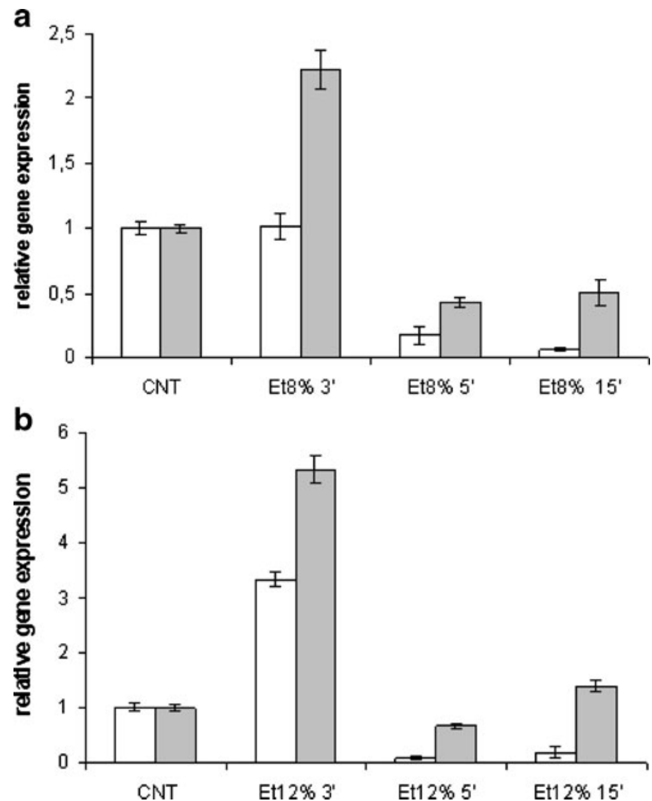

Fig. 3 Real-time RT-PCR analysis of *Lactobacillus brevis tdc* and *aguA1* relative gene expression (*tdc*, *white bar charts*; *aguA1*, *gray bar charts*) in response to 3, 5 and 15 min exposure to ethanol 8% (**a**) and 12% (**b**). Total RNA was extracted from exponentially growing *L. brevis* cultures before (control) and after stress. The expression levels observed in the stressed samples were normalized to those detected in the control unstressed sample. Data shown are mean ± standard deviations from three independent experiments

were found to be generally repressed after 3, 5 and 15 min (data not shown). While the repression observed under cold shock conditions might be addressed to drastical influence of temperature downshift on the metabolism, concerning the possible physico-chemical effects of sulphite on gene expression it is also more difficult to argue a hypothesis because of the poor literature that deals with this topic.

In conclusion, our observations indicate that *tdc* and *aguA1* genes are differently expressed during the growth cycle suggesting a different role in *L. brevis* IOEB 9809. In addition, both genes are transiently induced by ethanol (12% v/v) and acidic stresses (pH 3.2 and 5.0), while they are generally repressed by cold and sulphite stress condition.

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References

Ancin-Azpilicueta C, González-Marco A, Jiménez-Moreno N (2008) Current knowledge about the presence of amines in wine. Crit Rev Food Sci Nutr 48:257–275

Arena ME, Fiocco D, Manca de Nadra MC, Pardo I, Spano G (2007) Characterization of a *Lactobacillus plantarum* strain able to produce tyramine and partial cloning of a putative tyrosine decarboxylase gene. Curr Microbiol 55:205–210

Bodmer S, Imark C, Kneubühl M (1999) Biogenic amines in foods: histamine and food processing. Inflamm Res 48:296–300

Bover-Cid S, Izquierdo-Pulido M, Vidal-Carou MC (2001) Effect of the interaction between a low tyramine-producing *Lactobacillus* and proteolytic staphylococci on biogenic amine production during ripening and storage of dry sausages. Int J Food Microbiol 65:113–123

Coton E, Coton M (2009) Evidence of horizontal transfer as origin of strain to strain variation of the tyramine production trait in *Lactobacillus brevis*. Food Microbiol 26:52–57

Fernández M, Zúñiga M (2006) Amino acid catabolic pathways in lactic acid bacteria. Crit Rev Microbiol 32:155–183

Fiocco D, Pardo I, Palermo C, Massa S, Spano G (2007) Biogenic amine formation in fermented beveranges: targets, tools and triumphs. Invited review. Food 1:49–55

García-Villar N, Hernández-Cassou S, Saurina J (2009) Determination of biogenic amines in wines by pre-column derivatization and high-performance liquid chromatography coupled to mass spectrometry. J Chromatogr A 1216:6387–6393

Griswold AR, Jameson-Lee M, Burne RA (2006) Regulation and physiologic significance of the agmatine deiminase system of *Streptococcus mutans* UA159. J Bacteriol 188:834–841

Landete JM, Pardo I, Ferrer S (2006) Histamine, histidine, and growth-phase mediated regulation of the histidine decarboxylase gene in lactic acid bacteria isolated from wine. FEMS Microbiol Lett 260:84–90

Lonvaud-Funel A (2001) Biogenic amines in wines: role of lactic acid bacteria. FEMS Microbiol Lett 199:9–13

Lucas PM, Landete J, Coton M, Coton E, Lonvaud-Funel A (2003) The tyrosine decarboxylase operon of *Lactobacillus brevis* IOEB 9809: characterization and conservation in tyramine-producing bacteria. FEMS Microbiol Lett 229:65–71

Lucas PM, Wolken WAM, Claisse O, Lolkema JS, Lonvaud-Funel A (2005) Histamine-producing pathway encoded on an unstble plasmid in *Lactobacillus hilgardii* 0006. Appl Environ Microbiol 71:1417–1424

Lucas PM, Blancato VS, Claisse O, Magni C, lolkema JS, Lonvaud-Funel A (2007) Agmatine deiminase pathway genes in *Lactobacillus brevis* are linked to the tyrosine decarboxylation operon in a putative acid resistance locus. Microbiology 153:2221–2230

Marcobal A, Martín-Alvarez PJ, Polo MC, Muñoz R, Moreno-Arribas MV (2006) Formation of biogenic amines throughout the industrial manufacture of red wine. J Food Prot 69:397–404

McCabe-Sellers BJ, Staggs CG, Bogle ML (2006) Tyramine in foods and monoamine oxidase inhibitor drugs: a crossroad where medicine, nutrition, pharmacy, and food industry converge. J Food Compos Anal 19:58–65

Molenaar D, Bosscher JS, Ten Brink B, Driessen AJM, Konings WN (1993) Generation of a proton motive force by histidine decarboxylation and electrogenic histidine/histamine antiport in *Lactobacillus buchneri*. J Bacteriol 175:2864–2870

Montel MC, Masson F, Talon R (1999) Comparison of biogenic amine content in traditional and industrial French dry sausages. Sci Aliments 19:247–254

Moreno-Arribas V, Torlois S, Joyeux A, Bertrand A, Lonvaud-Funel A (2000) Isolation, properties and behaviour of tyramine-producing lactic acid bacteria from wine. J Appl Microbiol 88:584–653

Satomi M, Furushita M, Oikawa H, Yoshikawa-Takahashi M, Yano Y (2008) Analysis of a 30 kbp plasmid encoding histidine decarboxylase gene in *Tetragenococcus halophilus* isolated from fish sauce. Int J Food Microbiol 126:202–209

Saurina J (2009) Characterization of wines through the compositional profiles of biogenic amines and related compounds. Focusing on the description of toxicological and organoleptic features. In: O'Byrne P (ed) RedWine and health. Nova, New York, pp 1–24

Shalaby AR (1996) Significance of biogenic amines in food safety and human health. Food Res Int 29:675–690

Silla Santos MH (1996) Biogenic amines: their importance in foods. Int J Food Microbiol 29:213–231

Torriani S, Gatto V, Sembeni S, Tofalo R, Suzzi G, Belletti N, Gardini F, Bover-Cid S (2008) Rapid detection and quantification of tyrosine decarboxylase gene (tdc) and its expression in gram-positive bacteria associated with fermented foods using PCR-based methods. J Food Prot 71:93–101

Wolken WA, Lucas PM, Lonvaud-Funel A, Lolkema JS (2006) The mechanism of the tyrosine transporter TyrP supports a proton motive decarboxylation pathway in *Lactobacillus brevis*. J Bacteriol 188:2198–2206