International Dairy Journal 62 (2016) 53–62

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Review

Lactic acid bacteria decarboxylation reactions in cheese

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ARTICLE INFO

Article history:
Received 5 April 2016
Received in revised form
13 July 2016
Accepted 13 July 2016
Available online 22 July 2016

ABSTRACT

Fermentation in cheese comprises oxidation-reduction of carbohydrates to yield organic acids, alcohols and carbon dioxide. Furthermore, organic acid and amino acid metabolism produces a series of compounds that positively or negatively affect final cheese quality. Under the strong selective pressure of the acidic environment of cheese ripening, lactic acid bacteria have developed multiple stress-resistant strategies, including decarboxylase and deiminase reactions that play a main physiological role during the ripening process of cheese production. The control of the expression and activity of these enzymes is one active strategy for intracellular acid-base homeostasis. This review covers relevant pathways and aspects related to gene regulation of gene clusters present in starter or non-starter lactic acid bacteria that are involved in sensory changes such as flavour development. From the point of view of food safety the main decarboxylation pathways that lead to the formation of biogenic amines are described.

Contents

1. Introduction

Cheese is a very ancient food; and cheese making dates back to even before written history. Nowadays, there are more than a thousand cheese varieties worldwide with unique characteristics regarding flavour and texture (Beresford, Fitzsimons, Brennan, & Cogan, 2001). Consumption of these products has become a globally important phenomenon, and consumers are demanding higher quality products in terms of sensory characteristics, nutritional value and the absence of toxic compounds or health hazards. In cheese production, quality is closely related to sensory perception. Such perception is a complex process influenced by many factors, such as aromatic content, texture and appearance. Aromatic compounds associated with the natural aroma and flavour of food depend on many factors, e.g., rennet, milk, but basically they are related to microbiological cultures and various fermentation processes that occur during cheese formation (Beresford, 2003; Smit, 2003). These processes become more important during product ripening, when aroma is defined by the slow conversion of chemical and biochemical components present in milk (McSweeney, 2011). However, these processes can also lead to undesired compound

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http://dx.doi.org/10.1016/j.idairyj.2016.07.007
0958-6946/

formation, such as biogenic amines, that negatively affect sensory cheese properties and can lead to consumer health problems.

Cheese fermentation can be separated into two stages according to this process, in which lactic acid bacteria (LAB) represent the main group of microorganisms involved. Many LAB species have been used for centuries in the fermentation and storage of food and, therefore, are recognised as GRAS (Generally Recognized As Safe by the Food and Drug Administration, USA) or QPS (Qualified Presumption of Safety by the European Food Security Agency) and approved for their use in foods for human or animal consumption. However, species from the genera *Streptococcus* and *Enterococcus* (Franz, Stiles, Schleifer, & Holzapfel, 2003) with members considered to be opportunistic pathogens also fall into this classification.

In the first step carried out by starter LAB (S-LAB), milk composition and the manufacturing process are crucial. This period is particularly controlled by the speed and amount of acid produced by the microorganisms present in the starter culture, which in turn define the initial conditions of the subsequent step. In the second stage or ripening, accomplished by non-starter LAB (NS-LAB), the development of the sensory characteristics occurs. Moreover, in the last several years, an increasing number of publications referring to mesophilic lactobacilli and enterococci (defined and non-defined NS-LAB, respectively) strongly suggest a positive contribution to the sensory properties of cheese (Beresford, 2003; De Vuyst & Leroy, 2007; Franz et al., 2003; Giraffa, 2003; Ogier & Serror, 2008; Sarantinopoulos, Kalantzopoulos, & Tsakalidou, 2001; Suárez, Blancato, Poncet, Deutscher, & Magni, 2011). Ripening goes through a broad series of processes of microbiological, biochemical and physical nature, which varies widely between hard, medium-soft and soft cheeses (Fox & McSweeney, 2004; Fox, Guinee, Cogan, & McSweeney, 2000).

In this context, dairy processing involves many stress conditions that produce a phenotypical response of microorganisms during fermentation and storage. One such condition is the decrease in pH, and LAB encode specific genetic mechanisms that lead to stress responses producing physiological changes (Broadbent, Larsen, Deibel, & Steele, 2010; Lucas et al., 2007; Montanari, Sado Kamdem, Serrazanetti, Etoa, & Guerzoni, 2010; Welmer, 2011). Proton consuming pathways mediated by decarboxylase and deaminase enzymes are crucial in pH homeostasis (Lolkema, Poolman, & Konings, 1995). Some of the responses associated with regulatory elements lead to the expression of a large number of metabolic changes useful for acid neutralization and energy production from protein and amino acids along with the formation of flavour components or biogenic amines.

While the most important energy and carbon source for LAB are generally carbohydrates (lactose, galactose and glucose) many NS-LAB will survive in cheese under low lactose concentrations and, in some cases, they can grow during the ripening stage. Thus, NS-LAB have the capacity to obtain energy by metabolising organic and amino acids that are present in cheese curd (Coppola et al., 1997; Skeie, Kieronczyka, Næssa, & Østliea, 2008).

The development of molecular techniques has allowed systematic approaches to study positive and negative effects of decarboxylation pathways in LAB strains (Giannino, Marzotto, Dellaglio, & Feligini, 2009; Psoni, Kotzamanidis, Yiangou, Tzanetakis, & Litopoulou-Tzanetaki, 2007; Psoni et al., 2006; de Angelis et al., 2001). This review systematises recent investigations on these issues that will allow the rational development of adjunct microbiota with potential to influence cheese quality.

2. Decarboxylation pathways role in pH homeostasis

In LAB, substrate-level phosphorylation is the main mechanism for ATP generation. The weak acids produced in these fermentation processes display potent antimicrobial activity. They can freely pass through cell membranes, causing cytoplasm acidification and reducing cell viability (Cotter & Hill, 2003). Therefore, it is essential for bacteria to regulate the intracellular acid-base homeostasis to ensure optimal pH for all biological processes. The F_1F_0-ATPase links ATP production to the proton motive force (PMF) allowing ATP generation at its expense. A second active strategy for pH homeostasis involves controlling the expression and activity of enzymes participating in metabolic processes to remodel metabolic patterns.

Under acidic conditions, there is an increase in the expression of enzymes whose reactions consume cytoplasmic protons. One of the major metabolic responses associated with acid resistance is the arginine deiminase (ADI) system disseminated in different S-LAB and NS-LAB. This pathway produces ornithine, ATP, CO_2 and NH_4^+, which counteract internal acidification. Although agmatine deiminase (AgDI) pathway is less disseminated in LAB, its pathway produces ATP, CO_2, NH_4^+ and putrescine with a similar physiological role (see below). Additionally, organic acids (like citrate or malate) as well as the fermentation of some amino acids (such as tyrosine or histidine) contribute to the acid resistance in LAB but the fact that the presence of these gene clusters is more strain-specific suggests a recent niche adaptation of these microorganisms (Suárez, Espariz, Blancato, & Magni, 2013).

In organic acid metabolism, decarboxylation reactions acquire an important role as a detoxification mechanism. Decarboxylation reactions consume H^+ and produce CO_2; therefore, these reactions modify the environmental pH and contribute to buffer capacity due to the CO_2 dissolved balance. Proton concentration is affected by H^+ consumption as well as by the acidity of the end product compared with the substrate, particularly in the decarboxylation of organic acids. On the other hand, decarboxylation involves oxidative and non-oxidative reactions. In this sense, during NAD(P)H production, the redox potential is also modified. However, the energetic contribution of organic acid metabolism must be considered in decarboxylation systems, where the enzyme responsible for the decarboxylation reaction is associated with other enzymes and a transporter. In general, the transporter is an electrogenic system while the enzyme acts as a scalar proton consumption reaction; thus acting as a PMF generating pathway (Lolkema et al., 1995).

2.1. Positive effects of the decarboxylation pathway in cheese

Citrolactic, malolactic and amino acid fermentations play important roles in the organoleptic properties of fermented foods. Citrate fermentation is strain-dependent and is involved directly in cheese flavour and quality (Díaz-Muñiz et al., 2006; Gale, 2006). In LAB it proceeds through two main routes that are catalysed by a variety of enzymes and transporters. In a first step common to both pathways, citrate lyase (CL) catalyses the cleavage of internalized citrate into OAA and acetate. Then, two diverging pathways are described in LAB that produce either succinate or pyruvate. The route to succinate via malate and fumarate is found in many mesophilic NS-LAB lactobacilli (Dudley & Steele, 2005). The second route involves the decarboxylation of OAA to generate pyruvate, contributing to the central pool of pyruvate in the glycolytic pathway for carbohydrate/citrate co-metabolism (Fig. 1). In this pathway, citrate fermentation is associated with an OAA decarboxylase (OAD) and, therefore, with the generation of PMF (Lolkema et al., 1995). In LAB, two types of OAD enzymes exist, the soluble OAD belonging to the malic enzyme family and the OAD membrane complex, a biotin-dependent decarboxylase. The first enzyme is present in *Lactococcus lactis* (S-LAB) and *Weissella mesenteroides* (NS-LAB), among other LAB, it catalyses the conversion of oxaloacetate from citrate to pyruvate in the presence of divalent metals (Espariz et al., 2011; Sender, Martín, Peiru, & Magni, 2004).

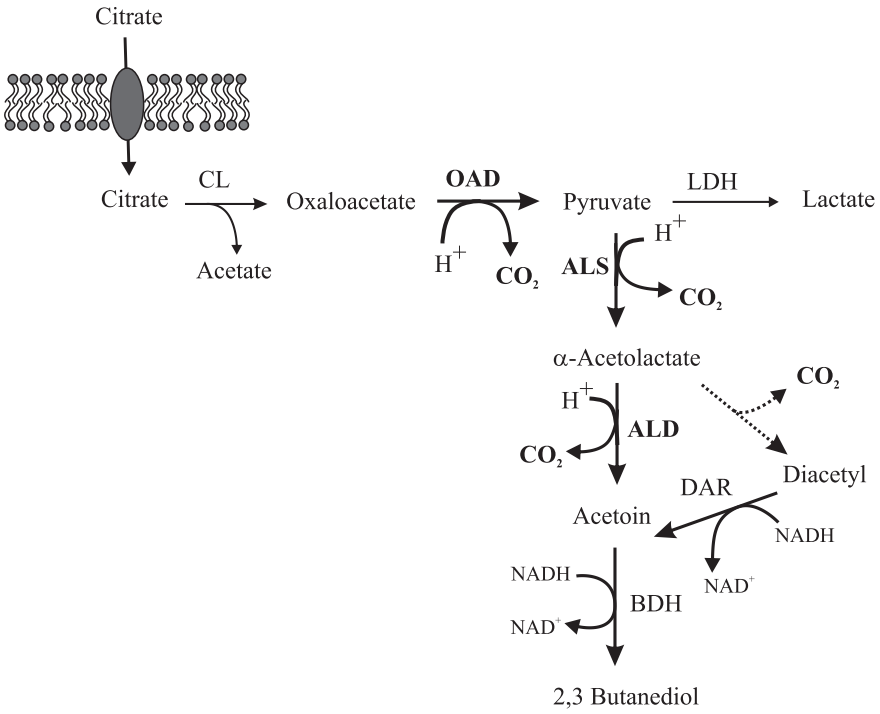

Fig. 1. Schematic representation of generation of flavour compounds by citric acid metabolism in LAB. Enzyme abbreviations are: CL, citrate lyase; OAD, oxaloacetate decarboxylase; LDH, lactate dehydrogenase; ALS, α-acetolactate synthase; ALD, α-acetolactate decarboxylase; DAR, diacetyl-acetoin reductase; BDH, butanediol dehydrogenase. Proton scalar consumption is indicated by H^+ and non-enzymatic oxidative decarboxylation with dotted line.

The second one, a membrane associated OAD consisting of four polypeptides, is found in *Enterococcus faecalis* (NS-LAB) and *Lactobacillus casei* (S-LAB) (Mortera, Pudlik, Magni, Alarcón, & Lolkema, 2013; Repizo, Blancato, Mortera, Lolkema, & Magni, 2013).

As shown in Fig. 1, pyruvate from citrate could be condensed to give a molecule of α-acetolactate (AL) by α-acetolactate synthase (ALS). The α-acetolactate formed can be converted to acetoin by the action of α-acetolactate decarboxylase (ALD) or diacetyl in a nonenzymatic oxidative decarboxylation reaction. This pathway also could be completed with additional enzymes such as acetoin-diacetyl reductase (DAR) and butanediol dehydrogenase (BDH).

From the two main species of microorganisms (*L. lactis* and *Streptococcus thermophilus*) that are widely used as starter cultures in the cheese industry, only *L. lactis* biovar. diacetylactis (*L. diacetylactis*) is able to metabolise citrate. The pathway increases the intracellular level of pyruvate which results in the production of aroma compounds (diacetyl, acetoin and butanediol). In *L. diacetylactis*, this metabolism has been extensively characterised over the last two decades (García-Quintans, Magni, de Mendoza & López, 1998; García-Quintáns, Repizo, Martín, Magni, & López, 2008; Lopez de Felipe, Magni, de Mendoza, & López, 1996; Magni, de Felipe, López, & de Mendoza, 1996; Magni, de Mendoza, Konings, & Lolkema, 1999; Martín, Sender, Peirú, de Mendoza, & Magni, 2004; Martín, Magni, de Mendoza, & López, 2005a; Zuljan, Repizo, Alarcón, & Magni, 2014).

Genes associated with citrate metabolism in *L. lactis* are organised in two operons, one involved in citrate transport (*citQRP* plasmidic operon) and the other in its conversion to pyruvate (*citM-citI-CDEFXG* operon) as Fig. 2A shows. The *citM-citI-CDEFXG* operon encodes citrate lyase (*citDEF*) and the accessory genes necessary to activate the holoenzyme, together with a soluble cytoplasmic oxaloacetate decarboxylase (CitM). Both operons are transcriptionally induced at low pH, by a still unknown mechanism (García-Quintans et al., 1998; Martín et al., 2004). Moreover, the genes involved in C4 compound production, the *als* monocistron (coding for the α-acetolactate synthetase), *aldB* and *aldC* (coding for two α-acetolactate decarboxylases) and the *butBA* operon (encoding a butanediol dehydrogenase and a diacetil/acetoin reductase, respectively) are specifically induced under acidic conditions (García-Quintáns et al., 2008). Thus, during citrate metabolism pyruvate is deviated towards the production of neutral compounds. This contribution to the acetoin/diacetyl pathway is evident at pH 4.5, a common acidic stress situation faced by *L. lactis* during growth (Zuljan et al., 2014). Experiments revealed that during co-metabolism of sugars and pyruvate under acidic conditions, the generation of aroma compounds plays an important role for bacterial growth. Genome-scale analysis suggests that this capacity to control internal pH through the production of flavour compounds was present in *L. lactis* prior to its differentiation into subspecies (Zuljan et al., 2014).

In *Weisella paramesenteroides* (NS-LAB) and *Leuconostoc mesenteroides* (S-LAB) the presence of a 23 kbp plasmid encoding the *cit* cluster was identified (Bekal et al., 1998; Bekal, Diviès, & Prévost, 1999; Martín, Corrales, de Mendoza, López, & Magni, 1999; Martín, Magni, López, & de Mendoza, 2000). Despite the strong identity between the operons present in the large and small plasmids found in starter or NS-LAB, in the latter we demonstrated that citrate is the operon inducer. Fig. 2 shows the transcriptional regulation of citrate metabolism. CitI was identified as the transcriptional factor (belongs to the DeoR family) required for activation of the operons (Fig. 2B). CitI binds to two AT-rich operator sites located between *citI* and the *citMCDEFGP* operon and its DNA-binding affinity is increased by citrate. Subsequently, this citrate signal leads to the activation of the *cit* operon through an enhanced recruitment of RNA polymerase to its promoters (Martín et al., 2005a). In *W. paramesenteroides* no effect of the sugar source (PTS or non PTS) on the expression of the *cit* operons was detected.

Furthermore, citrate metabolism was also studied in *E. faecalis* (NS-LAB) and *Lb. casei* (S-LAB). In these microorganisms citrate

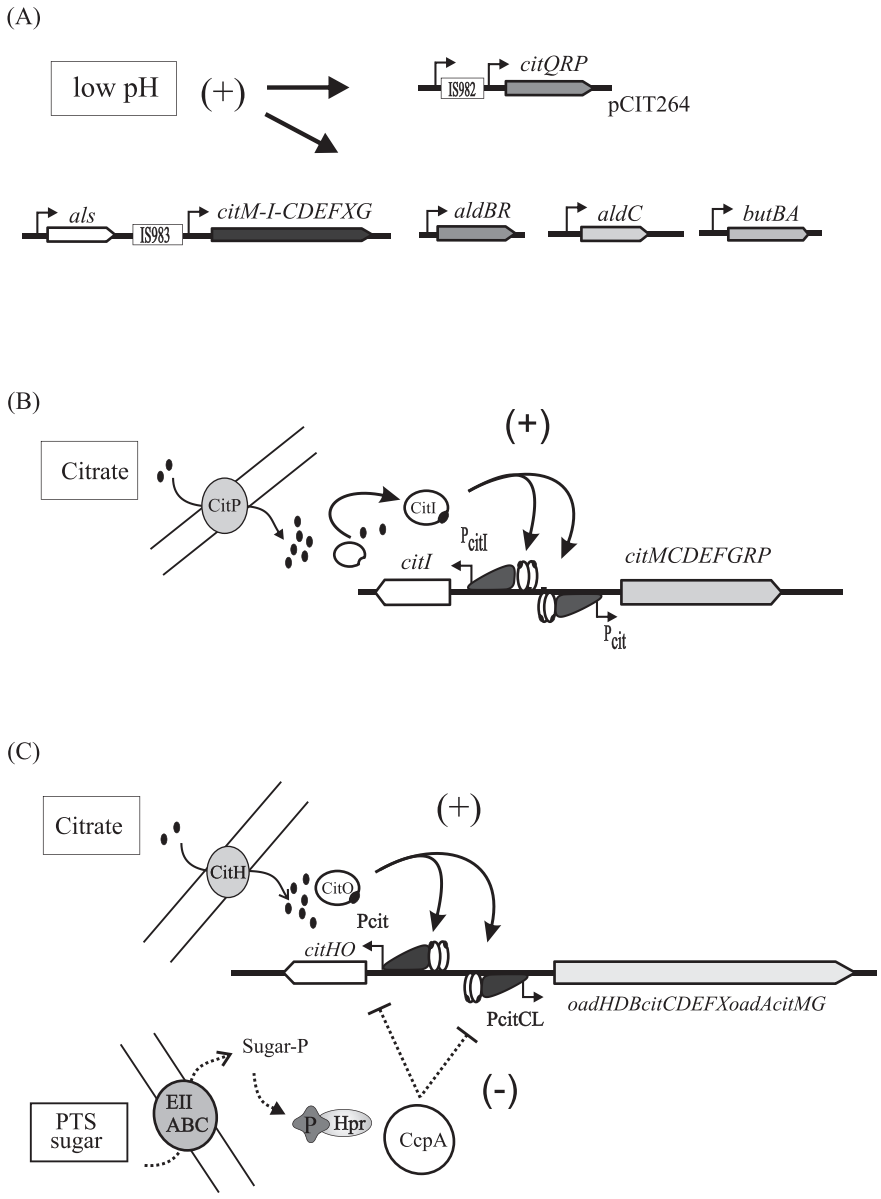

Fig. 2. Regulation of citrate operons in starter and non-starter LAB: (A) transcriptional induction of the genes involved in citrate metabolism and aroma production in Lactococcus lactis at low pH; (B) transcriptional induction of cit operon in Weisella paramesenteroides mediated by activator CitI and (C) positive and negative control of the cit operons in Enterococcus faecalis.

metabolism is regulated by the presence of citrate and also by PTS-sugars. In *E. faecalis*, the molecular mechanism for the induction by citrate and repression by carbon source was explored in depth. The molecular mechanism of citrate activation in *E. faecalis* required the activator CitO (a GntR superfamily member), which is able to sense intracellular citrate levels and induce the expression of the divergent operons (*citHO* and *oadHDBcitCDEFoadAcitXG*) (Blancato, Repizo, Suarez, & Magni, 2008) (Fig. 2C). Further studies have shown that CitO belongs to the FCD family and that the binding of Ni^{2+} or Zn^{2+} cations to the C-terminal domain of the protein improved CitO-citrate interaction (Blancato, Pagliai, Magni, Gonzalez, & Lorca, 2016).

The repressive effect of the PTS-sugars follows a redundant mechanism that could act through CcpA (Suárez et al., 2011). It was found that CcpA regulates the expression of *citHO* and *citCL* by binding to three *cre* sites (Fig. 2C). Therefore, this sophisticated control mechanism assures the regulation of the activator (CitO) together with the citrate transporter, reducing the level of intracellular inducer required by CitO. An additional regulatory step (*cre* site) was found in the *citCL* operon responsible for adjusting the level of catabolic enzymes encoded in the operon.

E. faecalis is able to utilise pyruvate as a carbon and energy source as well as to produce acetoin and diacetyl from this compound (Repizo, Mortera, & Magni, 2011). Excretion of C4 compounds has vital implications for the physiology of different microorganisms because, as described elsewhere, it is used to avoid acidification, participates in the regulation of the NAD^+/NADH ratio and functions as a carbon-storing strategy (Xiao & Xu, 2007). Bioinformatic studies revealed only the presence of two genes encoding ALS (*alsS*) and ALD (*alsD*) in the *E. faecalis* genome and no other genes encoding enzymes involved in C4 metabolism, such as DAR or BDH, were detected (Repizo et al., 2011). Transcriptional

analysis demonstrated that these genes form a single operon (*alsSD*) and their expression in the exponential phase is enhanced by the addition of pyruvate. Finally, a strain with a disruption of the *alsSD* operon showed susceptibility to increasing pyruvate concentrations under acidic conditions, which confirmed the connection between this metabolic route and the mechanisms of pH resistance (Repizo et al., 2011).

Recently, the citrate metabolism in *Enterococcus faecium* (NS-LAB) has been analysed (Martino, Quintana, Espariz, Blancato, & Magni, 2016). Diversity of the *cit* clusters showed that the micro-organisms can be classified as cit^- (no citrate metabolism genes), cit^+ type I and II according to the genes present in the operons. Type I encodes CitI regulator, CitM cytoplasmic soluble oxaloacetate decarboxylase and CitP citrate transporter (2-hydroxy-carboxylate transporter family); whereas type II encodes a CitO regulator, OAD membrane oxaloacetate decarboxylase complex and CitH citrate transporter (CitMHS family). In type I microorganisms, induction of the pathway seems to be mediated by CitI, in the presence of citrate and under catabolic response (Martino et al., 2016).

On the other hand, amino acid catabolism in LAB is depicted in Fig. 3, showing the reactions involved in decarboxylations and aroma production. The first step involves decarboxylation, deamination, transamination and desulphuration reactions. A second step involves the conversion of amines, including α-ketoacids and aldehydes. The third step involves reduction or oxidation of these aldehydes to alcohols and carboxylic acids (McSweeney & Sousa, 2000; Smit, Smit, & Engels, 2005b). Two types of non-oxidative decarboxylations can be identified (amino acid and α-ketoacids decarboxylation), both having fundamental effects on the final organoleptic characteristics of cheese. During decarboxylation reactions, a proton is consumed in the process, and the product is exported from the cell, resulting in an increase in intracellular pH (Fig. 3). Also, amino acid decarboxylations have an important role in sensory changes. The action of decarboxylases on free amino acids generates primary amines with CO_2 release (Fig. 3). Some of these amines are volatile compounds that contribute to flavour. Some cheese types, such as Camembert, contain numerous volatile amines: methylamine, ethylamine, n-propylamine, isopropylamine, n-butylamine, 1-methylpropylamine, n-amylamine, isoamylamine, n-hexylamine, ethanolamine, dimethylamine, diethylamine, dipropylamine and dibutylamine. Secondary and tertiary amines, which also contribute to flavour, are also found, but their production is not clear. Alkyl pyrazines and pyridine are present in various cheeses like parmesan, emmental and gruyere (Ardö & Varming, 2010; Fernández & Zúñiga, 2006; Smit et al., 2005b; Yvon, 2006). In addition, amino acid transamination reactions play the main role in the production of aromatic compounds. Transaminases are widely distributed in microorganisms and catalyse the conversion of an amino acid into α-ketoacids (Fig. 3). These enzymes have a specific activity that is related to two aminoacid groups, branched chain and aromatic amino acids (Rijnen et al., 2003; Tanous, Gori, Rijnen, Chambellon, & Yvon, 2005; Yvon, Chambellon, Bolotin, & Roudot Algaron, 2000). Catabolism of branched amino acids leucine, isoleucine and valine leads to the formation of α-ketoisocaproate, α-keto-β-methyl valerate and α-ketoisovalerate (Thierry, Maillard, & Yvon, 2002).

In lactococci, α-ketoglutarate is generally the amino group receptor, although pyruvate and oxaloacetate may also function as acceptors (Pudlik & Lolkema, 2013). When α-ketoglutarate is added to cheese coagulate it increases the amino acid conversion rate, leading to a greater concentration of α-ketoacids, α-hydroxy acids, and carboxylic acids (Banks et al., 2001; Yvon, Berthelot, & Gripon, 1998). This conversion is further magnified by the action of glutamate dehydrogenase, which produces α-ketoglutarate from glutamate. Introduction of the gene coding for this enzyme in strains of *L. lactis* results in an increase in transaminase activity and in the production of aromatic compounds. However, flavour production depends upon aminotransferase affinity for different amino acids (Tanous et al., 2005). As shown in Fig. 3, the central α-ketoacids are intermediaries in amino acid catabolism. After hydrogenation, they originate the corresponding α-hydroxyacid along with aldehydes by decarboxylation (Ardö & Varming, 2010; Smit et al., 2005b).

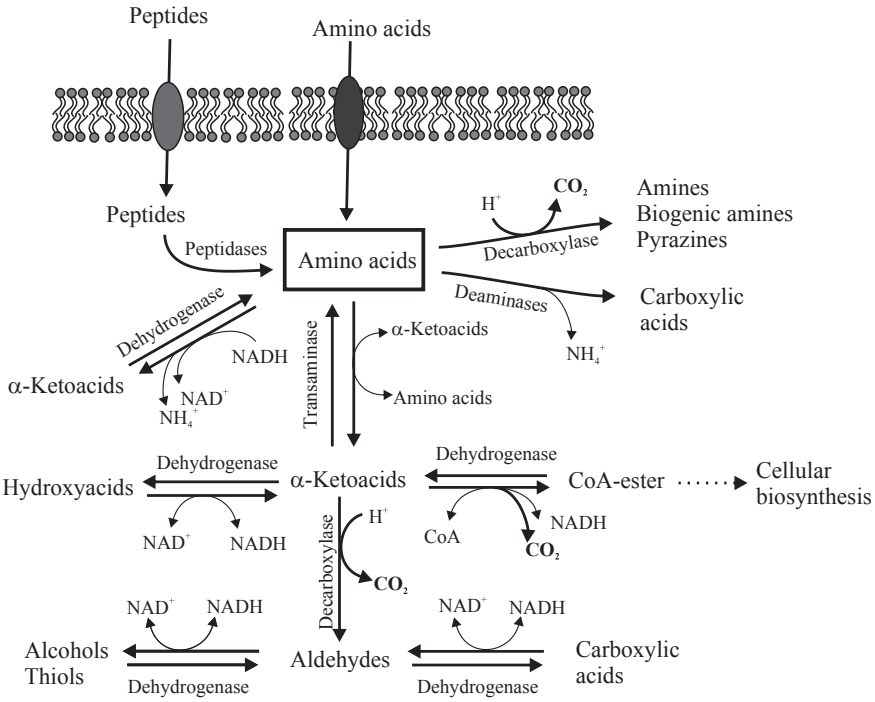

Fig. 3. Overview of amino acid catabolic reactions relevant for flavour compounds formation in cheese.

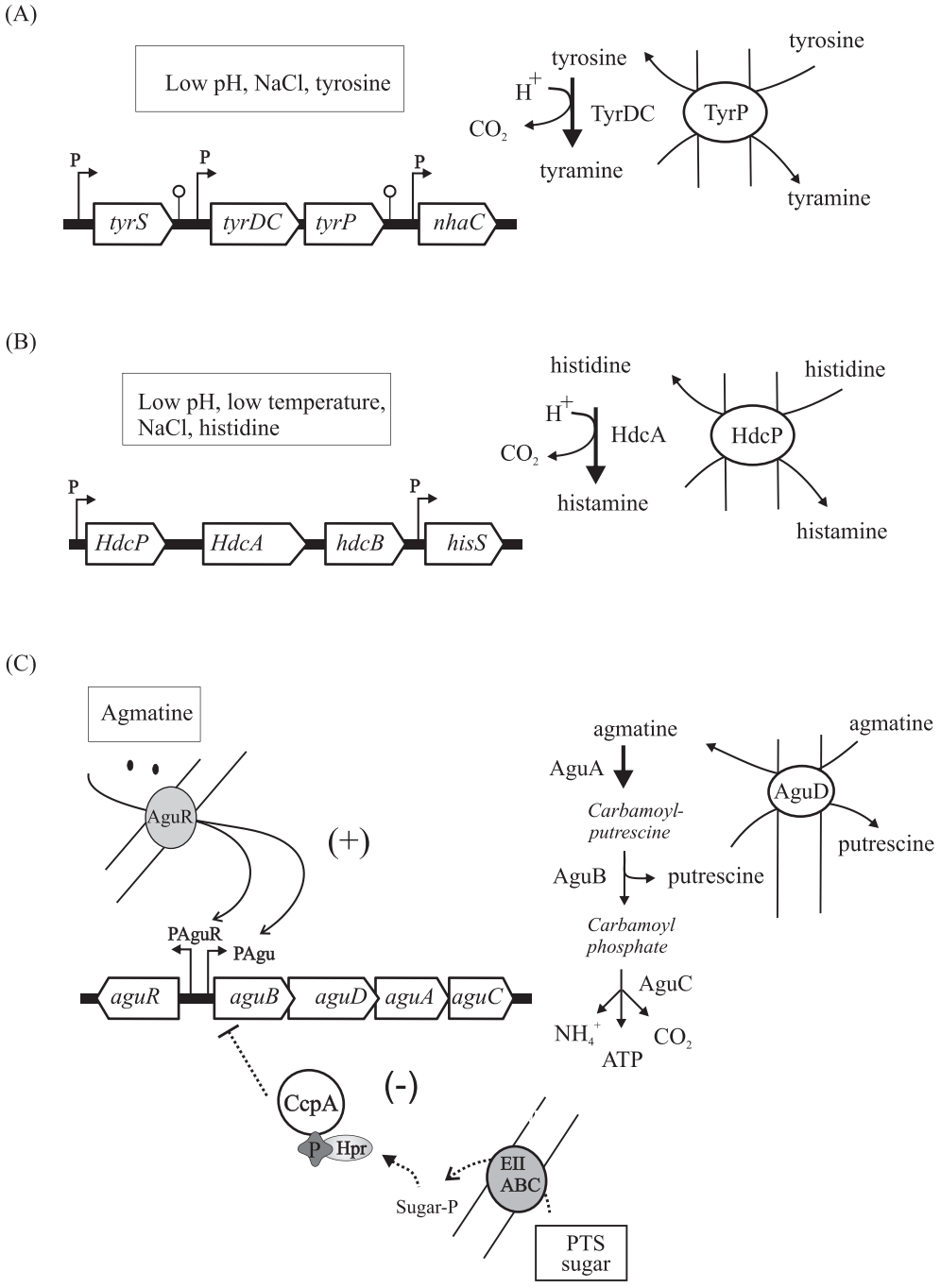

Fig. 4. Biogenic amine production in LAB: (A) tyramine:tyrosine/tyramine transporter (TyrP), tyrosine decarboxylase (TyrDC), *tyrS* and *nhaC* encoded aminoacyl tRNA synthetase and a putative Na^+/H^+ antiport; (B) histamine: histidine/histamine transporter (HdcP), histamine decarboxylase (HdcA), *hdcB* encoded enzyme required for activation of HdcA and *hdcS* encoded aminoacyl tRNA synthetase; (C) putrescine:agmatine/putrescine transporter (AguD), agmatine deiminase (AguA), putrescine carbamoyl phosphate transferase (AguB), carbamate kinase (AguC), and transcriptional activator, AguR.

Decarboxylation of α-ketoacids is likely to play a major role in flavour generation. α-keto derivative decarboxylation from branched chain amino acids leads to the formation of chocolate-flavoured aldehyde 3-methyl butanal, 2-methyl butanal, and 2-methyl propanal, which are generally produced by starter or NS-LAB species such as *L. lactis* and *Lb. casei* (Smit et al., 2005a, 2005b). Some lactococci produce small amounts of aldehydes, suggesting that there is weak gene expression associated with the decarboxylase or a very efficient metabolism with the subsequent formation of other products (Smit et al., 2005a, 2005b).

2.2. Negative effects of the decarboxylation pathway in cheese ripening

Amino acid decarboxylations are important reactions in sensory changes. However, from a food safety standpoint both reactions may also lead to the formation of biogenic amines (Fig. 4). These compounds are organic bases of low molecular weight with biological activity. The most common biogenic amines are tyramine, histamine, agmatine, putrescine, cadaverine, spermine, spermidine, phenylethylamine, and tryptamine. Although biogenic

amines are compounds commonly present in all living organisms (Dudkowska et al., 2003), ingestion of large amounts can cause health problems such as headaches, palpitations and vomiting (Silla Santos, 1996; Spano et al., 2010). Biogenic amines are detected in cheese and other fermented foods such as beer and wine while the highest amount of these compounds is found in fish and its derivatives (Coton et al., 2010; Linares et al., 2012; Mayer, Fiechter, & Fischer, 2010). Among LAB, strains of the *Enterococcus*, *Lactobacillus*, *Leuconostoc* and *Streptococcus* genera have been described as biogenic amine producers. These may belong to species that are normal microbiota of milk, introduced as contamination or indeed added as S-LAB or NS-LAB strains during cheese making (Linares et al., 2012; Spano et al., 2010). Biogenic amine content in cheese is of great significance not only for its potential health hazard but also from an economic point of view, since products exceeding recommended limits can be refused in some commercial transactions (EFSA, 2011; Suárez et al., 2012). Therefore, the risk of incorporating biogenic amine-producing strains should be avoided by using well-characterised cultures (EFSA, 2011; Linares et al., 2012).

The most important biogenic amines in dairy foods are histamine, tyramine (produced by enzymatic decarboxylation of histidine and tyrosine, respectively), and putrescine (synthesised via ornithine decarboxylation or agmatine deimination) (EFSA, 2011; Linares et al., 2012). The physiological role of biogenic amine metabolism in acid resistance has been extensively reported (Griswold, Jameson-Lee, & Burne, 2006; Lucas et al., 2007; Romano, Ladero, Alvarez, & Lucas, 2014; Suárez et al., 2013). Decarboxylation of histidine and tyrosine contributes to bacterial survival in acid stressed media by generation of chemiosmotic energy through the production of membrane potential and intracellular proton consumption through decarboxylation reactions (Konings et al., 1997; Pereira, Matos, Sao Roman, & Crespo, 2009). On the other hand, agmatine produces direct synthesis of ATP through level-substrate phosphorylation, NH_4^+ and CO_2, which lead to intracellular proton consumption (Fig. 4).

Defined S-LAB used in the dairy industry usually do not exhibit amino acid decarboxylation activity, therefore the main producers of biogenic amines are NS-LAB strains that belong to the genus *Enterococcus* and *Lactobacillus*. However, biogenic amines production is strain dependent, which explains why a particular group shows great differences in their production (Novella-Rodríguez, Veciana-Nogués, Trujillo-Mesa, & Vidal-Carou, 2002). In agreement with this, different biogenic amine pathways have been encoded in the same genomic cluster in several LAB species (Lucas et al., 2007; Romano et al., 2014; Suárez et al., 2012), and as shown in Fig. 4, each set of genes encoding for histidine, tyrosine and agmatine pathways are organised in their respective operons.

The ingestion of tyramine may develop a hypertensive crisis that could be potentially fatal in monoamine oxidase inhibitor users sometimes called “cheese reaction”. This reaction depends on the concentration of free tyrosine and the presence of microorganisms that possess the gene coding for tyrosine decarboxylase (*tyrDC*). It has been determined that other factors that affect tyramine production in bacteria are growth phase, pH, temperature and salt concentration. Tyrosine decarboxylase enzyme (TDC), like other decarboxylases, is dependent on the presence of coenzyme pyridoxal phosphate and is induced by reaction substrate, i.e., tyrosine. The gene cluster encoding TDC was first characterised in *E. faecalis* (Connil et al., 2002) and is transcribed in an operon that consists of TDC itself (*tyrDC*), substrate/product transporter (*tyrP*), aminoacyl tRNA synthetase and Na^+/H^+ antiporters (*nhaC-2*), are suggested to have been transferred in bacteria as a single mobile genetic element (Fig. 4A) (Lucas, Landete, Coton, Coton, & Lonvaud-Funel, 2003). In *Lactobacillus brevis* (NS-LAB strain), it was shown that tyrosine transporter (TyrP) catalyses the tyrosine/tyramine exchange with high efficiency and a net charge is translocated through the membrane during the exchange, generating proton motive force (Wolken, Lucas, Lonvaud-Funel, & Lolkema, 2006). The genetic organisation of the genes of the TDC route is conserved in several species of NS-LAB such as *E. faecalis*, *E. faecium* and *Lb. brevis*. Moreover, transcriptional activation in response to low pH has been reported for genes encoding tyramine decarboxylase (*tdcA*) and tyramine transporter (*tyrP*) from *Enterococcus durans* (Linares, Fernández, Martín, & Alvarez, 2009).

Histamine is the most important toxicological compound present in cheese (EFSA, 2011). However, histidine decarboxylation is an uncommon feature among Gram-positive bacteria. This trait may be associated to chromosomal or plasmid elements and are strain specific (Trip, Mulder, Rattray, & Lolkema, 2011). It was shown that in *Lactobacillus buchneri* and other NS-LAB species, four genes are responsible for histamine synthesis: *hdcA, hdcC, hisS* and *hdcB* (Martín, Fernández, Linares, & Alvarez, 2005b) (Fig. 4B). These genes encode for a pyruvoyl-dependent histidine decarboxylase, a histidine/histamine antiporter, a histidyl-tRNA synthetase and an enzyme involved in the activation of HdcA, respectively (Trip et al., 2011). Similar to the observations for the TDC cluster, a specific aminoacid -tRNA synthetase was also present. These enzymes may be acting as regulators of biogenic amine synthesis in several bacteria.

Putrescine is frequently isolated from cheese. However, this diamine could be generated by ornithine decarboxylase present mainly in Gram-negative (EFSA, 2011). In LAB, putrescine is produced by AgDI. This pathway was described in the cariogenic microorganism *Streptococcus mutans* and also in *E. faecalis*, and *Lactobacillus hilgardii* (Alberto, Arena, & Manca de Nadra, 2007; Griswold et al., 2006; Llácer et al., 2007).

In *E. faecalis*, agmatine is used an energy source for growth (Llácer et al., 2007). The genetic organisation of the cluster of genes coding for enzymes involved in the metabolism of agmatine in *E. faecalis* is shown in Fig. 4C. The genes are organised in an operon that consists of the *aguB*, *aguD*, *aguA* and *aguC* genes, and divergently a gene corresponding to *aguR* regulator is found (Suárez et al., 2013). It was reported that these genes were induced by the presence of agmatine; it was also reported that catabolite repression by glucose-mediated and arginine is operative on the gene cluster (Suárez et al., 2013). This pathway was more detailed in *S. mutans*, wherein the AgDI system is encoded by the operon *aguBDAC* (Fig. 4C). Agmatine is transported into the cell by agmatine-putrescine exchanger AguD; it is then hydrolysed to N-carbamoylputrescine and ammonium by agmatine deiminase. The N-carbamoylputrescine undergoes phosphorolysis reaction catalysed by the enzyme putrescine carbamoyl transferase (*aguB*), giving putrescine and carbamyl. Finally, the phosphate is transferred from carbamyl phosphate to ADP by the kinase carbamate (AguC) generating ATP, CO_2 and NH_4^+. Putrescine is then exchanged via agmatine exchanger (Fig. 4C).

In Fig. 4C, the mechanisms of induction and catabolite repression involved in regulating both *aguBDAC* operons in *L. lactis* (Linares et al., 2013) and *E. faecalis* (Suárez et al., 2013) are summarised. Agmatine may interact with the transcriptional regulator, activating transcription AguR *aguBDAC* operon, which codes for enzymes involved in the metabolism of agmatine AguA, AguB and AguC) and transport (AguD). The PTS sugars are transported into the cell via the PTS, these sugars can exert catabolite repression through two mechanisms, one dependent and one independent of CcpA. The first mechanism uses a *cre* site, while the second mechanism would exert its action through interaction of PTS glucose/mannose system and an undetermined regulatory protein (Lamberti et al., 2011; Landete, Pardo, & Ferrer, 2008; Suárez et al, 2013).

3. Conclusion

Cheese as a bioreactor represents a very complex ecosystem where the final product represents a balance between physical, chemical and biochemical processes. Most of these processes are directly caused or influenced by the fermentation process. In the dairy industry, lactic acid bacteria (LAB) have been used as natural starter cultures (non-selected) for hundreds of years. More recently selected microorganisms have been introduced to improve the cheese quality.

In the last decade the main properties involved in the generation of desirable quality have been explored and selections of LAB for specific starters have been performed. Citrate metabolism is one of the most common and desirable generator of organoleptic characteristics, as it generated not only holes but also diacetyl/acetoin. *L. lactis* biov. diacetylactis is one of the globally used starter LAB bacteria and represents a domesticated microorganism in which citrate and acetoin/diacetyl pathway is coordinated and expressed at low pH. It is important to note that the acetoin/diacetyl pathway is essential for the generation of aroma compounds, which give a characteristic butter tone to cheese. In other *L. lactis* used as starters, the pathways present intense genetic rearrangements that include the presence of redundant function and modifications by insertion. Some genetic traits, such as citrate transport and lactose metabolisation, are encoded in plasmids but genes that code for pyruvate metabolisation towards C4 compounds and its subsequent pH homeostasis are conserved in the chromosome of all *L. lactis* strains.

During the ripening process, non-starter microorganisms play a dual role in positive and negative properties. In these microorganisms, citrate metabolism is a common property found in genera such as *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Weissella* and others. The regulation of gene expression is different and adapted to the specific microorganism. Transcriptional activation is mediated by different transcriptional factors (DeoR and GntR family) in the presence of the substrate and catabolic repression is also observed in *Enterococcus* strains but not in *Weissella* and *Leuconostoc*.

Only in the last years a number of undesirable groups of genes has emerged, which is associated with public health and potential risk of the presence of biogenic amines. A set of recommendations given by food protection agencies about the use and quality of the raw milk and the hygienic quality of the cheese production process is intended to reduce the risk. The use of selected or defined S-LAB, that do not produce biogenic amines is a good, inexpensive practice for factories. Available molecular techniques for characterisation and selection of non-biogenic amine producing strains are widely described in references in the bibliography. Its utilisation should be a standard step in an industry concerned to produce safer foods. The situation is more complex in artisanal cheese production, where utilisation of natural starter and nonstarter is not defined. In this context, a better understanding about the probable genetic profile of strains used in artisanal cheese making and the conditions under which traits are expressed should help to develop safer production strategies.

In summary, the selection of components of the microbiota with desirable features could be a solution to the potential risk of presence of the biogenic amines during a long period of ripening. The use of adjuncts with desirable features could be a solution to inhibit undesirable microbiota. Thus, selected strains could be used as adjunct to natural starters to diminish the growth of undesirable microorganisms without affecting the sensorial quality of the cheese. In this direction novel strategies are under investigation that include the use of lactic acid bacteria able to degrade biogenic amines or the use of phages to reduce the number of specific undesirable microorganisms present in the cheese matrix. For example, Herrero-Fresno et al. (2012) developed a strategy based on the use of selected *Lb. casei* strains that showed a high biogenic amines degradation rate. Consequently the use of these strain as non-starter culture in the manufacture of raw-milk cheese produces a high reduction of tyramine content in mini-Cabrales cheese.

Undoubtedly, our better understanding of the decarboxylation reactions and of gene regulation mechanisms that operate in these pathways, will allow us in the near future to develop starter cultures based on a rational selection of microorganisms and strains to generate safe and high quality cheeses.

Acknowledgements

We would like to thank Agencia Nacional de Promoción Científica y Tecnológica (ANPyCT, PICT 2014-1513 and PICT 2014-3482), CONICET (PIP Nº 0718 and 11220150100855CO) and the European Union (BIAMFood, contract KBBE-211441) for financial support. FAZ and PM are fellows of CONICET; SHA, VB, ME and CM are researchers of the same institution. We also thank the staff from the English Department (Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR) for language correction of the manuscript.

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