Comprehensive
REVIEWS
in Food Science and Food Safety

Biogenic Amines in Dairy Products: Origin, Incidence, and Control Means

Noreddine Benkerroum

Abstract: Biogenic amines (BAs) are toxic compounds produced by a number of microorganisms (bacteria, yeasts, and molds) as a result of the metabolism of some amino acid, usually decarboxylation reactions. BA-producing microorganisms are not necessarily pathogenic, such as lactic acid bacteria, which are, on the contrary, among the most beneficial microbiota to human beings and some of which even have probiotic properties. However, the incidence of BAs in dairy products and their possible implication in serious dairy-borne intoxications has long been overlooked. Consequently, the implementation of control measures to limit such an incidence has not been considered among the priorities of the food safety authorities. Nonetheless, there is a growing concern with regard to the presence of BAs in dairy products, because their toxicological status as toxins that may have serious acute and/or chronic adverse health effects is becoming increasingly evident and well-documented. The main BAs associated with dairy products are reviewed herein from the perspective of their incidence in these food products, and to draw the attention of readers to the shortage in data to perform pertinent risk assessment, which is considered to be a key action to provide efficient control means and to help decision makers issue appropriate legislative and regulatory measures.

Keywords: biogenic amines, biosynthesis, control, dairy products

Introduction

Dairy products are important components in the diet of human beings around the world. Their current consumption is relatively high and is expected to increase steadily during the next 2 decades (Gerosa and Skoet 2013). Therefore, the provision of wholesome and safe dairy products to consumers is expected to be more challenging with the anticipated increased consumption, as the risk increases with the exposure to hazards, such as biogenic amines (BAs), potentially present in the product; the exposure is a function of the intake, which is positively correlated with the consumption. The increase in consumer demand for minimally processed dairy products and those prepared from raw milk adds to this challenge.

However, milk provides an adequate medium for the growth of virtually all microorganisms, including those producing toxic metabolites due to its rich and balanced chemical composition. Microbial growth and subsequent *in situ* production of metabolites with putative toxicological effects is favored by the limited inhibitory activity of the naturally occurring antimicrobial substances in milk (Benkerroum 2008, 2010; Claeys and others 2013).

Therefore, dairy products have been frequently associated with foodborne intoxications due to contamination with preformed toxins of microbial origin, including bacterial exotoxins, mold mycotoxins, and BAs. The latter toxic compounds continue to raise concern due to their frequent detection at high levels in various types of dairy products, especially ripened cheeses, and to increased awareness of their actual or potential adverse health effects. Also, the fact that BAs are produced not only by microbial dairy contaminants of different origins but also by the technological microbiota used in the fermentation and/or ripening of dairy products, including lactic acid bacteria, yeasts, and molds, complicates their control by conventional means.

This review focuses on BAs that can occur in various types of dairy products as a result of the metabolism of some amino acids. The precursor amino acids occur naturally in milk or are generated by hydrolytic activities of proteases, peptidases, and/or aminopeptidases on milk proteins during cheese-making (fermentation, maturation, and/or storage of the product). The BAs are reviewed herein from the perspective of their incidence in dairy products, their origin, and biosynthesis pathways for their generation and accumulation in dairy products, and to suggest possible means to control their presence in these products. Some emphasis is put on the need to implement surveillance programs in order to generate the necessary data for pertinent risk assessment studies.

Origin of BAs in Dairy Products

BAs represent a group of toxic compounds, which has been classically associated with seafood (Shalaby 2000). However, the presence of these natural toxicants in dairy products is raising increased concern regarding food safety. BAs are low molecular weight basic substances, which are structurally related to alkaloids, and they are analogs of naturally occurring amines that play important physiological roles in animals and plants (Smith 1971;

MS 20160396 Submitted 13/3/2016, Accepted 27/4/2016. *Author is with Inst. Agronomique et Vétérinaire Hassan II, Dépt. des Sciences Alimentaires et Nutritionnelles, BP 6202, Instituts, 10101-Rabat, Morocco. Direct inquiries to author Benkerroum (E-mail: n.benkerroum@gmail.com).*

doi: 10.1111/1541-4337.12212

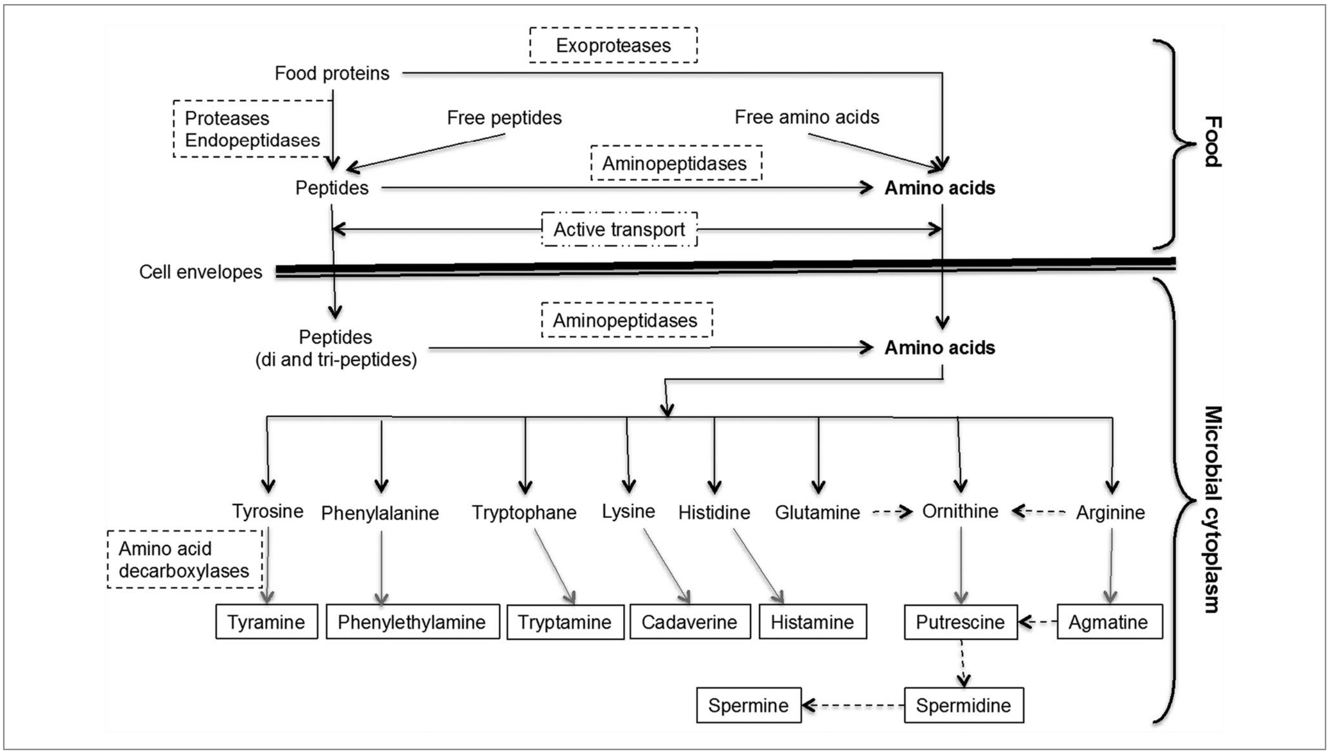


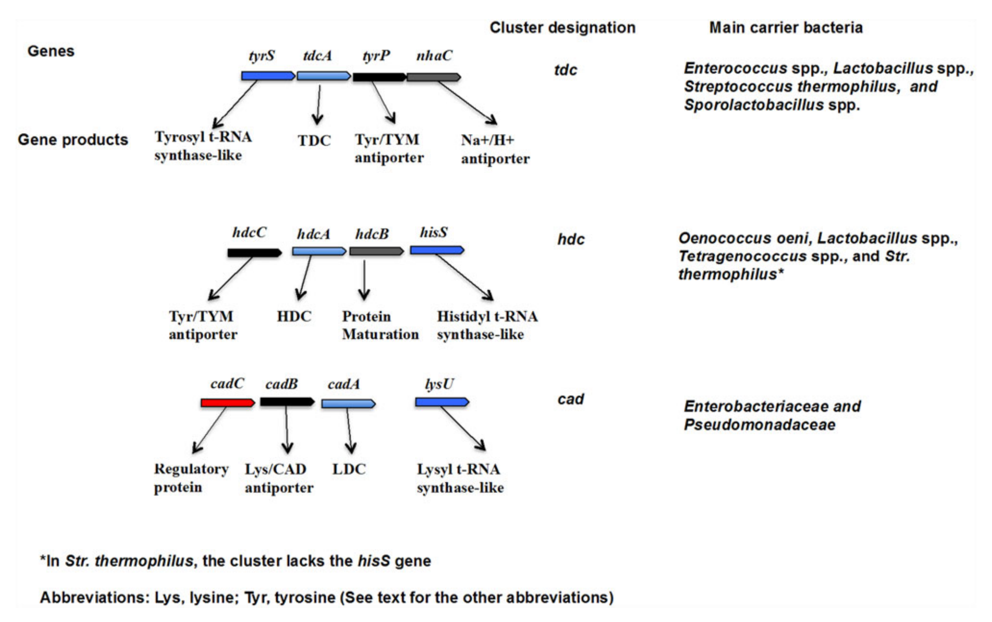
Figure 1–Formation of biogenic amines in food as a result of microbial metabolic activities. Gray arrows represent decarboxylation reactions leading directly to the formation of biogenic amines, and the dashed arrows indicate biogenic amines produced through pathways that differ from a 1-step decarboxylation reaction (see Figure 2 and 3 for detailed reactions and enzymes involved in polyamine biosynthesis). Adapted from Ruiz-Capillas and Jimenez-Colmenero (2004)

Medina and others 2003). For example, the so-called "natural BAs" (they are biosynthesized *de novo*) play vital roles in the bioregulation of cell growth and gene expression, protein synthesis, membrane division and stabilization, tissue repair, and modulation of intracellular signaling pathways and ion channels (Kusano and others 2008; Galgano and others 2012). In addition, polyamines (agmatine, spermine, and spermidine) and diamines (putrescine and cadaverine [CAD]; also considered as polyamines by some authors) play an important role in the regulation of membrane-linked enzymes, as they can interact with the anionic phospholipids of the membrane owing to their polycationic nature (Moinard and others 2005; Igarashi and Kashiwagi 2010). However, these nitrogen compounds are designated as biogenic or exogenous amines (as opposed to endogenous amines synthesized physiologically) when they are formed in foods by microorganisms where they play physiological roles. In microorganisms, BAs contribute to the provision of metabolic energy through proton-motive force when released outside the cell via antiporter systems (Molenaar and others 1993) or at substrate level when produced via pathways involving carbamate kinase (CK) enzyme (Cunin and others 1986). In addition, BAs play direct roles in acid tolerance (Romano and others 2014; del Rio and others 2015b) and in the regulation of osmotic and oxidative stresses (Fernández and Zúñiga 2006). Therefore, BAs are expected to be found in fermented foods and beverages of both animal or plant origin, especially in foods with high protein content (fish and fish products, meat and meat products, eggs, and dairy products) where they are released upon microbial/enzymatic hydrolysis of the proteins. The main BAs occurring in dairy products are produced by microbial metabolism consisting essentially of a decarboxylation reaction of specific cationic or aromatic amino acids (Figure 1). In some instances, aliphatic amines can derive from the amination and transamination of aldehydes and ketones (Koutsoumanis and others 2010).

Biosynthesis Pathways of BAs

A one-step decarboxylation reaction

Histamine (HIM), tyramine (TYM), phenylethylamine (PEA), and CAD are produced by a 1-step decarboxylation reaction from their respective precursor amino acids histidine, tyrosine, phenylalanine, and lysine. The production of these BAs in the cytoplasm, followed by their excretion outside the cell, requires systems for active transport and amino acid decarboxylase enzymes. The transport of precursor amino acids into the cytoplasm occurs generally via an antiporter protein in exchange for the resulting BA, with the known exception of tyrosine, which can use a uniporter transport system, although less efficiently than the antiporter system using TyrP protein. Following its intake, the precursor amino acid is decarboxylated by pyridoxal phosphate-dependent decarboxylases. The most studied of such decarboxylases are histidine decarboxylase (HDC), tyrosine decarboxylase (TDC), and lysine decarboxylase (LDC) produced by various bacteria where they are coded by the respective genes *hdcA*, *tdcA*, and *cadA*. These decarboxylase-coding genes are organized in clusters with other genes involved in other steps of the BA production process, such as transport and maturation of the enzyme, as shown in Figure 2. The specificity of these decarboxylases has long been debated, and it is now well established that a decarboxylase can decarboxylate different structural analogs. For example, TDC of the *Enterococcus*, *Lactobacillus*, and *Staphylococcus* genera decarboxylates phenylalanine and tyrosine to produce PEA and TYM, respectively (EFSA 2011; Marcobal and others 2006b, 2012). However, in some lactic acid bacterial species, such as *Lb. brevis*, TDC is specific for


Cluster designation
Main carrier bacteria
Genes
tyrS
tdcA
tyrP
nhaC
tdc
Enterococcus spp., *Lactobacillus* spp., *Streptococcus thermophilus*, and *Sporolactobacillus* spp.
Gene products
Tyrosyl t-RNA synthase-like
TDC
Tyr/TYM antiporter
Na+/H+ antiporter
hdcC
hdcA
hdcB
hisS
hdc
Oenococcus oeni, *Lactobacillus* spp., *Tetragenococcus* spp., and *Str. thermophilus**
Tyr/TYM antiporter
HDC
Protein Maturation
Histidyl t-RNA synthase-like
cadC
cadB
cadA
lysU
cad
Enterobacteriaceae and *Pseudomonadaceae*
Regulatory protein
Lys/CAD antiporter
LDC
Lysyl t-RNA synthase-like

*In *Str. thermophilus*, the cluster lacks the *hisS* gene

Abbreviations: Lys, lysine; Tyr, tyrosine (See text for the other abbreviations)

Figure 2–Organization of gene clusters involved in the production of biogenic amines by a 1-step decarboxylation reaction of precursor amino acids. Adapted from Linares and others (2011) Abbreviations: *tdc*, tyrosine decarboxylase; *hdc*, histidine decarboxylase; *cad* lysine decarboxylase; *nha*, sodium/hydrogen antiporter gene.

tyrosine, although it is less efficient than the TDC with a dual activity on both tyrosine and the structural homolog phenylalanine (Moreno-Arribas and Lonvaud-Funel 2001). Conversely, isolates of *Staphylococcus carnosus* were shown to produce significant amounts of PEA without producing TYM (de Las Rivas and others 2008), suggesting the existence of another mechanism or a specific decarboxylase to produce PEA from phenylalanine. Likewise, the transport system was shown not to be always specific, as lysine can enter the cell by either the *cadB* gene product of the *cad* cluster (Figure 2) or by the homologous *pot* gene product, which primarily codes for putrescine/ornithine antiporter system (see below for further details). No data are available in the literature, to our knowledge, on specific transport systems and decarboxylating enzymes of phenylalanine and tryptophan, which may otherwise use a common mechanism for aromatic amines, as is the case for TYM and PEA.

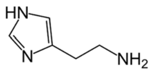
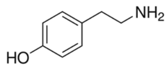
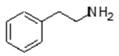
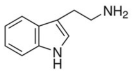
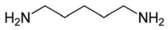
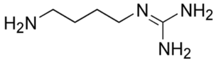
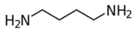
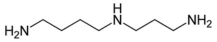
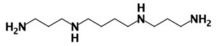
Biosynthesis of polyamines

Polyamines such as putrescine, agmatine, spermidine, and spermine are formed through various pathways (Figure 2 and 3) involving different enzymes coded by gene clusters which may be either species-specific or strain-specific, that is they are acquired by horizontal gene transfer (Ladero and others 2011a; Linares and others 2011; Marcobal and others 2012; Wunderlichová and others 2014).

Putrescine. Putrescine is one of the most abundant and frequently found polyamines in dairy products where it is produced by various LAB of the starter or adjunct starter cultures, or by microbial contaminants (Table 1 and 2). Concentrations of up to 2.5 g of putrescine per kg of cheese have been recorded recently (Linares and others 2013). Ornithine and agmatine are the main direct precursors of this BA via different pathways depending on the producer bacterium, genes/enzymes it possesses, and the ecological niche from which it originates (Liu and others 1995; Nannelli and others 2008; Figure 2). Alternatively, arginine is indirectly used as a putrescine precursor after being hydrolyzed or decarboxylated into ornithine or agmatine, respectively (Cunin and others 1986).

Biosynthesis of putrescine directly from ornithine. Ornithine undergoes a single-step decarboxylation pathway by ornithine decarboxylase (ODC) enzyme to yield putrescine and carbon dioxide (Figure 3E). The resulting putrescine is excreted via an antiporter protein in exchange for ornithine. Ultimately, this pathway results in the alkalinization of the cytoplasm and the generation of a proton motive force, as a means for the producing bacterium to resist acid stress and ensure ATP provision in order to survive nutrient shortage (Romano and others 2014). The ODC pathway is especially common in the Gram-negative enterobacteria and pseudomonads that possess the so-called "decarboxylation system" typically encoded by a gene cluster containing 2 adjacent genes: (i) *speC* encoding a biosynthetic/constitutive form of the ODC enzyme and (ii) *potE* encoding the transmembrane substrate/product exchanger protein. In several ODC-positive strains of *Enterobacteriaceae*, such as *E. coli*, *Salmonella* spp., and *Morganella morganii,* the ODC cluster includes *speF* gene encoding an inducible/biodegradative form of ODC in lieu of the *speC* gene (Applebaum and others 1975; de las Rivas and others 2007; Linares and others 2011). Nevertheless, some authors use *odc* referring to the gene encoding the ODC enzyme regardless of its metabolic form (Marcobal and others 2006a; Coton and others 2010a; Romano and others 2014). Gram-positive bacteria, however, have been infrequently reported to possess an ODC enzyme; and those that do have only the biodegradative form (*speF* product), and are not relevant to dairy products (Cunin and others 1986). Notable putrescine-producing Gram-positive strains via the ODC pathway are essentially, although not exclusively, derived from a wine environment, and they include strains of LAB belonging to the species *Lactobacillus saerimneri*, *Lactobacillus brevis* (Romano and others 2012, 2014), *Lactobacillus mali* (Coton and others 2010b), and *Œnococcus œni* (Marcobal and others 2006a). In a few instances, however, ODC-positive strains of *S. epidermidis* (Coton and others 2010a) and *Weissella halotolerans*

Table 1–Chemical properties of biogenic amines encountered in dairy products and their producer microorganisms.

Biogenic amine	Classification	Amino acid precursor	Producing microorganisms of dairy relevance	Chemical structure
Histamine	Heterocyclic/ monoamine	Histidine	**Gram-positive bacteria**: *Streptococcus thermophilus Lactobacillus buchneri, Lactobacillus parabuchneri, Lactobacillus curvatus, Lactobacillus helveticus, Lactobacillus sakei, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus rossiae, Clostridium perfringens, Staphylococcus xylosus* **Gram-negative bacteria**: *Morganella morganii, Klebsiella pneumonia, Kl. oxytoca, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter gergoviae, Hafnia alvei, Serratia liquefaciens, Ser. marcescens, Serratia liquefaciens Proteus vulgaris, Pseudomonas putrefaciens, Aeromonas hydrophila* **Yeast**: *Debaryomyces hansenii*	
Tyramine	Aromatic/ monoamine	Tyrosine	**Gram-positve bacteria**: *Lactococcus lactis subsp. lactis, L. lactis subsp. cremoris, Str. thermophiles, Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus durans, Lactobacillus brevis, Lb. curvatus, Lactobacillus divergens, Lb. buchneri, Lactobacillus alimentarius, Lactobacillus plantarum, Lb. curvatus, Lactobacillus bavaricus, Lactobacillus reuteri, Lb. sakei, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus bavaricus, Lactobacillus bifermentans, Lactobacillus casei, Lactobacillus johnsoni, Lactobacillus paracasei subsp. paracasei, Pediococcus spp., Sporolactobacillus. Carnobacterium divergens, Carnobacterium gallinarum Carnobacterium piscicola, Leuconostoc mesenteroides* **Gram-negative bacteria**: *Pseudomonas putida, Citrobacter freundii, E. coli, Hafnia alvei, Citrobacter braakii, Raoultella ornithinolytica, E. gergoviae, Ser. liquefaciens* **Yeast**: *Yarrowia lipolytica*	
Phenyl ethylamine	Aromatic/ monoamine	Phenylalanine	**Gram-positive bacteria**: *Lb. brevis, Carnobacterium divergens, Ent. faecalis, Ent. faecium, Ent. hirae, Bacillus cereus, Staphylococcus spp.*	
Tryptamine	Heterocyclic/ monoamine	Tryptophan	*Lb. curvatus, Lb. bulgaricus, B. cereus, Clostridium sporogenes*	
Cadaverine	Aliphatic/ diamine	Lysine	**Gram-positve bacteria**: *Lb. brevis, Lb. curvatus, Lb. casei, Lb. paracasei* ***Gram-negative bacteria***: *Pseudomonadaceae and Enterobcteriaceae (similar to putrescine below)* **Yeast**: *Y. lipolytica*	
Agmatine	Aliphatic/ polyamine	Arginine	**Gram-positive bacteria**: *Ent. fæcalis, B. cereus, B. subtilis* **Gram-negative bacteria**: *Enterobacteria (e.g., E. coli, Klebsiella aerogenes, Salmonella Typhimurium), pseudomonads (e.g., Ps. aeruginosa), and Aeromonas spp.*	
Putrescine	Aliphatic/ diamine	Arginine[a],[b]	**Gram-negative bacteria**: *Lb. brevis, Lactobacillus acidophilus, Lactobacillus collinoides, Lb. brevis, La ctobacillus mali, Lactobacillus paracollinoides, Lactobacillus fructivorans, Lb. curvatus, Lb. sakei, Lb. fermentum, Lb. lactis, Lactobacillus paracasei, Lb. plantarum, Lactobacillus rhamnosus, Lactobacillus sanfranciensis, Enterococcus casseliflavus, Enterococcus durans, Ent. faecalis, Ent. faecium, Enterococcus hirae, Leuconostoc mesenteroides subsp. mesenteroides, Leuconostoc lactis, Str. thermophilus, Streptococcus mutans, Pediococcus parvulus, Pedicoccus pentosaceus, Lactococcus lactis, Listeria monocytogenes, Bacillus subtilis, Bacillus licheniformis, Bacillus cereus* **Gram-negative bacteria**: *Escherichia coli, Escherichia fergusonii, Citrobacter freundii, Citrobacter braakii, Cronobacter sakazakii, Enterobacter aerogenes, E. cloacae, Enterobacter georgoviae, Hafnia alvei, Klebsiella oxytoca, Klebsiella terrigena, Mor. morganii, Pr. mirabilis, Pr. vulgaris, Providencia spp. Salmonella enterica, Serratia grimesii, Serratia liquefaciens, Serratia marcescens, Yersinia enterocolitica, Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas lundensis, Pseudomonas luteola , Ps. putida, Pseudomonas agglomerans, Psychrobacter celer, Acinetobacter spp., R. ornithinolytica* **Yeast**: *Y. lipolytica, Candida intermedia, Candida incospicua, Kluyveromyces marxiamus var. lactis, and Debaryomyces hansenii, Saccharomyces cerevisiae*	
Spermidine[c]	Polyamine	Arginine	*Corynebacterium sp., Bacteroides spp. Lb. plantarum, Lb. acidophilus, E. coli, B. subtilis, Enterobacter sp. E. coli, Ps. aeruginosa, Str. mutans* **Dairy-borne pathogens**: *Bacillus anthracis, Haemophilus influenzae, Salmonella Paratyphi, Salmonella Typhi, Salmonella Typhimurium, Shigella boydii, Shigella sonnei, Shigella dysenteria, Shigella flexneri, Streptococcus pneumoniae, Streptococcus pyogenes, Yersinia pestis, helocobacter pylori, Mycobacterium tuberculosis, Mycobacterium bovis* **Yeasts**: *Sac. cerevisiae, Schysoaccharomyces pombe, Sporomyces roseus, Candida albicans, Kluyveromyces lactis*	
Spermine[c]	Aliphatic/ polyamine	Arginine	**Gram positive bacteria**: *B. stearothermophilus, B. thermodenitrificans, B. acidocaldarius* **Yeast**: *Sac. cerevisiae, Candida albicans, Kluyveromyces lactis*	

[a] In some species, the production of putrescine may be strain specific, and hence dairy strains may not produce the BA.

[b] See text for the pathway used by the main producer microorganisms of putrescine from arginine (ADI or ADC via AgDI or ARG).

[c] Although the production of these polyamines by the dairy-borne microorganisms listed is well established, no correlation has been established between these microorganisms and the presence of the BAs in dairy products (Marino and others 2000).

Data compiled from: Bover-Cid and Holzapfel (1999); Bowman and others (1973); Buňková and others (2009); Cunin and others (1986); Davis (1986); Galgano and others (2012); Griswold and others (2004); Hosoya and others (2004); Komprda and others (2008a); Ladero and others (2011a); Lorencová and others (2012); Maifreni and others (2013); Marcobal and others (2012); Nout (1994); Pegg and Michael (2010); Rimaux and others (2012); Sekowska and others (1998); Spano and others (2010); Tabor and Tabor (1985); Vrancken and others (2009); Williams and others (2014); Wunderlichová and others (2014).

Table 2–Average and/or range (lower-higher concentration) of biogenic amine contents (mg/kg) in dairy products.

	Dairy products (well-established industrial cheeses)								
Biogenic amines	Parmesan	Parmesan	Mozzarella	Cheddar	Blue-veined cheese	Feta cheese	Emmentaler	Camembert	Gouda and Edam
TYM	103.0	3.6	1.8	44.5	(ND-1585.4)	246.0	(ND-917)	(>10.0–210)	(>0.1-670.0)
HIM	148.0	1.4	0.0	29.4	(ND-376.6)	84.6	(69.0–650.0)	(ND-480.0)	(ND-450.0)
AG	2.0	–	–	–	–	–	–	–	–
TRY	0.0	–	–	–	(ND-128.8)	5.7	–	(ND-60.0)	(ND-200.0)
PHM	8.6	ND	0.0	2.3	(ND-39.7)	4.9	<0.1	–	<0.1
CAD	12.7	1.2	0.2	4.5	(ND-2101.4)	82.8	16.0	–	(17.0-48.0)
SPD	2.5	30.7	17.5	26.6	–	–	–	–	–
SPM	0.3	ND	0.00	6.4	–	–	–	–	–
PUT	6.1	3.6	0.9	2.9	(3.0–257.2)	193.0	<0.5	–	(7.0-20.0)
TBA	–	40.5	20.3	116.4	–	617.0	–	–	–
References	Custódio and others (2007)	Min and others (2004)			Novella-Rodríguez and others (2003)	Valsamaki and others (2000)	O'Brien and others (2004)		

	Dairy products (specialty cheeses)							
Biogenic amines	Fresh cheese	Hard ripened cheeses	Hard ripened cheeses from raw milk	Goat milk cheese	Goat milk cheese	Goat milk fresh cheese	Raw goat milk ripened cheese	Blue-veined cheese from ovine milk[1]
TYM	(ND-0.6)	(ND-301.4)	(ND-609.4)	(ND-830.5)	735.4	11.3	207.1	52.20
HIM	ND	(ND-163.6)	(ND-391.4)	(ND-88.4)	23.2	ND	ND	–
AG	–	–	–	–	ND	–	–	–
TRY	ND	(ND-45.1)	(ND-33.8)	(ND-17.4)	4.6	–	–	71.11
PHM	ND	(ND-32.0)	ND-29.7	(ND-11.7)	6.5	–	–	61.44
CAD	ND-1.5	(ND-710.1)	0.9-368.5	(ND-88.7)	83.4	ND	149.0	–
SPD	–	–	–	–	6.1	–	–	–
SPM	–	–	–	–	2.0	ND	ND	–
PUT	ND-3.1	(ND-611.7)	(ND-670.1)	(ND-191.8)	34.4	ND	ND	32.97
TBA	–	–	—	–	–	(ND–32.7)	356.1	–
Reference	Novella-Rodríguez and others (2003)		–	–	Novella-Rodríguez and others (2002)	Bunkova and others (2013)		Calzada and others (2013)

(*Continued*)

Table 2–Continued.

	Dairy products (specialty cheeses)							
			Pecorini Abruzzeses					
Biogenic amines	Ewe's milk fresh cheese	Ewe's milk Pasta filata type cheese	A	B	Fromaggio di Fossa	Pecorino Del Parco Di Migliarino-San Rassore	Chilean Gouda	Dutch semi-hard cheese
TYM	(10.2–11.1)	ND	185.0	230.0	461.6	1300.1	(27.9–59.0)	(5.0–392.0)
HIM	ND	ND	261.0	76.0	24.1	32.4	ND	(22.0–59.0)
AG	–	–	–	–	–	–	–	–
TRY	–	–	–	–	–	–	–	–
PHM	–	–	35.0	305.0	173.0	–	–	–
CAD	(11.4–35.8)	ND	18.0	75.0	1302.9	22.4	(1.8–3.8)	–
SPD	–	–	–	–	–	–	–	–
SPM	ND	13.2	–	–	–	–	–	–
PUT	(55.3–118.2)	ND	80.0	163.0	579.6	173.0	–	(1.0–132.0)
TBA	(ND–140.3)	(ND–13.2)	697.9	1086.0	2557.7	1578.7	(4.4–87.6)	–
Reference	Bunkova and others (2013)		Martuscelli and others (2005)		Mascaro and others (2010)	Forzale and others (2011)	Brito and others (2014)	Komprda and others (2008a)

	Dairy products (specialty cheeses)							
Biogenic amines	Civil (Turkish)	Urfa	Mihalic	Kasar (Ripened)	Kasar (fresh)	Orgu	Otlu peynir	Otlu peynior
TYM	1381.6 (925.1–1951.6)	115.0 (0.0–324.3)	135.5 (108.3–183.9)	309.6 (0.0-931.1)	109.5 (32.1–194.9)	9.8 (0.0–30.2)	182.4 (47.4-263.8)	360.3 (18.0- 1125.5)
HIM	947.6 (912.3–996.5)	ND	126.4 (109.1–157.3)	45.9 (9.8-188.0)	35.2 (0.0–61.3)	25.4 (0.0–71.9)	17.4 (0.0-52.5)	197.9 (ND-681.5)
AG	–	–	–	–	–	–	–	–
TRY	ND	ND	ND	ND	ND	ND	ND	103.2 (ND-172.6)
PHM	ND	2.9 (0.0–14.5)	ND	ND	3.7 (0.0–0.48)	ND	ND	33.6 (ND-100)
CAD	489.6 (134.2–830.8)	161.0 (0.0–912.0)	8.5 (0.0–26.3)	26.5 (2.5-99.2)	42.6 (11.6–76.4)	26.3 (0.0–76.2)	8.0 (0.0-34.5)	288.4 (ND-1844.5)
SPD	ND	373.0 (115.3–831.4)	129.8 (914.0–170.3)	114.8 (0.0-197.6)	75.7 (0.0–169.3)	22.3 (0.0–30.2)	115.7 (41.8-168.9)	–
SPM	ND	ND	ND	ND	ND	ND	–	–
PUT	674.3 (359.6–990.5)	ND	208.7 (184.7–255.5)	88.1 (65.2-113.1)	34.7 (6.3–60.6)	48.3 (0.0–88.2)	24.3 (0.0-46.7)	192.5 (ND-847.0)
TBA	3493.1	651.9	608.9	584.9	201.4	132.1	347.8	–
Reference	Durlu-Ozkaya (2002)							Andic and others (2010)

(*Continued*)

Table 2–Continued.

Biogenic amines	Dairy products (fermented milks)					
	Stirred yogurt	Fermented cream	Kefir	Fermented cow milk	Fermented goat milk	Buttermilk
TYM	–	(5.5–15.4)	ND-9.6	249.6	337.1	(4.6–5.0)
HIM	(0.6-21.2)	–	(ND-1.6)	17.8	53.9	–
AG	–	–	ND	–	–	–
TRY	–	–	ND	–	–	–
PHM	0.1-5.2	–	ND	–	–	–
CAD	–	ND	(0.6-2.2)	29.1	22.3	ND
SPD	ND-1.2	–	0.6-4.5	82.9	34.9	–
SPM	0.3–4.8	–	ND	–	–	–
PUT	(ND-0.6)	(8.6–8.8)	(0.3–12.1)	20.3	22.9	ND
TBA	(2.5–26.7)	(5.5–15.4)	(2.4–35)	398.9	470.8	(4.6–5.0)
Reference	Min and others (2004)	Bunkova and others (2013)	Ozdestan and Uren (2010)	Costa and others (2015)		Bunkova and others (2013)

Abbreviations: TYM, tyramine; HIM, histamine; AG, agmatine; TRY, tryptamine; PHM, phenylethylamine; CAD, cadaverine; SPD, spermidine; SPM, spermine; PUT, putrescine; TBA, total biogenic amines; ND, not detected; -, not tested.

Pecorino is an Italian cheeses made exclusively from pure sheep milk characterized by a high fat content and is mainly produced by traditional procedures from raw or pasteurized milk. There exist many varieties of Pecorino cheese (including Fromaggio di Fossa and Pecorino Del Parco Di Migliarino-San Rassore) and have different ripening periods ranging from 21 d to 8 mo).

A: Cheese made from raw ewe's whole milk without added starter culture.

B: Cheese made from thermized ewe's whole milk with added starter culture.

1 After 360 d of ripening

Dairy products are obtained from pasteurized cow milk unless otherwise specified.

(Pereira and others 2009) were isolated from fish and meat products, respectively. Human isolates of *Lactobacillus acidophilus* (Azcarate-Peril and others 2004), *Lactobacillus johnsonii* (Wegmann and others 2009), and *Staphylococcus lugdunensis* (Tsoi and Tse 2011) were also reported to decarboxylate ornithine. Although the ODC gene cluster in the above-mentioned Gram-positive bacteria is also composed of *potE* and *odc/speF* genes, similar to that of Gram-negative bacteria, *Lactobacillus gasseri* ATCC33323, and *Lactobacillus casei* ATCC334 were shown to possess a putative ODC system having a unique dual specificity for ornithine and L-2,4-diaminobutyric acid (DABA; Romano and others 2012). This cluster has another particular feature consisting of the unidirectional uptake of the substrate (ornithine or DABA) and, as a consequence, the resulting diamine (putrescine or diaminopropane) remains within the cytosol (Romano and others 2012) to be further metabolized via different pathways including the synthesis of higher polyamines (Tabor and Tabor 1985; Cunin and others 1986). Moreover, Romano and others (2014) described an ODC gene cluster located on an acid resistance locus in the *L. brevis* IOEB 9906 genome. This newly described ODC cluster contains, in addition to *odc* and *potE* genes, a putative inducible transcriptional regulator (TR) gene of the *Lac*I family. However, the function of this TR gene with respect to *odc* and *potE* genes, and hence to putrescine biosynthesis, remains to be clarified. Considering the fact that none of the presently known ODC-positive LAB strains is of dairy relevance (Nannelli and others 2008; Ladero and others 2011a; Linares and others 2011), the accumulation of putrescine in dairy products via the ODC pathway is believed to be mainly due to contaminating Gram-negative bacteria of the *Enterobacteriaceae* and *Pseudomonadaceae* families. Therefore, the level of putrescine in these food products has been suggested to be used as a yardstick to assess their hygienic quality (Pattono and others 2008; Ladero and others 2010). Nonetheless, LAB including advantageous ones, or those used as starter or adjunct starter cultures, can use other means to produce putrescine in dairy products, and hence putrescine levels may not always be a valid spoilage indicator in dairy products.

Biosynthesis of putrescine directly from agmatine. Use of agmatine as the starting substrate to produce putrescine follows either one of 2 pathways depending on the bacterial species or strain: (i) agmatinase pathway (AGM) or (ii) agmatine deiminase (AgDI) pathway. The former is a biosynthetic route wherein agmatine is directly converted into urea and putrescine by the action of the agmatinase enzyme (AGM), a *speB* gene product (Figure 3F), and the resulting putrescine may be excreted or further metabolized to produce, for example, spermine or spermidine (Cunin and others 1986). Although this pathway is essentially common in *Enterobacteriaceae* (Tabor and Tabor 1985), it has also been reported in other dairy-borne contaminants such as *Bacillus* spp. (Sekowska and others 1998; Ivanova and others 2003) and *Pseudomonas* spp. (Ohji and others 2014; Ichise and others 2015). The prevalence of agmatinase pathway in such dairy contaminants provides an additional support to the assumption that high levels of putrescine in dairy products would represent an indication of nonhygienic manipulations during processing and/or storage.

The 2nd pathway for putrescine production from agmatine, the AgDI pathway, is biodegradative and mostly common in *Pseudomonas* spp., *Aeromonas* spp., and lactic bacteria. Here, agmatine undergoes the sequential action of 3 enzymes: (i) AgDI, (ii) putrescine carbamoyltransferase (PCT), and (iii) CK. The AgDI enzyme deiminates agmatine to yield an ammonium ion and N-carbamoyl putrescine which is, in turn, phosphorylated by

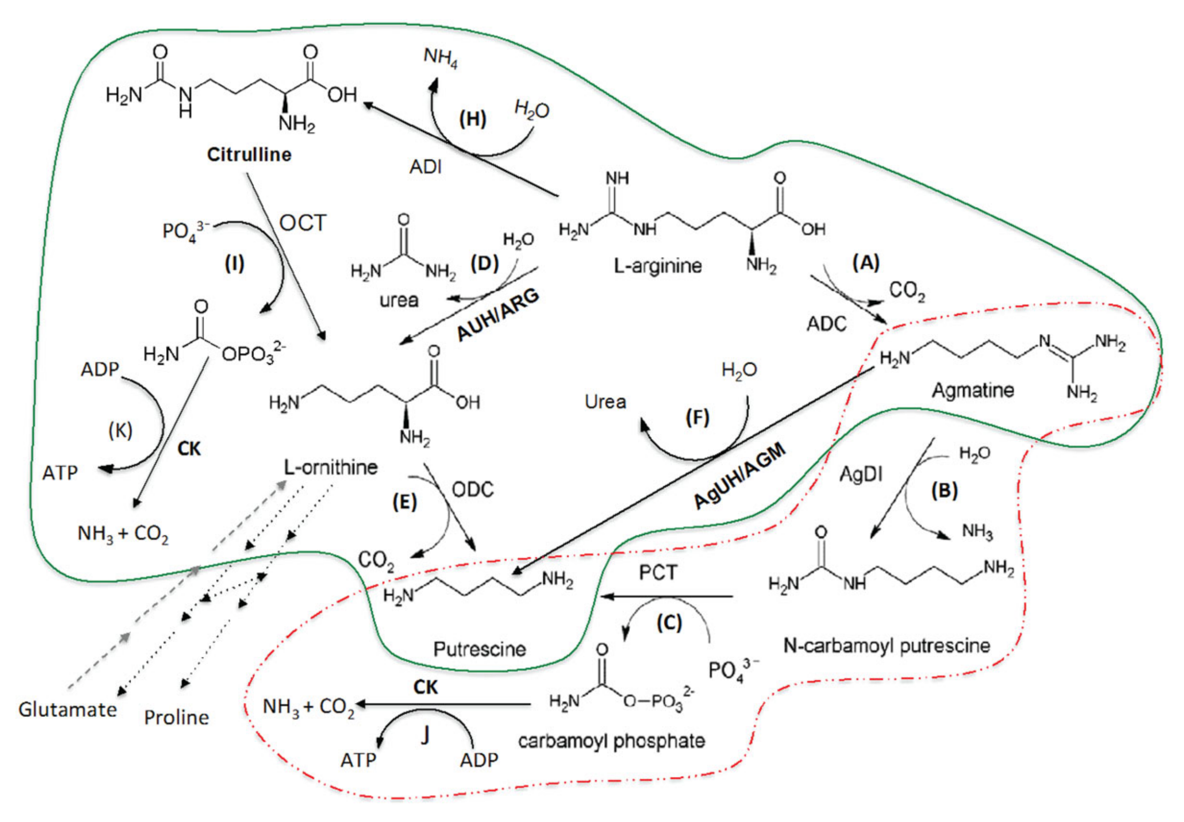


Figure 3–Biosynthesis pathways of putrescine in Gram-positive and Gram-negative bacteria. The biogenic amine (BA) can be produced in food by microorganisms via at least 5 distinct metabolic pathways: (1) arginine decarboxylase pathway (A to C), (2) arginase pathway (D and E), (3) agmatinase pathway (A and F), (4) arginine deiminase pathway (A, H, and I), and (5) ornithine decarboxylase (ODC; E). The dashed grey arrow shows that glutamate is converted, in arginine biosynthetic pathways (not discussed in this review), by some microorganisms into ornithine used as an intermediate in putrescine biosynthesis; for further reading, see Cunin and others (1986) and Lu (2006). The black small dashed arrows indicate the fate of ornithine produced from arginine via the catabolic arginase pathway in some bacilli and yeasts, which leads to other end products than putrescine (see text). Pathways circled in solid green line are found in Gram-positive and Gram-negative bacteria susceptible to contaminate dairy products, and the red dashed line circle indicates the only route presently known for putrescine biosynthesis in dairy LAB bacteria, although some lactococci may also use the ADI pathway (Budin-Verneuil and others 2006). Abbreviations: ARG/AUH, arginase/arginine ureohydrolase; ADC, arginine decarboxylase; AgDI, agmatine deiminase; PCT, putrescine carbamoyl transferase; ODC, ornithine decarboxylase; AGM/AgUH, agmatinase/agmatine ureohydrolase; OCT, ornithine carbamoyl transferase; ADI, arginine deiminase; CK, carbamate kinase; ADP, adenosine diphosphate; ATP, adenosine triphosphate.

the PCT to produce putrescine and carbamoyl phosphate (CP; Figure 3B, C, and J). Although the produced putrescine is excreted via a specific agmatine/putrescine antiporter (AgmP), CP is used as a substrate for ADP phosphorylation by CK to give an ATP and another ammonium ion. Therefore, the outcome of this pathway is the production of metabolic energy in the form of ATP at the substrate level and the alkalinization of the growth medium with concomitant accumulation of putrescine. However, despite the medium alkalinization as a result of ammonia and putrescine excretion, this pathway appears to be primarily used to promote the growth of the producing bacterium after nutrient depletion rather than a means for acid tolerance (del Rio and others 2015b), contrary to what has been demonstrated earlier in *Streptococcus mutans* and *Lb. brevis* (Griswold and others 2004; Lucas and others 2007; Liu and others 2009; Spano and others 2010). Indeed, the growth of a putrescine-producing *Lactococcus lactis* in the presence of agmatine was accelerated after the stationary phase, in a typical diauxic growth pattern, and it caused putrescine to accumulate in the external medium. Also, the growth promotion by agmatine after the stationary phase was not impaired when the pH was maintained constant at 5.6 (del Rio and others 2015b). These results are in line with those of a previous study showing that the AgDI gene cluster is not induced by low pH in *Enterococcus fœcalis*, although the alkalinization of the growth medium following agmatine catabolism alleviates the effect of acidity on the growth of the bacterium (Suarez and others 2013). It is worth mentioning that some strains may have more than 1 gene cluster encoding putrescine production via different pathways to cope effectively with unfavorable conditions of low pH and nutrient shortage. For example, a wine isolate, *Lb. brevis* IOEB9906, was shown to harbor both AgDI and ODC gene clusters next to each other on an acid resistance chromosomal locus (Romano and others 2014). Occurrence, in the same strain, of different gene clusters encoding the production of more than 1 BA has also been suggested to be used as a response to stressful conditions of high acidity and/or nutrient depletion (Tabor and Tabor 1972; Driessen and others 1988; Lucas and others 2007; Pereira and others 2009; Romano and others 2013; del Rio and others 2015b). In dairy LAB, TDC cluster, coding TYM production rather than the ODC, was frequently found next to the AgDI cluster on an acid tolerance locus (Lucas and others 2007; Romano and others 2014). In fact, no ODC cluster has as yet been described in dairy LAB which, however, use the AgDI

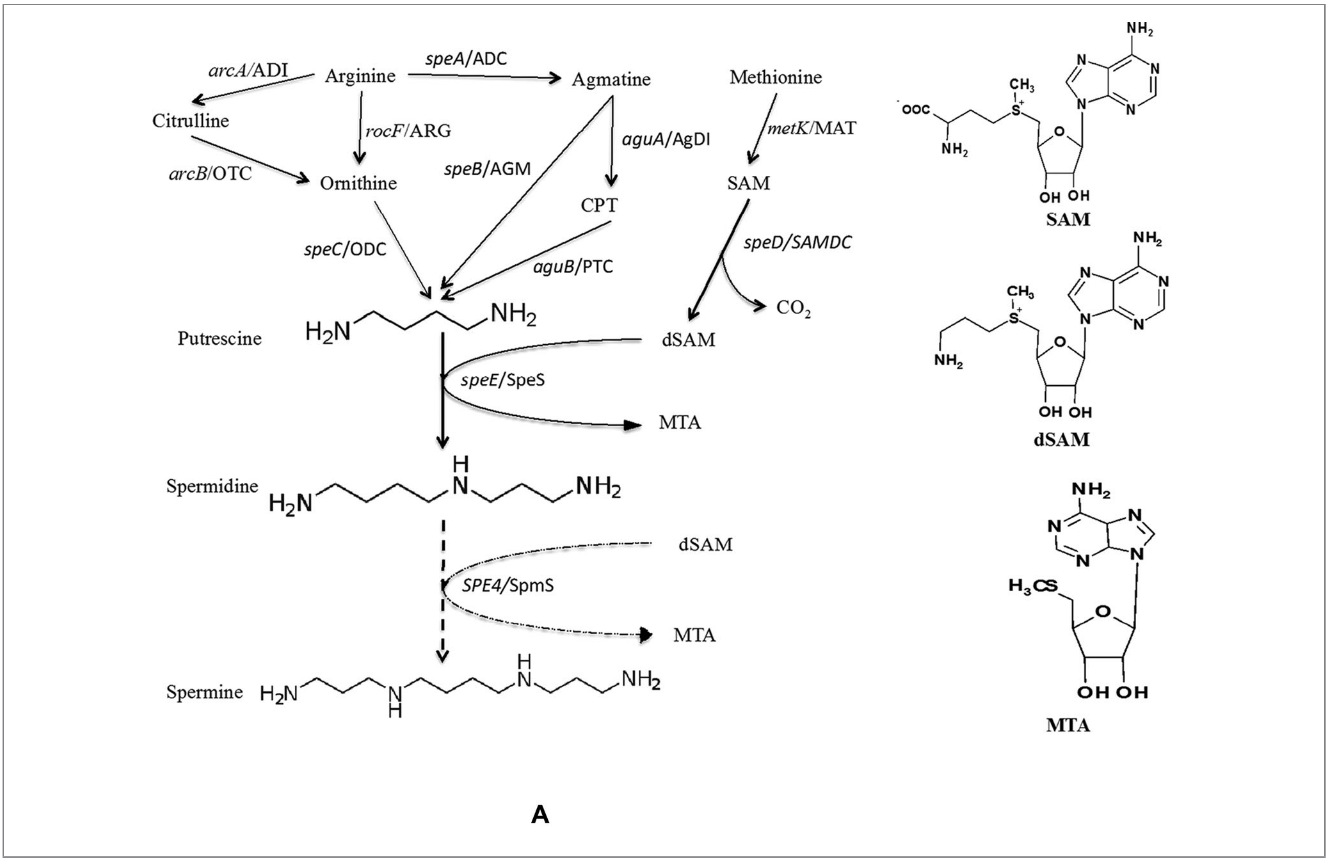


Figure 4A–Formation of spermidine and spermine from putrescine in microorganisms. Dashed arrows indicate the pathway of spermine biosynthesis in yeasts, and it ends at the spermidine level in bacteria lacking the spermine synthase (Tabor and Tabor 1985).

pathway as the only means to produce putrescine; and species such as *Ent. faecalis*, *Enterococcus hirae*, *Lb. brevis*, *Lactobacillus curvatus*, and *L. lactis* are known to be the main putrescine producers in dairy products (Ladero and others 2012a, 2012b). Indeed, many strains of these species were shown to harbor gene clusters encoding the components of the AgDI pathway consisting of the *aguR* gene encoding a TR plus 4 other genes organized in 1 operon encoding the following catabolic components of the pathway: *aguD* encoding AgmP, *aguA* encoding AgDI enzyme, *aguB* encoding PCT, and *aguC* encoding CK (Figure 5). Although AgDI clusters of different putrescine-producing species were shown to have the same panel of genes and share many structural and functional properties, they also bear significant differences that distinguish an AgDI cluster of one species or strain from another. For example, the AgDI clusters in *Lactobacillus spp., Lis. monocytogenes*, and *P. penstosaceus* contain a duplicate of *aguA* gene, *aguA2* (Lucas and others 2007; Ladero and others 2011a; Romano and others 2014), which appears to code for an AgDI devoid of catalytic activity (Lucas and others 2007; Cheng and others 2013). A duplicate of *aguD* gene was also reported in AgDI clusters of *Lb. sakei* subsp. *sakei* 23K strain (Landete and others 2010; Rimaux and others 2012). No data are available, to our knowledge, on the functionality of duplicate *aguD* product. In addition, the location and orientation of the *aguR* gene within AgDI clusters vary among bacterial species, as it can be positioned upstream or downstream the cluster, and oriented in the same or opposite direction as the genes of the *AguBDAC* operon (Figure 5). Moreover, some AgDI gene clusters have been shown to carry insertion elements (IS) in 1 or 2 locations thereby interrupting the transcription process and, consequently, inactivating the whole gene cluster (Ladero and others 2011a). This is particularly the case of *L. lactis* strains reported to have IS982 and/or IS983 inserted within *aguR* and/or between *aguD* and *aguA* genes (Figure 5). Further differences between AgDI clusters in putrescine-producing species/strains reside in the structure and function of *aguR* gene product, but these differences remain insufficiently documented. For example, the AguR in *Lb. brevis, Lactobacillus sakei*, *Lb. casei*, *Lis. monocytogenes*, and *P. pentosaceus* was reported to contain a DNA-binding helix–turn–helix (HTH) motif at the N-terminal domain, and was claimed to belong to the RpiR family of TRs (Lucas and others 2007). The characterization of this AguR was essentially based on sequence similarities as determined by computational algorithms; and it is not clear whether or not the C-terminal domain of the AguR possesses the sugar phosphate-binding domain and if a response regulator is involved besides AguR, as is the case of known RpiR family of TRs. However, *Str. mutans* and *Ent. faecalis* were reported to have homologous AguR genes belonging to the LuxR family of TRs which function as a two-component system (TCS; Suarez and others 2013). Here again, neither the putative response regulator nor phosphorylation cascade that characterizes the TCS has been identified, and no alternative mechanism of action was proposed (Griswold and others 2006; Liu and others 2009). In fact, some authors suggest that the AguR of *Str. mutans* and *Ent. faecalis* would belong to the LysR rather than to the LuxR family of TRs (Lucas and others 2007). At the structural level, the AguR of *Str. mutans* was predicted to have 4 N-terminal membrane-spanning domains with portions exposed to the extracellular environment, and a cytosolic DNA-binding HTH motif at

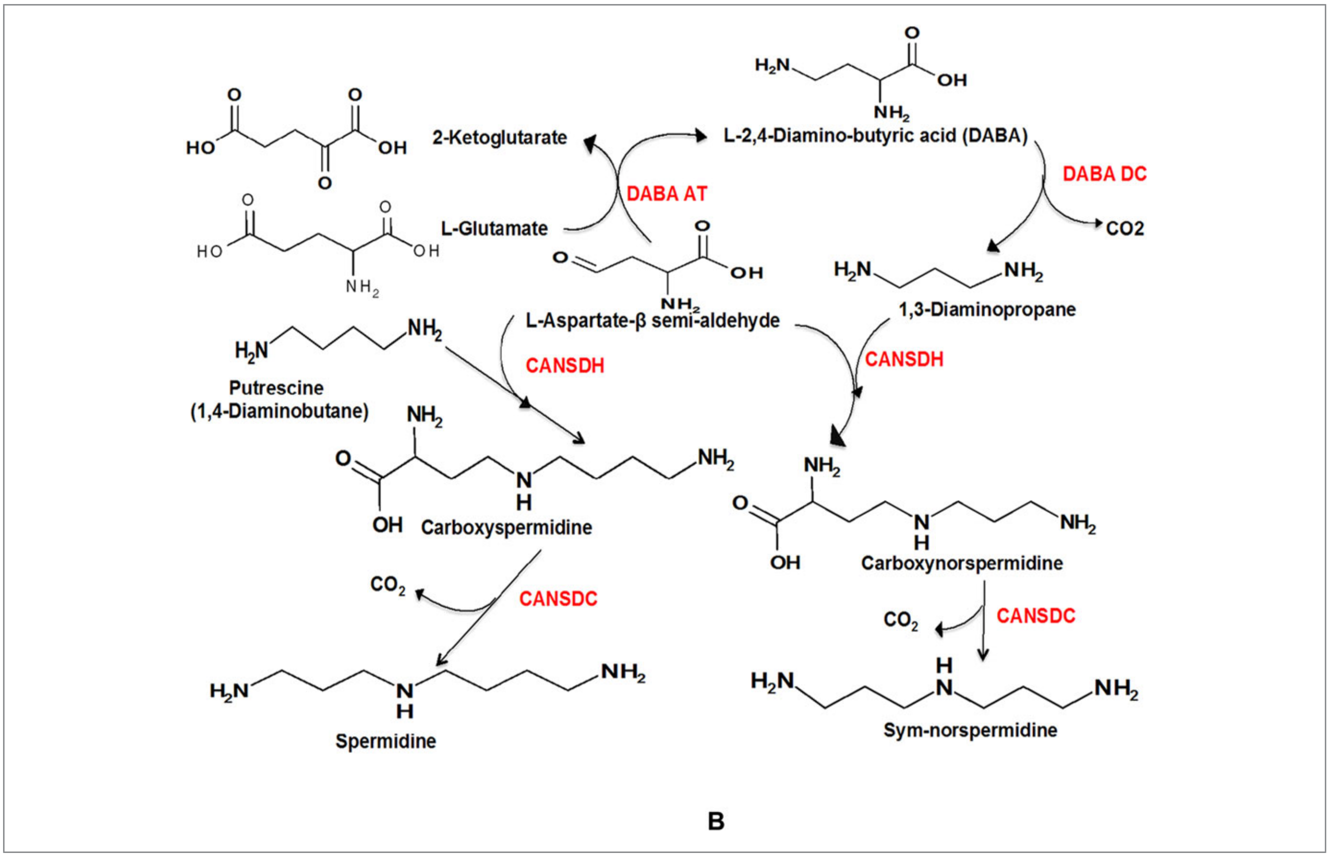


Figure 4B–Alternative pathways of the biosynthesis of spermidine and unusual analogs. The enzymes involved in the pathway are in bold red fonts. Adapted from Hobley and others (2014). Notes:
1. The pathway for the production of putrescine from arginine, ornithine, or agmatine in Figure 4A, and whether putrescine will be excreted or further used in higher polyamine biosynthesis depends on the microorganism and the "genes/enzymes" it possesses (see text).
2. Gene designations may vary according to the host organism.
Abbreviations: SAM, S-adenosylmethionine; SAMDC, S-adenosylmethionine decarboxylase; MAT, methionine adenosyl transferase; dSAM, decarboxylated S-adenosylmethionine; MTA, methylthioadenosine; CPT, N-carbamoylputrescine; SpeS, spermidine synthase; SpmS, spermine synthase; ADC, arginine decarboxylase; ADI, arginine deiminase; AgDI, agmatine deiminase; AGM, agmatinase; ARG, arginase; OTC, ornithine transcarbamoylase; PTC, putrescine transcarbamoylase; ODC, ornithine decarboxylase; DABA, L-2,4-diaminobutyrate; DABA AT, DABA aminotransferase; DABA DC, DABA decarboxylase; CANSDH, carboxynorspermidine dehydrogenase; CANSDC, carboxynorspermidine decarboxylase; APT, adenine phosphoribosyltransferase.

the C-terminal domain (Liu and others 2009). Likewise, a recent *in silico* structure analysis predicted the AguR of a *L. lactis* subsp. *cremoris* cheese isolate to be a transmembrane protein with a typical LuxR_C-like HTH DNA-binding cytoplasmic C-terminal motif (Linares and others 2015). This predicted AguR differs from that of *Str. mutans* by having 7 N-terminal domains embedded in the plasma membrane instead of 4, and an N-terminal tail pointing outside the cell membrane contrary to that of *Str. mutans* AguR which is cytoplasmic. Further characterization of the lactococcal AgDI cluster showed that *aguR* gene is expressed constitutively at low levels into AguR protein which functions as a 1-component transduction system (Linares and others 2015). These authors suggested that the lactococcal AguR would play a pivotal role in both sensing the presence of agmatine in the extracellular medium and interacting with the *aguB* promoter (P_{aguB}) to activate the transcription of the *aguBDAC* operon into polycistronic mRNA to be translated into the 4 catabolic genes of the AgDI system. In such a way, the extracellular portion of the AguR would sense the exogenous agmatine concentration and transduce the signal to the HTH DNA-binding domain. However, the exact mechanism for signal-sensing and transduction, as well as the possible roles of the substrate (agmatine) and product (putrescine) in this regulatory system which is in addition subject to carbon catabolic repression mediated by glucose (Linares and others 2013) and lactose (del Rio and others 2015a), remains poorly understood.

Biosynthesis of putrescine indirectly from arginine. Arginine is widely used as an indirect precursor of putrescine in bacteria that are able to convert it into ornithine or agmatine, each of which may then be used as an intermediate for putrescine production (Cunin and others 1986).

The conversion of arginine into ornithine is widespread among Gram-positive and Gram-negative bacteria, and is carried out via 2 different pathways: arginine deiminase (ADI) and arginase (ARG) pathways. The ADI pathway consists of a transport step allowing the uptake of arginine via antiport exchanger (arginine/agmatine), followed by hydrolysis by the ADI enzyme into citrulline and CP. The resulting citrulline is then converted into ornithine and ammonia by an ornithine-carbamoyl transferase (OCT), whereas the CP is used by a CK to produce ATP from ADP with concomitant release of ammonia and carbon dioxide (Figure 3H, I, and K). The ornithine produced, as one of the end products, is either

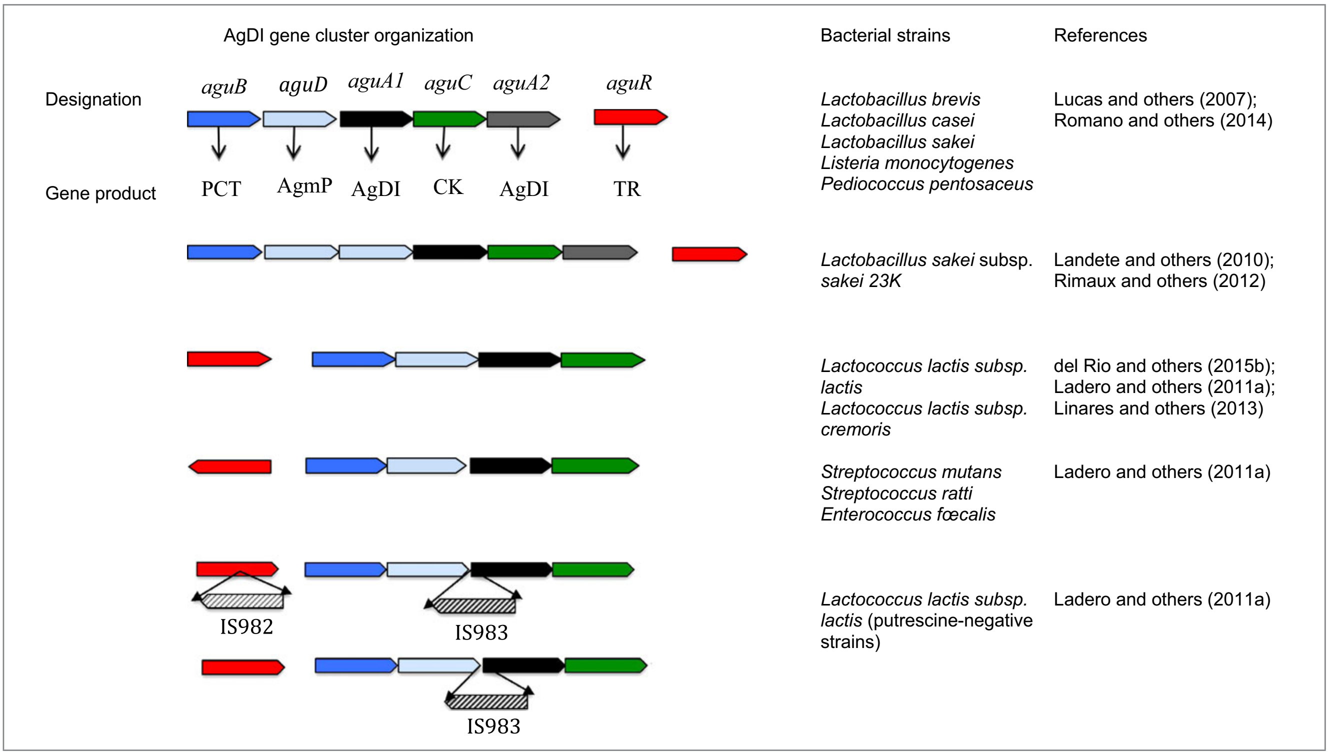

AgDI gene cluster organization
Bacterial strains
References
Designation
aguB
aguD
aguA1
aguC
aguA2
aguR
Gene product
PCT
AgmP
AgDI
CK
AgDI
TR
Lactobacillus brevis
Lactobacillus casei
Lactobacillus sakei
Listeria monocytogenes
Pediococcus pentosaceus
Lucas and others (2007);
Romano and others (2014)
Lactobacillus sakei subsp. sakei 23K
Landete and others (2010);
Rimaux and others (2012)
Lactococcus lactis subsp. lactis
Lactococcus lactis subsp. cremoris
del Rio and others (2015b);
Ladero and others (2011a);
Linares and others (2013)
Streptococcus mutans
Streptococcus ratti
Enterococcus fœcalis
Ladero and others (2011a)
IS982
IS983
Lactococcus lactis subsp. lactis (putrescine-negative strains)
Ladero and others (2011a)
IS983

Figure 5–Schematic genetic organization of AgDI gene clusters in different putrescine-producing bacteria. Abbreviations: PTC, putrescine transcarbamoylase; AgmP, agmatine/putrescine antiporter; AgDI, agmatine deiminase; CK, carbamate kinase; TR, transcriptional regulator; IS, insertion element. Note: Gene drawings are not in scale.

released in the extracellular environment in exchange for arginine via the antiporter system arginine/ornithine (Driessen and others 1988; Liu and others 1995; Barcelona-Andres and others 2002) or is used as an intermediary substrate in other metabolic routes, including the ODC pathway (3E) leading to putrescine formation discussed above (Cunin and others 1986; Nakada and others 2001; Pereira and others 2009). When excreted in exchange for arginine, ornithine accumulates in the external medium, whereas arginine is internalized to be metabolized via the ADI pathway. The ADI pathway is generally characterized by an abundant ornithine excretion, indicating that only the guanidino group of arginine is used (Cunin and others 1986). The excreted ornithine will then be taken up and decarboxylated by ODC-positive microorganisms present in the same food product. This is particularly relevant to dairy products whose proteins are devoid of ornithine (Verbeke and others 1968), and which are usually contaminated with Gram-negative bacteria that are capable of metabolizing ornithine into putrescine. LAB, *Bacillus* spp., *Pseudomonas* spp., *Aeromonas* spp., and clostridia, usually associated with dairy products, have been reported to operate via the ADI pathway, and some of them also possess the ODC pathway (Cunin and others 1986). In particular, many LAB belonging to the genera *Enterococcus* (Mackey and Beck 1968; Barcelona-Andres and others 2002), *Lactobacillus* (Arena and others 1999; Spano and others 2007; Vrancken and others 2009), *Lactococcus* (Crow and Thomas 1982; Budin-Verneuil and others 2006; Ryan and others 2009), *Leuconostoc* (Liu and others 1995), *Œnococcus* (Liu and others 1995; Divol and others 2003), and *Weissella* (Pereira and others 2009) were shown to possess the ADI pathway, but they were generally claimed to be of wine origin (Liu and others 1995; Ammor and Mayo 2007), with the exception of the lactococcal strains reported to be isolated from dairy products (Budin-Verneuil and others 2006). Although it is well admitted that dairy LAB produce putrescine exclusively from agmatine via the AgDI pathway (Linares and others 2011), heterofermentative lactobacilli and leuconostocs of wine environment were suggested to produce putrescine exclusively from arginine via the ADI pathway (Liu and others 1995). Such findings were used to explain the selective effect of the ecological niche on the type and pathway of BA biosynthesis by a given microorganism (Nannelli and others 2008). However, recent reports demonstrated the occurrence of ADI-positive LAB of the genera *Enterococcus* (Kaur and Kaur 2015) and *Weissella* (Kaur and Kaur 2012) in dairy products. Although dairy LAB are not expected to produce putrescine directly from arginine via the ADI pathway, because they lack the ODC enzyme, they can contribute to the accumulation of putrescine in dairy products by supplying the precursor ornithine to ODC-positive contaminants of enterobacteria and pseudomonads. The ADI-positive microorganisms possess the operon *arcABCTDR* encoding the following respective components: ADI (*arcA*), ornithine carbamoyltransferase (*arcB*), carbamate kinase (*arcC*), putative transaminase (*arcT*), arginine/ornithine antiporter (*arcD*), and a regulatory protein (*arcR* product; Broman and others 1978; Vrancken and others 2009; Rimaux and others 2012). In fact, both of the ADI and AgDI pathways may be present in a single bacterium as is the case for *Ent. faecalis* (Simon and Stalon 1982; Simon and others 1982) and *Lb. sakei* (Rimaux and others 2012), making such bacteria susceptible to contribute directly and indirectly to the accumulation of putrescine in dairy products.

The 2nd pathway for ornithine production from arginine is the ARG pathway that uses the ARG enzyme, a product of *rocF/aga/cargA* gene, to cleave arginine into ornithine and urea

(Figure 3D). The resulting ornithine can be an intermediary substrate in the biosynthesis of putrescine (Figure 3E) and/or other polyamines such as spermine and spermidine, as illustrated in Figure 4 (Cunin and others 1986; Lu 2006), proline, or glutamic acid (Davis 1986; Davis and others 1970; Calogero and others 1994; Maghnouj and others 1998). It should be mentioned, however, that there is no evidence for the occurrence of ARG in LAB, although it has been extensively reported in *Bacillus* spp. (Maghnouj and others 1998; Yu and others 2013), *Proteus* spp. (Prozesky and others 1973), and yeasts including *Sac. cerevisiae* and *N. crassa* (Davis 1986; Borkovich and Weiss 1987; Davis and others 1970; Green and others 1990). These ARG-positive microorganisms are commonly found in dairy products as either contaminants, advantageous, or part of the beneficial microbiota (such as *Sac. cerevisiae* in kefir). Therefore, they are the most likely to contribute directly or indirectly to putrescine production in dairy products via the ARG pathway. Nevertheless, this may not represent a significant concern for most dairy products, as the ornithine resulting from the ARG hydrolysis of arginine is mainly used in the biosynthesis of glutamine and/or proline (Cunin and others 1986; Davis 1986; Lu 2006). In *B. subtilis,* for example, arginine catabolism via the ARG pathway is governed by the expression of a *roc* regulon, comprised of 2 separate operons, *rocABC* and *rocDEF*, in addition to a *rocR* gene located upstream of the *rocDEF* operon and encoding TR RocR. The expression of these *roc* operons is sigL-dependent and under complex control of RocR protein belonging to the *NtrC*/NifA family of TRs. Accordingly, to activate both *roc* operons, RocR regulator should interact with another activator protein, AhrC, in the presence of an inducer, which is in this case ornithine or citrulline (Gardan and others 1997). Therefore, the *rocF* gene encoding the ARG enzyme is cotranscribed with the genes encoding the other components of ARG pathway leading to glutamate and proline biosynthesis as end products (Calogero and others 1994; Gardan and others 1997; Lu 2006). This dissimilates the ornithine released from arginine and, hence, limits its availability for putrescine formation (Cunin and others 1986). A similar situation was described in *Sac. cerevisiae* and *N. crassa*, which, however, can still use ornithine for putrescine biosynthesis, as they possess *spe-1/put-1* gene encoding ODC enzyme (Davis and others 1970; Davis 1986; Schwartz and others 1995).

Agmatine as an intermediate substrate in putrescine biosynthesis. Arginine can also generate putrescine indirectly via agmatine as an intermediate molecule. In this case, arginine is decarboxylated by arginine decarboxylase enzyme (ADC) into agmatine which then undergoes either the AgDI (Figure 3B, C, and J) or AGM (Figure 3A and F) pathway described above (Nakada and others 2001). Both pathways start with arginine decarboxylation carried out by biosynthetic or biodegradative ADC enzyme encoded by *speA* or *adiA* gene, respectively (Tabor and Tabor 1985; Cunin and others 1986; Forouhar and others 2010; Wunderlichová and others 2014). Therefore, the presence of one or both of these genes in a strain is a prerequisite for its ability to use arginine via the ADI or AGM pathway. Although the occurrence of ADC is widely distributed in enterobacteria, pseudomonads, and *Aeromonas* spp. (Tabor and Tabor 1985; Cunin and others 1986), there is no evidence for its existence in LAB, with the notable exception of the wine isolate *Lactobacillus hilgardii* X_1B (Arena and Manca de Nadra 2001).

Biosynthesis of spermidine, spermine, and related/unusual polyamines. The biosynthesis of higher polyamines proceeds by the addition of propylamine residues to the putrescine released from ornithine or agmatine via the ODC or ADC pathways, respectively. Although it is generally admitted that ornithine is the most common precursor of polyamines in living organisms, and is even the only 1 in *Sac. cerevisiae* (Tabor and Tabor 1985), many bacteria can produce polyamines from agmatine (Shah and Swiatlo 2008). In the process of polyamine synthesis, putrescine is 1st converted into spermidine by the addition of a propylamine residue. Subsequently, another propylamine is added to spermidine forming spermine (4A). These 2 consecutive condensation reactions are catalyzed by spermidine synthase (*sepE* product) and spermine synthase (for example, *SPE4* product, in *Sac. cerevisiae*), respectively. Simultaneously, an S-adenosylmethionine (SAM) derived from methionine is decarboxylated by the S-adenosylmethionine decarboxylase (SAMDC), a *speD* product, to yield a decarboxylated S-adenosylmethionine (dSAM), which serves as a donor of the propylamine residues for spermidine and spermine biosynthesis (Figure 4A). However, it has been generally admitted that most bacteria do not produce spermine, even if they produce its precursor spermidine due to the lack of spermine synthase gene in their genome (Tabor and Tabor 1985; Panagiotidis and others 1987; Sekowska and others 1998). Pegg and Michael (2010) argued that "contentions that bacteria do not produce spermine are an incorrect generalization," as this polyamine was shown to be widely distributed among bacteria of Clostridiales and Bacillales orders (Hosoya and others 2004), and in some cases in higher amounts than its precursor spermidine (Hamana and others 1989). However, no information was provided in the latter studies, as to whether or not the spermine was excreted after being formed within the cell. Alternative pathways leading to the formation of unusual spermidine analogs such as sym-homospermidine, sym-norspermidine, and thermospermidine have been described in many bacteria, especially those of intestinal origin, including foodborne pathogens such as *Vibrio cholera, Campylobacter jejuni*, and *Yersinia pestis* (Lee and others 2009; Hanfrey and others 2011), as well as in dairy contaminants, such *as Bacillus subtilis* (Hobley and others 2014). Polyamine synthesis via an alternative pathway (L-aspartate-β semi-aldehyde pathway) was reported to result from an adaptation phenomenon of microorganisms that possess neither the spermidine synthase gene (*speE*/S) nor the SAMDC gene (*speD*; Hanfrey and others 2011). Instead, these bacteria possess the genes/enzymes to catalyze the production of spermidine from putrescine by using the aspartate-semialdehyde in lieu of dSAM as the aminopropyl group donor. Diaminopropane can also be used as a precursor, but the end-product is sym-norspermidine (a 1-carbon shorter chain compared with spermidine) instead of spermidine (Figure 4B). In this case, sym-spermidine is formed by the condensation of diaminopropane with the aspartate semi-aldehyde catalyzed by a carboxynorspermidine dehydrogenase (CANSDH) enzyme, followed by a decarboxylation of the resulting carboxynorspermidine catalyzed by a carboxynorspermidine decarboxylase (CANSDC) enzyme (Figure 4B). However, the relevance to dairy products of the newly described pathways and the resulting polyamines remain insufficiently investigated and controversial. Few reports, however, suggest the absence of unusual polyamines in the LAB species *L. acidophilus, Lactobacillus bulgaricus, Lb. casei, Lb. rhamnosus*, *Streptococcus thermophilus*, and *Ent. faecalis* (Hamana and others 1989; Chipeva and others 1995; Hanfrey and others 2011). Yet, the wide distribution of such alternative pathways in the gut microbiota (Hanfrey and others 2011), including members of the Proteobacteria and Firmicutes phyla, which encompass many of the Gram-negative and Gram-positive dairy contaminants, does not exclude the production of spermidine or unusual polyamines in dairy products via alternative pathways.

Irrespective of the biosynthesis pathway, various dairy contaminants, including pathogens, have been shown to produce spermine and spermidine, or to harbor the corresponding synthase genes (Table 1). Conversely, LAB appear not to contribute significantly to the accumulation of these polyamines in dairy products (Hamana and others 1989), because they lack the ODC and ADC enzymes, as well as spermidine synthase (Raynaud and others 2005). Also, there is no evidence that they produce unusual polyamines via an alternative pathway. On the other hand, dairy-borne pathogens susceptible to produce spermine and spermidine do not reach high enough counts to cause significant accumulation of polyamines. For these products to be safe for consumption, pathogens should be either absent or present at low counts due to the inherent health risk associated with their presence in food, whereas polyamine production is contingent on a critical mass of the producer microorganisms (Joosten and Northolt 1987). Nevertheless, the prevalence of spermine and spermidine in dairy products is well documented (Table 2). This may be partly explained by the high level of contamination with polyamine-producing spoilage microorganisms (such as Gram-negative bacteria and bacilli) or by the natural occurrence of polyamines in milk (Novella-Rodríguez and others 2003; Spano and others 2010; Linares and others 2012). Yeasts are another potential source for these polyamines in dairy products when used as part of the technological microbiota and/or allowed to reach high counts. Similarly, molds may contribute to the accumulation of spermine and spermidine in mold-ripened cheeses as was suggested earlier for *P. roqueforti* in view of the significantly higher content of these polyamines in blue cheeses compared with unripened and semi-hard and hard cheeses (Eliassen and others 2002; Novella-Rodríguez and others 2003; Komprda and others 2008b). However, this assumption remains to be substantiated with a causal link between the increase in polyamine content in cheese and specific mold species/strains.

Contamination of dairy products with BAs

Conditions for BA production in dairy products. The presence and amount of a BA in food depends on many factors among which the availability of the precursor amino acid(s) is a limiting factor. The precursor amino acids may be naturally present in milk in a free state or be released from milk proteins by hydrolysis. Proteolytic activities leading to the formation of precursor amino acids in dairy products may result from different sources acting independently or in combination, such as: (a) proteolytic strains of microorganisms present in dairy products, (b) the milk-native heat-stable protease plasmin, (c) proteases used for coagulating milk in cheese-making, and (d) other proteases liberated from somatic cells (Tsakalidou 2011; Calzada and others 2013). Subsequently, BA-producing microorganisms will continue the formation process of BAs (Figure 1), which are then released into the matrix of dairy products. It should be noted, however, that although dairy contaminants such as *Enterobacteriaceae* and pseudomonads are known to be major BA-producing microorganisms, they are not exclusively responsible for BA accumulation in dairy products. Lactic acid bacteria of the starter cultures, as well as nonstarter LAB (NSLAB) predominating during the manufacture and/or storage of fermented milks and cheeses, are the main BA-producing bacteria in the final products (Spano and others 2010; Linares and others 2011). Dairy strains of lactobacilli, enterococci, lactococci, pediococci, streptococci, and leuconostocs have been associated with high levels of BAs in cheese and other dairy products (Table 1), and genetic studies have revealed that many of these strains harbor genes or operons coding for decarboxylating enzymes or other enzymes implicated in various pathways for BA biosynthesis or catabolism (Komprda and others 2008a; Nout 1994; Koutsoumanis and others 2010; Wunderlichová and others 2014). Yeast species that contribute to the fermentation and/or maturation of many cheese varieties and fermented milks (Benkerroum and Tamime 2004) also produce BAs (Table 1). In fact, cheese constitutes an ideal environment for the production and accumulation of these natural toxicants due to the fact that the main factors influencing the formation of BAs are usually optimal in these products which, therefore, may contain hazardous BA levels. Such factors include the availability of precursor amino acids, the presence of amino acid-decarboxylating microorganisms and cofactors, and adequate pH, temperature, and water activity. Indeed, the presence of free amino acids and microbial decarboxylase activity have repeatedly been shown to correlate positively with high levels of BAs (Nout 1994; Ruiz-Capillas and Jimenez-Colmenero 2004; Ruiz-Capillas and Moral 2004; Ozdestan and Uren 2010; Costa and others 2015); and cheese, especially the ripened varieties, contain adequate levels of free amino acids to generate significant amounts of BAs (Calzada and others 2013). The availability at sufficient amounts of pyridoxal phosphate (Edwards and Sandine 1981), a required cofactor for the activity of amino acid decarboxylase, and the pH of cheese (5.0 to 6.5) provide additional suitable conditions for BA formation in cheese. The water activity levels of cheese (0.90 to 1.00) are also optimal for the growth of BA-producing bacteria (Marcos 1993), although the a_w in cheese has been shown to decrease with an increase in fat content, which inhibits proteolytic bacteria, thereby limiting the availability of precursor amino acids, and eventually reducing BA formation (Ruiz-Capillas and Jimenez-Colmenero 2004). Numerous microorganisms, advantageous, intentionally added as starter or adjunct starter cultures, or contaminants, have been reported to produce BAs in dairy products (Table 1). Moreover, the usual temperatures of fermentation (25 to 44 °C) and maturation (10 to 20 °C) can be favorable to proteolysis and BA formation that may continue during storage under temperature-abuse conditions or even under refrigeration due to the activity of BA-producing psychrophilic and psychrotrophic bacteria (such as *Pseudomonas* spp. and *Proteus* spp.). During fermentation, the proteolytic activity is important for the provision of energy and source for carbon, essential amino acids, and nitrogen in order to ensure active growth of the starter culture and to accelerate milk acidification and gelation. During the maturation stage, microbial proteolytic and lipolytic activities are essential biological means for the development of sensory attributes and structural characteristics of cheese, and they are carried out by LAB (starter culture and NSLAB) or molds. In addition to the provision of amino acid precursors (the substrates) by proteolytic activities, intrinsic, and extrinsic environmental conditions during fermentation and maturation are not only optimal for the growth of BA-producing bacteria, but also for the enzymatic activity of their amino acid decarboxylases.

Incidence of BAs in dairy products. Optimal conditions for BA formation in dairy products are met in traditional matured cheeses where the highest concentrations of the total BAs (TBA) and specific type of BAs have been recorded (Table 2). A survey of the concentrations of BAs in the most common cheese types and other dairy products in the EU showed high values for the TBA with an overall mean of 177 to 334 mg/kg. For mean calculations, when BA was not detected or quantified, a value of 0 mg/kg was considered yielding the lower mean value;

alternatively, the limit of detection (LOD) or the limit of quantification (LOQ) was used to give the upper mean value. Acid curd cheese recorded the highest mean value (1460 mg/kg), followed by washed rind cheese (220 to 388 mg/kg), blue cheese (188 to 351 mg/kg), hard cheese (167 to 318 mg/kg), and fresh cheese (32.1 to 172 mg/kg); for the sum concentrations in cheese, there was an equal contribution from TYM and CAD followed by putrescine and HIM. BA concentrations in yogurt and miscellaneous cheeses were 3.1 to 6.3, respectively, hence they are not raising a health concern with regard to BA content (EFSA 2011). In general, fermented milks and yogurt appear to be less exposed to BA accumulation due to their short processing time and shelf-life; and they have been consistently reported to contain little or undetectable levels of BAs (Table 2).

The incidence of a specific BA in food products varies according to the type of BA formed and the food. For example, HIM, which is usually considered as the prototype of BAs, is essentially found at high levels in fish products, mainly fish of the scombroid family (tuna and mackerel). However, it is well established that TYM is the predominating BA in dairy products and the most frequently associated etiological agent with BA-mediated dairy-borne intoxications designated as "cheese reaction" (Komprda and others 2008a; Ten Brink and others 1990; Costa and others 2015). This situation is illustrated in Table 2 showing that TYM is encountered in most dairy products at generally high concentrations, yet this BA is absent or detected at too low concentrations to raise safety concerns in other cheese varieties and fermented milks. BAs such as putrescine, HIM, and CAD are also commonly detected in cheeses, and sometimes in amounts exceeding those of TYM (Novella-Rodríguez and others 2003; Martuscelli and others 2005; ; Custódio and others 2007; Bunkova and others 2013). Conversely, the same table shows that agmatine, tryptamine, PEA, and spermine are infrequently found in dairy products, and their concentrations in positive samples are too low to raise serious health concerns. This is probably due to the absence or presence at low counts of species/strains able to produce these BAs which may also be degraded or involved in other metabolic pathways as short-lived intermediary compounds. To date, few of the dairy-borne bacteria producing tryptamine or PEA have been identified (Marcobal and others 2006b), and the accumulation of agmatine, which is an intermediate for putrescine and higher polyamine formation, appears to be unlikely. Also, due to the interconvertibility of polyamine metabolism, their accumulation may be prevented through an oxidative biodegradation pathway reported to be inducible by product accumulation (Linares and others 2011). Table 2 also shows that the same cheese may exhibit highly different profiles and concentrations of BAs, depending on the sample analyzed. Such discrepancies are explained by inconsistencies in the hygienic and sanitary conditions under which each batch of cheese is produced with a consequent variability of its microbiological quality. High concentrations of HIM, putrescine, and CAD, for example, are primarily attributed to the presence of Gram-negative bacteria, essentially represented by members of *Enterobacteriaceae* and pseudomonads (Wunderlichová and others 2014). Members of these bacterial families with high potential to produce CAD, isoamylamine, HIM, and putrescine have been reported to occur frequently in mold-ripened and smear cheese varieties, and even to play a role in their maturation (Coton and others 2012). However, elevated levels of TYM are essentially associated with high counts of LAB of the starter culture and advantageous enterococci, known as the main TYM-producing bacteria (Komprda and others 2008a). Therefore, heavy contamination with HIM, putrescine, or CAD would indicate that the cheese was produced under poor sanitary conditions and/or inadequately stored. In particular, occurrence of CAD at high concentrations in dairy products strongly suggests insufficient hygiene practices during cheese processing and storage. Conversely, high levels of TYM are not necessarily related to faulty hygienic conditions during cheese processing, and hence corrective measures relying solely on the improvement of hygiene and sanitation may not reduce effectively the content of this BA in the cheese and other fermented dairy products.

Significance of BAs in food

Because of their microbial origin, BAs have been proposed for use as indicators of the hygienic quality and degree of microbial alteration of food. For this purpose, a number of the so-called BA indices (BAIs) or quality indices (QIs) have been defined using a single BA or multiple BAs. The QIs proposed aim to determine values that would rank foods according to the extent of their spoilage as "good," "acceptable," or "deteriorated" (unfit for human consumption). The definition of such indices varies according to many factors, including the food product, implicated microorganisms, and the nature and fate of BAs during processing or storage (Koutsoumanis and others 2010). This makes it difficult to set intervals that denote the extent of spoilage of food. Most BAIs use ratios of BAs whose concentrations increase to those whose concentrations decrease during processing or storage. Although this approach proved to be useful for some specific raw foods (meat and fish), it yielded imprecise results in others of the same category, and it was inapplicable for fermented foods including dairy products. Recently, Costa and others (2015) suggested the use of TYM as a QI for fermented dairy products, because this BA was predominating in milk and was the only BA whose concentration increased in 2 fermented milks during fermentation and storage. However, many dairy products may contain no or only low concentrations of TYM, and yet they are highly contaminated with other BAs such as HIM, CAD, or putrescine (Table 2). Also, the fact that TYM is mainly produced by LAB makes it a controversial indicator of the hygienic quality of cheese, as these bacteria may be part of the starter culture or natural flora of some cheese varieties, especially those having a PDO status.

From a safety standpoint, foods containing high concentrations of BAs may represent a serious threat to public health, as BAs may cause severe toxicological effects. BA-mediated intoxications have been reported in various countries to be associated with different cheese varieties, including Gouda, Swiss cheese, Cheddar, Gruyere, grated cheese, and Cheshire (Rauscher-Gabernig and others 2009; EFSA 2011). At physiological concentrations, BAs play many crucial roles in humans either in the nervous system as neurotransmitters (psychoactive), or in the vascular system as vasoactive substances, among other roles stated above. However, further intake of exogenous BAs at elevated amounts may result in toxicological effects with various degrees of severity, from a limited headache to organ failure and death (Table 3). The role of putrescine and CAD in potentiating cancer by reacting with nitrite to form carcinogenic nitrosamines in heat-treated foods is well documented (Seiler 1990; Seiler and others 1990; Shalaby 1996; Medina and others 2003; Koutsoumanis and others 2010). In fact, polyamines including putrescine and CAD (occasionally considered to be polyamines) can be converted *in vivo* by bacteria of the GIT into stable carcinogenic N-nitroso compounds (such as nitrosopyrrolidine), and they have been shown to enhance the growth of chemically induced aberrant crypt foci in

Table 3–Biogenic amines (BAs) found in dairy products, and their main toxicological properties.

Biogenic amine	Mechanism	Symptoms	Threshold toxic level[a] (mg)	Acceptable levels in cheese (mg kg^{-a})
Histamine (HIM)	Vasoactive and psychoactive: Binds to cell membrane receptors of skin, respiratory, cardiovascular, gastrointestinal, nervous, and immunology systems via histamine receptors H1, H2, and H3:		75-100	50–400
	• Vascular: Attachment on the peripheral blood vessels, predominantly arteries mediated by recpetors H1 and H2 causing dilation and increased capillary permeability	Hypotension, flushing tachycardia and headache, edema, urticaria, hemoconcentration and increased blood viscosity		
	• Heart: Attachment on the extravascular smooth mucle mediated by H1 or H2 recptors causing contraction (H1) or relaxation (H2)	Palpitations, extrasystoles, blood, pressure disorders, increased cardiac output, tachycardia		
	• Gastrointestinal: Attachment on extravascular smooth muscles mediated by H2 recpetors causing contraction and gastric secretions	Abdominal cramps, nausea, flatulence, vomiting and diarrhea		
	• Neurological: Attachment on sensory and motor neurons mediated by histamine receptors H1 and H3	Pain, itching, headache, migraine, oral burning sensation, peppery taste, nausea, swelling of tongue		
	• Respiratory: Bronchoconstrictor via histamine H1-receptor activation	Bronchospasms and respiratory distress		
	• Attachment to skin and immune cells, and to motor neurons of the brain mediated by H3 receptor causing disregulation on the neurotransmission	Oxidative stress, schizophrenia, immunopathology, skin cancer		
Tyramine (TYM)	Vasoactive: Interaction with the sympathetic noradrenergic nerve terminals innervating cardiac and vascular smooth muscle tissues: Alteration of the release and/or reuptake of catecholamine neurotransmitters, displacement norepinephrine (NE)/noradrenaline (NA) from neuronal storage vesicles, and increased activity of the central vagal pre-ganglionic neurons causing peripheral vasoconstriction and neurological disorders	Hypertensive crisis: Increased heart rate and blood pressure, migraine, nausea and sometimes vomiting, perspiration, palpitations, dilatation of the pupils, lacrimation, salivation, pulmonary edema, and eventually intracranial hemorrhage, and cardiac failure. Schizophrenia, Parkinson's disease, depression, and Reyes' syndrome[c]	600[b]	100–800
	Enhancement of adherence of the bacterial pathogens to intestinal epithelial cells (as yet unkown mechanism)	Increase in susceptibility to bacterial infections		

(*Continued*)

Table 3–Continued.

Biogenic amine	Mechanism	Symptoms	Threshold toxic level[a] (mg)	Acceptable levels in cheese (mg kg^{-a})
Phenylethylamine (PEA)	Vasoactive: Alteration of the release and/or reuptake of neurotransmitters including NA thereby causing vasoconstriction and neurological disorders (same as tyramine, except that PEA does not appear to displace NA and accumulate in neuronal storage vesicles)	Initiation of hypertensive crisis: headache, dizziness, hypertension, and discomfort Depression, schizophrenia, phenylketonuria, Reye's syndrome, Parkinson's disease, attention deficit hyperactivity disorder, Tourette's syndrome, epilepsy and migraine[c]	30	
Tryptamine (TRY)	Vasoactive: Alteration of sensitivity of adrenergic receptors: Stimulation of NA release from nerve terminals and inhibition of its uptake leading to vasoaconstriction, and neuronal and brain cell death (autophagy)	Hypertension, migraine, and fever, nausea and sometimes vomiting, perspiration, brain hemorrhage and heart failure. Schizophrenia, depression, hepatic encephalopathy[d]	NA	NA
Putrescine (PUT)	Vasoactive substance (Weak acute toxicity)	Increased cardiac output, dilation of the vascular system, hypotension, and bradycardia possibly leading to heart failure and cerebral hemorrhage, lockjaw and paresis of the extremities	>2000	180
	Indirect toxic effects: • Inhibition of DAO and histamine-N-methyl transferase (HMT) detoxifying enzymes, and disruption of the physical barrier function of the small intestine to facilitate passage of HIM into the blood circulation	Potentiation of histamine toxicity		
	• Formation of carcinogenic nitrosamines (N-nitrosopyrrolidine) in presence of nitrites in heat-treated foods or in vivo in the gastro-intestinal tract foods	Gastric or intestinal cancer diseases		
	• Promotion of swarming phenotype in pathogens such as Proteus mirabilis	Enhancement of bacterial pathogenesis		
Cadaverine (CAD)	Same as putrescine with some specificities, e.g., the nitrosamine produced in presence of nitrites is N-nitrosopiperidine	Same as for putrescine	>800[a]	540
Polyamines (PA): Agmatine (AG), spermidine (SPD), and spermine (SPM)	Critical physiological functions requiring stringent control of PA concentrations in different tissues. Dietary intake that alters physiological concentrations of PAs lead to dysregulation of vital functions including cell proliferation (uncontrolled) and homeostasis Precursors of the most potent carcinogenic N-nitrosamines Helicobacter pylori gastric infection upregulates orinithine decarboxylase (ODC) activity of host cells, facilitating tumor progression	Ischemia, muscular distrophy, epilepsy, Alzheimer'disease, psoriasis, cystic fibrosis, and cancer	NA	NA

[a] Values for healthy adults not being under medication.

[b] Lethal dose (LD_{50}) as determined in rats is 2000 mg/kg of body weight.

[c] No evidence for dietary BAs as causative agent of these diseases.

NA, not available.

Data compiled from: Nout (1994), Shalaby (1996), Koutsoumanis and others (2010), Rauscher-Gabernig and others (2012), Rodriguez and others (2014), Medina and others (2003), EFSA (2011), Herrera and others (2006), FAO/WHO (2014), Naila and others (2010), Narang and others (2011), Narang and others (2014), Lyte (2004), Shah and Swiatlo (2008).

the intestine (Paulsen and others 1997; Eliassen and others 2002). Also, direct implication of putrescine in cancer development was demonstrated by Pegg and others (1995), who showed that the reduction of ornithine activity of decarboxylase (ODC), an enzyme involved in the formation of putrescine (Figure 3), suppresses the growth of tumor cells. In addition, colorectal and *Helicobacter pylori*–induced gastric cancers have been directly related to the alteration of intracellular polyamine concentration in mammalian cells (Alam and others 1994; Wallace and Caslake 2001; Gerner and Meyskens 2004). Moreover, BAs have been associated with other debilitating diseases including immunopathologies, oxidative stress, schizophrenia, ischemia, muscular dystrophy, epilepsy, Alzheimer's disease, psoriasis, cystic fibrosis, Parkinson's disease, depression, and hepatic encephalopathy (Table 6; for a review see Medina and others 2003). The latter diseases indicate that, besides being well-established causative agents of acute health disorders, BAs would also have chronic or subchronic effects upon repeated exposures. Therefore, assessment of the risk associated with the dietary intake of BAs should take into account the chronic and subchronic effects that these toxicants may have severe health implications that have been overlooked so far, as they are not considered to be related to dietary intake of BAs.

Although it is beyond a doubt that the presence of BAs in food represents a significant risk to consumers, the dietary intake of a BA or a combination of different BAs that would trigger clinical symptoms remains undefined with certainty. This is because the susceptibility to BAs is highly variable among individuals depending not only on the amount and nature of the BA, but also on other factors inherent in consumers, including age, gender, and the efficacy of detoxifying activity mainly in the gastrointestinal tract, liver, or kidneys. In fact, exogenous BAs are normally detoxified in intestinal mucosa cells, mainly via oxidative deamination pathways using amine oxidases including monoamine oxidases (MAOs; -A and -B isoforms) and diamine oxidases (DAOs), also found in the liver and muscles (mainly the MAO-B isoform), and kidneys (Medina and others 2003). These enzymes play key roles in the detoxification pathways of monoamines and diamines including TYM, PEA, HIM, CAD, putrescine, and tryptamine. Spermidine and spermine, whose metabolic pathways are interconvertible, undergo oxidative biodegradation via pathways involving polyamine oxidases (PAOs) and spermidine/spermine-N-acetyltransferases (SSATs) to yield back putrescine which is, in turn, detoxified via specific pathways using either DAO or MAO (Medina and others 2003; Wunderlichová and others 2014). In humans, the extent of detoxification activity of BAs is a genetic trait whose performance varies widely among individuals. Yet, even the highest detoxifying activity does not ensure an absolute protection against toxicological effects of BAs. Too high an intake of one or more BAs will invariably result in acute symptoms, because ingested BAs will not be fully metabolized, and any unmetabolized BAs will rapidly gain access to the bloodstream and, thereafter, to various organs, including the central nervous system where they can induce severe health disorders (Medina and others 2003). The situation is more dramatic for human groups at risk, generally those with weakened BA oxidative activity, including children, the elderly, women during pregnancy or menstruation, people suffering from an allergy or gastrointestinal diseases (gastritis, inflammatory bowel diseases, and gastric ulcers), or those under medication with monoamine or DAO inhibitors (for example, antidepressants and anti-Parkinson's disease drugs); alcohol intake and smoking were also reported to inhibit MAOs and, consequently, amplify toxicological effects of BAs (Silla Santos 1996; McCabe-Sellers and others 2006; EFSA 2011; Wunderlichová and others 2014).

As for the toxicity of BAs taken individually, it is well established that HIM and TYM are the most active due to their relatively low threshold toxic levels in addition to the severity of symptoms they may cause (Table 3). These BAs, recognized to be of major concern, are the most frequently encountered in dairy products, especially ripened cheeses (Rauscher-Gabernig and others 2009). Therefore, toxic levels for healthy or susceptible persons have been suggested for HIM and TYM in order to help determine the safe/unsafe doses in foods and, consequently, regulatory standards have been set. On the basis of available information in the literature from documented outbreaks and case reports, the ingestion of 100 mg HIM by healthy individuals is generally considered to cause typical threshold symptoms (flushing and headache) of "HIM intolerance," whereas oral administration of >1000 mg results in a severe acute intoxication (Edwards and Sandine 1981; Rauscher-Gabernig and others 2009; Koutsoumanis and others 2010) referred to as "HIM poisoning" characterized by a critical endpoint of an allergy-like reaction (Table 3). However, a recent human challenge study showed that doses between 25 and 50 mg have no clinical effects, although 75 mg caused mild symptoms and was thus considered as the toxicological threshold level (Wohrl and others 2004). The dose of 50 mg was then considered as the No-Observable-Adverse-Effect-Level (NOAEL) and used in a deterministic model to quantify the risk of HIM intoxication associated with the consumption of dairy products in a number of European countries (EFSA 2011). This study concluded that a cheese containing a HIM concentration of 200 mg/kg or less would be safe for consumption according to "the worst case scenario" of a high exposure (95th percentile). A similar study conducted in Austria using the threshold level of 100 mg as a reference dose, concluded that a concentration of 400 mg/kg cheese or less can be ingested safely via cheese, which is reasonably achievable by the dairy industry, and a concentration of 1170 mg/kg would result in mild symptoms (Rauscher-Gabernig and others 2009). In fact, both of these levels (200 and 400 mg) appear to be realistic with regard to the maximum intake of HIM in the studied European countries (32.1 mg), which is further corroborated by documented outbreaks where HIM concentrations in implicated cheeses ranged between 850 and 1870 mg/kg (Taylor and others 1982; EFSA 2011). A cumulative intake of HIM from different food sources in a typical Austrian meal with cheese as the main dish showed that HIM intake may vary between 1.1 and 38 mg per serving (Rauscher-Gabernig and others 2009). A more conservative figure of 50 to 100 mg/kg as a tolerable level of HIM in fermented foods has been proposed on the basis of available information in the literature and toxicological studies (Nout 1994). Nevertheless, based on the hazard level (NOAEL) of 50 mg and the upper value for a serving size (m) of 270 g per day (EFSA 2011), the maximum level of HIM (L) in a serving that would not cause an adverse health effect would be 185 mg/kg as calculated according to Eq. 2 used for a deterministic risk characterization:

$$L = \frac{\text{NOAEL}}{m} \tag{2}$$

$$L = \frac{50\,\text{mg}}{270\,\text{mg}} = 185\,\text{mg/kg}$$

As the values for a serving size (m) vary, depending on the country, region, or even locality, the maximum tolerable level of 185 mg/kg may not be applicable to all countries, although cheese consumption in the EU is among the highest worldwide. Therefore, this level can be regarded as conservative and would apply to other countries outside the EU. It is worth mentioning, however, that these levels are not valid for children and groups at risk. For HIM-intolerant persons who display clinical symptoms, even when exposed to small amounts of HIM, only food with HIM levels below the detectable limits can be considered safe (EFSA 2011).

Similarly, different threshold toxic doses of TYM have been defined by monitoring the increase in systolic blood pressure (SBP) as the yardstick for the onset of toxicological effects upon ingestion of this BA. Accordingly, doses of TYM from 600 to 2000 mg were reported to be necessary to cause a significant increase in the SBP in healthy persons (Korn and others 1988a, 1988b; Zimmer and others 1990; Patat and others 1995). Furthermore, a dose-response curve showed that 1100 mg of TYM corresponded to the effective dose (ED_{50}) at which 50% of healthy individuals not taking MAO inhibitors (MAOIs) experienced an SBP increase of at least 30 mmHg as evidence for the causal effect (Patat and others 1995). Such toxic doses are significantly reduced (>100 times) when ingested in combination with either the classical or new-generation MAOIs. Indeed, in patients under treatment with classical MAOIs the ingestion of 6 to 10 mg TYM in 1 or 2 servings causes mild symptoms, whereas 10 to 25 mg provokes a severe reaction (McCabe 1986). This was explained by the irreversible and nonselective inhibition of both MAO-A and MAO-B isoforms by the classical MAOIs. However, medication with the new-generation MAOIs, which inhibits either MAO-A or MAO-B in a selective and reversible manner, thus termed RIMA for "reversible inhibitor monoamines," appear to have less impact on the BA-detoxifying activity of patients who can tolerate up to 150 mg of TYM when concomitantly taking RIMA at low dosage (Zimmer 1990; Patat and others 1995; McCabe-Sellers and others 2006). These findings were corroborated by the recent quantitative risk assessment conducted in the EU showing that the intake of 600 mg TYM per meal had no adverse effects in healthy individuals not taking MAOIs, whereas this dose decreased to 6 or 50 mg in those taking classical or new-generation MAOIs, respectively (EFSA 2011). The same study concluded that TYM intake of 600 mg per meal would not be exceeded even by a combined high intake (95th percentile) of 5 food sources of TYM in the same meal. On the contrary, the doses of 6 and 50 mg, especially the former (6 mg) can be easily exceeded by the consumption of fermented foods. This would put consumers under MAOIs medication at high risk, regardless of the nature of MAOIs the patients are taking. In fact, a concentration of TYM varying between 100 and 800 mg/kg has been considered to be acceptable for fermented foods on the basis of case reports and outbreak data (Nout 1994). However, the adequacy of such sources of information as a basis for the definition of tolerable levels remains controversial, because only doses greatly exceeding the threshold levels are recorded in case reports and outbreaks (EFSA 2011), and hence they would lead to an underestimation of the risk.

In contrast to HIM and TYM, putrescine, CAD, PEA, spermidine, and spermine are the least toxic (Koutsoumanis and others 2010), and they have thus attracted little attention as foodborne toxins. Yet, their impact on food safety should not be overlooked, and they should be given due attention for 2 main reasons: (i) it is well established that these BAs can potentiate toxicity of other BAs, including HIM and TYM, as is the case for putrescine and CAD which enhance the toxicity of HIM by inhibiting diaminooxidase (DAO) and HIM-N-methyltransferase (HMT), both involved in the oxidative biodegradation pathway of HIM (Stratton and others 1991; Al Bulushi and others 2009) potentiating HIM toxicity by putrescine or CAD and, presumably, TYM, which may also be explained by disruption of the physical barrier function of the small intestine, thereby facilitating the transit of HIM into the blood (Paik Jung and Bjeldanes 1979); and (ii) their implication in many debilitating chronic diseases, including cancer and neurodegenerative diseases (Table 3), is of paramount concern, especially with regard to the chronic effects due to repeated low-level intake with fermented foods that are part of culinary habits; this is an issue that has not yet been duly investigated. The presently available information is insufficient to identify concentrations of CAD, putrescine, spermine, spermidine, and the polyamines which will directly cause acute adverse health effects and/or potentiate the toxic effects of other BAs; and, therefore, tolerable levels of these BAs in food have so far not been established. However, despite the lack of information, a recent study conducted by Rauscher-Gabernig and others (2012) attempted to determine tolerable levels of putrescine and CAD in cheese on the basis of toxicological threshold levels, occurrence of these diamines in food, and consumption patterns in Austria. According to this study, maximum daily intakes of putrescine and CAD via cheese in Austria were estimated to be 19.2 and 23.1 mg per person, respectively. Accordingly, the authors proposed the respective maximum tolerable levels of 180 and 540 mg/kg for putrescine and CAD in cheese. Considering the reported concentrations of these BAs in various dairy products (Table 2), the proposed levels are exceeded in a number of cheeses and fermented milks. Therefore, further studies in different countries or regions of the world are needed to obtain a clearer insight in this regard.

Apart from HIM and TYM, there is a vacuum in terms of tolerable levels of BAs in cheese that can be used to establish regulatory provisions. This is essentially due to the lack of information on toxicity (toxicological threshold and intake causing severe intoxications), as well as concentrations and nature of specific potentiating BAs. In particular, the lack of precise definition of the threshold levels of all dietary BAs, individually or in combination, is recognized to be the limiting factor to produce meaningful and credible quantification of health risks associated with the dietary intake of BAs (EFSA 2011; FAO/WHO 2014). The common occurrence of more than 1 type of BAs in the same food and the limited knowledge of BA interactions is another significant limitation to the accuracy and feasibility of related risk assessments. In fact, this issue has been considered and the use of TBA instead/along of/with specific types of BAs has been proposed as a an alternative to define safe/unsafe levels with 750, 900, or 1000 mg/kg as maximum tolerable TBA levels (Ten Brink and others 1990; Spanjer and van Roode 1991; Silla Santos 1996).

As for regulatory aspects regarding the occurrence of BAs in foods, there are no established standards, with the exception of HIM for which the maximum acceptable levels are set in some countries for selected food commodities. For example, the maximum legal limits are only set in some countries for HIM in fish species with a high content of histidine. For example, a maximum limit of 50, 100, or 200 mg/kg is acceptable in the United States (Food and Drug Administration 2011), EU (Commission Regulation 2005), and South Africa (government notice N° R490), and Australia (Standard 2.2.3), respectively. Credible assessment of the risk associated with BAs in food would certainly help food safety authorities define maximum tolerable levels on a sound scientific basis in order to ensure effective consumer protection without imposing unnecessary restrictions to the food industry. Consequently, more countries are expected to regulate BAs in foods and feeds as this issue is being widely recognized as a major concern to public health, which may reshape food trade at national and international levels in the future.

Control of BA accumulation in dairy products

In view of the known or potential health risks associated with the presence of BAs at high levels in dairy products, sustained efforts have been made to reduce such levels to a minimum. To this aim, different strategies have been proposed, all of which emphasize, 1st and foremost, the need to improve the sanitary conditions during production and storage. In addition to good hygiene practices other measures should be implemented for optimal results. These include the inhibition of BA-producing bacteria, reduction of the number of BA producers via pasteurization of the cheese milk, reducing the amount of proteolytic activity to limit the availability of precursor amino acids, by reducing ripening times, addition of mono- and di-amine oxidases, use of appropriate starter or adjunct starter cultures, and so on. In some cases, however, the characteristics of fermented foods render these strategies inapplicable, difficult to follow, or too onerous to implement. Traditionally, measures including chilling or freezing have been used to limit microbial growth during storage, and hence BA formation. However, this effort may be of limited value, as significant amounts of BAs may already exist in the raw material or be formed during processing of fermented foods (Gonzaga and others 2009; Chen and others 2010). Therefore, alternative secondary control measures to prevent BA formation in foods or to reduce BA levels once formed have been suggested. Such approaches include hydrostatic pressure, irradiation, controlled atmosphere packaging, and the use of BA-degrading adjunct starter cultures or food additives (for a review, see Naila and others 2010). In fact, no matter how effective such techniques may be, the application of an appropriate quality assurance program, and the use of selected BA-negative or BA-oxidizing strains of the starter cultures, remain crucial to limit BA levels in fermented dairy products. These aspects are discussed below.

Proper hygiene practices. Improvement of the sanitary conditions throughout the entire production chain is necessary to attain a significant reduction of the BA content in dairy products. This can only be achieved by the implementation of quality assurance programs based on a holistic approach from farm to fork. In addition to the beneficial microbiota (such as LAB and some yeasts), raw milk is invariably contaminated with a wide variety of spoilage microorganisms comprising mesophilic (enterococci and coliforms), psychrotropic/psychrophilic (*Pseudomonas*, *Acinetobacter*, *Enterobacteriacea*), and thermoduric species of enterococci, *Corynebacterium*, *Microbacterium*, *Micrococcus*, and *Alcaligenes*, as well as spores of *Clostridium*, and, sometimes, various pathogens, including *Bacillus*, streptococci, *Staphylococcus, Campylobacter*, *Mycobacterium*, *Salmonella*, and *Listeria* (Chambers 2002; Hill and others 2012; Gleeson and others 2013; Murphy 2015). Members of all of these microbial groups can produce different types of BAs, from different substrates, and via different pathways (Figure 4A). Depending on the hygienic conditions during milking, the initial microbial load of milk varies between 10^3 and 10^5 cfu/mL (Chambers 2002), but this may increase under certain conditions to exceed 10^7 cfu/mL before transformation (Ravanis and Lewis 1995; Benkerroum and Tamime 2004). The higher this total count (TC) in raw milk, the more diverse and numerous are the BA-producing contaminants it contains. In addition, the use of milk with too high TC leads to fermented dairy products of poor hygienic quality, even if the milk is pasteurized before fermentation (Gleeson and others 2013). In dairy products, and regardless of the temperatures used during processing and storage, there will always be a group of microorganisms with the potential to grow and produce BAs (Linares and others 2012). Therefore, the production of raw milk with the lowest possible TC should be considered as a so-called "performance objective" in a holistic strategy aiming at the control of BAs via the implementation of a food safety objective (FSO) approach (van Schothorst and others 2009). Gram-negative bacteria, mainly *Enterobacteriaceae*, usually present at high counts in raw milk drawn under poor hygienic conditions, are able to survive the cheese-making process and produce BAs. In Montasio cheese, for example, this microbial group was shown to survive for up to 120 d of ripening and to produce HIM, putrescine, and CAD (Maifreni and others 2013). Also, a positive correlation was found between CAD concentration and the counts of *Enterobacteriaceae* in blue-veined cheese (Marino and others 2000). The effect of the initial TC in milk and occurrence of BAs in the cheese is also evidenced by the fact that cheese made from raw milk usually contains more BAs than that obtained from pasteurized milk (Novella-Rodriguez and others 2004; Fernandez and others 2007). Conversely, raw milk with a low TC (<5000 cfu/mL) was shown to sporadically contain HIM- and TYM-producing strains in numbers lower than 100 cfu/mL (Bachmann and others 2011). Nonetheless, even with such low numbers, the milk yielded cheese with HIM and TYM contents ranging between 1.0 and 2.0 g/kg after a 12-mo period of ripening. This indicates that good hygienic practices during milk harvest, although necessary, are not sufficient to ensure safe levels of BAs in end products, especially in cheese types with long ripening periods. Additional measures such as those discussed below are to be considered to add a safety factor.

Pasteurization of milk. It is well established that milk pasteurization generally improves the safety of dairy products derived thereof, and cheeses obtained from pasteurized milk were consistently shown to contain lower BA concentrations than those obtained from raw milk (Stratton and others 1991; Schneller and others 1997; Novella-Rodriguez and others 2004). This was usually explained by the substantial reduction in the TC and specific spoilage bacteria including *Enterobacteriaceae* considered as one of the most implicated microbial groups for BA production in dairy products (Maifreni and others 2013). In this regard, Novella-Rodriguez and others (2004) showed that pasteurization of goat milk reduced the TC by 1.46 log units and the enterobacteria counts to below the detectable limit in a 1-mL sample. Consequently, this study showed that the cheese made from pasteurized milk contained significantly lower concentrations of TYM, HIM, β-PEA, tryptamine, CAD, and putrescine than that obtained from its unpasteurized counterpart. For some authors, the low levels of BAs in cheese made from pasteurized milk is more related to the reduction in the cofactors needed for the decarboxylation reactions that generate BAs from precursor amino acids than any reduction in the numbers of BA-producing microorganisms (Joosten and Northolt 1987). Contrary to pathogens, which are eliminated by pasteurization, the counts of other milk-borne bacterial groups are only reduced to a certain degree by the same treatment. Survivors, including BA-producing microorganisms, may grow in dairy products during processing and/or storage and adversely affect their organoleptic properties, shelf-life, or safety. In particular, pasteurization does not eliminate thermoduric bacteria, such as enterococci and some lactobacilli, which have long been known to contribute significantly to the build-up of BAs in dairy products (Ladero and others 2011b). Therefore, the lower levels of BAs recorded in dairy products obtained from pasteurized milk compared with those obtained from raw milk may be due to the concurrent effect of reduced initial microbial load and depletion of decarboxylation reaction cofactors.

Irradiation. Among other physical treatments used in food preservation, irradiation appears to be a promising means to both reduce the counts of BA-producing microorganisms and inactivate preformed BAs in dairy products. Despite the believed reluctance of consumers to accept irradiated foods due to uncertainties regarding their safety (Roberts 2014), irradiation is gaining popularity as a technique that can efficiently control pathogenic and spoilage bacteria, viruses, molds, parasites, and insects, as well as toxic chemicals, such as nitrite, nitrosamines, and BAs in foods, thereby enhancing their safety and keeping quality (Wei and others 2009; Rabie and others 2010; Rabie and Toliba 2013). Ionizing radiations x and γ are currently legally approved for food preservation in about 50 countries around the world. This number is increasing steadily, because more scientific evidence is being built-up demonstrating that the benefits of food irradiation outweigh its potential risks. Different doses of γ radiation (1 to 30 kGy) are permitted for food preservation, depending on the nature of the food, the target microorganisms to kill, and the objective of the treatment (shelf-life extension, partial or total elimination of microbial contaminants, and ripening and sprouting of vegetable foods). Although no legal status exists regarding the irradiation of fermented foods, including dairy products, many studies have demonstrated the effectiveness of irradiation in reducing the BA content in such foods. Exposure of various cheese types to 1 to 6 kGy of γ irradiation reduces the microbial counts and BAs in a dose-dependent manner (Aly and others 2012; Shalaby and others 2016). However, concerns have been raised with regard to possible adverse effects on the nutritional quality of foods after irradiation. The main of such concerns were related to the free radical formation and lipid oxidation leading to alterations of the chemical composition of fat- and protein-rich food products when exposed to doses higher than 6 kGy (Chong and others 2011). Such doses have even been reported to increase the level of some BAs (PEA, spermidine, CAD, and tryptamine) in meat products (Wei and others 2009), which could also be the case for dairy products. Nonetheless, doses below 6 kGy were shown to affect neither the chemical composition nor the gustatory quality of dairy products while reducing BA contents and microbial counts to different extents (Aly and others 2012). In fact, the treatment of Ras cheese (an Egyptian hard-ripened cheese) with different doses of γ radiations (5, 10, and 15 kGy) reduced its BA content and microbial counts, and improved its gustatory quality after 6 mo of storage at 5 °C (Shalaby and others 2016). Therefore, the irradiation of dairy products may be especially useful in cheese where it can be applied after ripening to reduce BA content and, at the same time, prevent over-ripening of some cheese varieties where it concomitantly reduces the microbial counts.

Actions on starter cultures. Apart from microbial contaminants that produce BAs, advantageous or intentionally added LAB of the starter or adjunct starter cultures also contribute to BA accumulation in dairy products (Linares and others 2012). In contrast, some strains of LAB were shown to reduce the content of BAs in various foods including dairy products (Naila and others 2010). Therefore, selection of strains to be used as starter or adjunct starter cultures on the basis of decarboxylase-negative (not producing BAs) and/or BA-oxidizing (degrading BAs) activities has been suggested as a means to reduce the BA content in foods (Linares and others 2011).

LAB of the genera *Lactobacillus* and *Enterococcus* with decarboxylating activity are the most implicated in BA accumulation in dairy products (Ladero and others 2010). Species of both of these genera are consistently present in raw milk where they can survive pasteurization and develop as secondary microbiota during fermentation and/or ripening (Novella-Rodriguez and others 2002). In addition, lactobacilli are part of many commercial starter cultures used in the dairy industry, and they have been reported to contribute to BA accumulation in dairy products (Stratton and others 1991; Burdychova and Komprda 2007; La Gioia and others 2011). Therefore, beside the provision of cheese milk with good microbiological quality, the use of starter cultures composed of BA-negative strains helps reduce the amount of BAs in fermented dairy products more efficiently than each of these measures separately. This strategy was reported to be more efficient when a mixed starter or adjunct starter culture was used, as the mixed-strain cultures act synergistically in the control of BAs and result in a large pH decrease that may be an additional factor contributing to reducing BA accumulation (Hu and others 2007). Different mixtures of pediococcal, lactobacilli, and staphylococcal strains were shown to suppress BA production in different fermented food products of different origins (Fernandez-Garcia and others 2000; Bover-Cid and others 2001; Špička and others 2002; Hu and others 2007; Nieto-Arribas and others 2009; Lu and others 2015). This strategy can be efficient and practical, provided appropriate combinations of dairy strains are used.

Despite the good hygienic quality of milk and the use of selected starter culture, BAs may still be formed, and sometimes at relatively high levels, in dairy products, especially in cheeses relying on the natural microbiota for fermentation and/or ripening (Forzale and others 2011; Schirone and others 2012). In addition, BAs may be formed from sources other than starter cultures or bacterial contaminants, such as yeasts and molds used as a secondary microbiota, or may be naturally present in milk, such as spermine, spermidine, putrescine, and PEA (Gloria and others 2011). Consequently, means to remove pre-formed BAs from dairy products should be envisaged as an improvement rather than as a preventive measure to ensure safe levels of these toxic compounds. An emerging strategy, in this regard, appears to be the use of BA-degrading microbial strains as adjunct starter cultures. Detoxifying oxidation of BAs has been demonstrated *in vitro* in many bacteria of potential use in dairy products such as *Micrococcus varians* (Leuschner and Hammes 1998a), *Brevibacterium linens* (Leuschner and Hammes 1998b), *Lb. sakei* and *Lb. curvatus* (Dapkevicius and others 2000), *Staphylococcus xylosus* (Mah and Hwang 2009), and *Lb. casei* and *Pediococcus* spp. (Garcia-Ruiz and others 2011). For example, strains of *Lb. casei* isolated from Zamorano, Cabrales, and Emmentaler cheeses have been shown to degrade TYM and HIM *in vitro* and effectively reduce their contents in experimental models (Herrero-Fresno and others 2012). Dairy isolates of this species were reported to reduce HIM in a laboratory medium by 50% of its initial concentration (Naila and others 2012). Similarly, *Lactobacillus plantarum* reduced the content of putrescine and TYM in wine (Capozzi and others 2012), and TYM, CAD, and putrescine in Nhem, a Thai fermented meat (Valyasevi and Rolle 2002). Surface inoculation of Munster cheese with *Brevibacterium linens* reduced its content in TYM and HIM by 55% to 70% during a 4-wk ripening period (Leuschner and Hammes 1998b). Lu and others (2015) demonstrated a synergistic action between BA-degrading LAB strains of the species *Lb. sakei* and *S. xylosus,* and plant extracts to suppress the formation of tryptamine, putrescine, CAD, HIM, and TYM in traditional Chinese smoked horsemeat sausage during ripening and storage. These data provide a strong indication of the potential for the application of BA-negative and BA-oxidizing bacteria to help prevent the accumulation, or even reduce the levels, of pre-formed BAs in dairy products. However, for such bacteria to be effective, they must be able to grow optimally on the dairy matrix and dominate BA-producing and other contaminating bacteria (Xu and others 2010). They should, therefore, be selected on the basis of their compatibility to grow together in the dairy product where they are intended to be used, and for their ability to degrade BAs *in situ* before being validated for such utilization.

Conclusions

Milk and dairy products continue to raise concerns with regard to their contamination with microbial toxins of various origins, and their potential to cause foodborne disease outbreaks, which can result in heavy economic loss and a public health burden. Great efforts have been made worldwide to reduce the incidence of foodborne diseases; however, the effectiveness of such efforts will remain hampered by the huge gap in food safety practices and policies between developing and industrialized countries, since it is being made more evident than ever that food safety is a global issue.

The presence of BAs in dairy products is a rather common cause for foodborne intoxications, although underreported and largely overlooked. Effective control of the incidence of these toxins in dairy products will certainly contribute to alleviate the global foodborne disease impact. To achieve such a goal, a new food safety approach should be adopted in the face of the changing world and the increased demand by consumers for minimally processed and safe food products. Such challenge requires comprehensive scientific knowledge of these toxins, the routes of contamination, conditions for their production and/or inactivation, toxicological effects and the possible interactions between each other to enhance or reduce such toxicological effects, and so on. In fact, it is necessary to perform a comprehensive survey of all BAs contaminating dairy products and to make a quantitative estimate, if possible, of the risk associated with the combination BA/dairy commodity in order to efficiently target control measures. Developing robust and effective epidemiological and surveillance programs is another prerequisite to reduce the incidence of BAs in dairy products and to subsequently assess the efficacy of the measures applied. Few studies have been done to assess the risk of BAs in dairy products, and they remain insufficient to adequately estimate their impact on the safety of dairy products worldwide to ultimately suggest means for their management and control.

Conventional means (heat treatment, use of chemical additives, acidification, and fermentation) to reduce the overall contamination of dairy products with BAs have been found to be of limited value, as significant amounts of BAs may be formed during processing of fermented foods using processes that may encourage the growth of BA-producing bacteria. Therefore, secondary control measures to prevent toxin formation in dairy products, or to reduce their levels once formed, have been suggested as alternatives. However, such approaches may face difficulties related to practicability and cost-effectiveness. In fact, no matter how effective such techniques may be, the use of selected microorganisms (LAB, yeasts, molds) as starter cultures (LAB) or dairy fermentations and ripening, and the application of good manufacturing practices and appropriate quality assurance programs during processing, storage, and even retailing remain unavoidable to enhance the safety of fermented dairy products.

References

Al Bulushi I, Poole S, Deeth HC, Dykes GA. 2009. Biogenic amines in fish: roles in intoxication, spoilage, and nitrosamine formation—a review. Crit Rev Food Sci Nutr 49:369–77.

Alam K, Arlow FL, Ma CK, Schubert TT. 1994. Decrease in ornithine decarboxylase activity after eradication of *Helicobacter pylori*. Am J Gastr 89:888–93.

Aly SA, Farag DE, Galal E. 2012. Effect of gamma irradiation on the quality and safety of Egyptian Karish cheese. J Am Sci 8:761–6.

Ammor MS, Mayo B. 2007. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: an update. Meat Sci 76:138–46.

Andic SH, Genccelep H, Kose S. 2010. Determination of biogenic amines in herby cheese. Int J Food Prop 13:1300–14.

Applebaum D, Sabo DL, Fischer EH, Morris DR. 1975. Biodegradative ornithine decarboxylase of *Escherichia coli*. Purification, properties and pyridoxal 5′-phosphate binding site. Biochemistry 14:3675–81.

Arena ME, Manca de Nadra MC. 2001. Biogenic amine production by *Lactobacillus*. J Appl Microbiol 90:158–62.

Arena ME, Saguir FM, Manca de Nadra MC. 1999. Arginine, citrulline, and ornithine metabolism by lactic acid bacteria from wine. Int J Food Microbiol 52:155–61.

Azcarate-Peril MA, Altermann E, Hoover-Fitzula RL, Cano RJ, Klaenhammer TR. 2004. Identification and inactivation of genetic loci involved with *Lactobacillus acidophilus* acid tolerance. Appl Environ Microbiol 70:5315–22.

Bachmann HP, Fröhlich-Wyder MT, Jakob ERE, Wechsler D. 2011. Raw milk cheeses. In: Fuquay J, Fox P, Roginski H, editors. Encyclopedia of dairy science. London: Elsevier. p 652–60.

Barcelona-Andres B, Marina A, Rubio V. 2002. Gene structure, organization, expression, and potential regulatory mechanisms of arginine catabolism in *Enterococcus faecalis*. J Bacteriol 184:6289–300.

Benkerroum N. 2008. Antimicrobial activity of lysozyme with special relevance to milk. Af J Biotechnol 7:4856–67.

Benkerroum N. 2010. Antimicrobial peptide generated from milk: a survey and prospects for application in the food industry: a review. Int J Dairy Technol 63:320–38.

Benkerroum N, Tamime AY. 2004. Technology transfer of some Moroccan traditional dairy products (lben, jben and smen) to small industrial scale: a review. Food Microbiol 21:399–413.

Borkovich KA, Weiss RL. 1987. Purification and characterization of arginase from *Neurospora crassa*. J Biol Chem 262:7081–6.

Bover-Cid S, Holzapfel WH. 1999. Improved screening procedure for biogenic amine production by lactic acid bacteria. Int J Food Microbiol 53:33–41.

Bover-Cid S, Izquierdo-Pulido M, Vidal-Carou MC. 2001. Effectiveness of a *Lactobacillus sakei* starter culture in the reduction of biogenic amine accumulation as a function of the raw material quality. J Food Prot 64:367–73.

Bowman WH, Tabor CW, Tabor H. 1973. Spermidine biosynthesis: purification and properties of propylamine transferase from *Escherichia coli*. J Biol Chem 248:2480–6.

Brito C, Cid N, Muñoz O, Báez A, Horzella M. 2014. Biogenic amine content in Chilean Gouda cheese: physico-chemical and microbiological factors that may influence this content. Int J Dairy Technol 67:554–61.

Broman K, Lauwers N, Stalon V, Wiame JM. 1978. Oxygen and nitrate in utilization by *Bacillus licheniformis* of the arginase and arginine deiminase routes of arginine catabolism and other factors affecting their syntheses. J Bacteriol 135:920–7.

Budin-Verneuil A, Maguin E, Auffray Y, Ehrlich DS, Pichereau V. 2006. Genetic structure and transcriptional analysis of the arginine deiminase (ADI) cluster in *Lactococcus lactis* MG1363. Can J Microbiol 52:617–22.

Bunkova L, Adamcova G, Hudcova K, Velichova H, Pachlova V, Lorencova E, Bunka F. 2013. Monitoring of biogenic amines in cheeses manufactured at small-scale farms and in fermented dairy products in the Czech Republic. Food Chem 141:548–51.

Buňková L, Buňka F, Hlobilová M, Vaňátková Z, Nováková D, Dráb V. 2009. Tyramine production of technological important strains of *Lactobacillus*, *Lactococcus* and *Streptococcus*. Eur Food Res Technol 229:533–8.

Burdychova R, Komprda T. 2007. Biogenic amine-forming microbial communities in cheese. FEMS Microbiol Lett 276:149–55.

Calogero S, Gardan R, Glaser P, Schweizer J, Rapoport G, Debarbouille M. 1994. RocR, a novel regulatory protein controlling arginine utilization in *Bacillus subtilis*, belongs to the NtrC/NifA family of transcriptional activators. J Bacteriol 176:1234–41.

Calzada J, del Olmo A, Picon A, Gaya P, Nuñez M. 2013. Proteolysis and biogenic amine buildup in high-pressure treated ovine milk blue-veined cheese. J Dairy Sci 96:4816–29.

Capozzi V, Russo P, Ladero V, Fernandez M, Fiocco D, Alvarez MA, Spano G. 2012. Biogenic amines degradation by *Lactobacillus plantarum*: Toward a potential application in wine. Front Microbiol 3:122. doi: 10.3389/fmicb.2012.00122. Available from:http://www.ncbi.nlm.nih.gov/pubmed/22485114. Accessed 2016 April 8.

Chambers JV. 2002. The microbiology of raw milk. In: Robinson RK, editor. Dairy microbiology handbook: the microbiology of milk and milk products. 3rd ed. Chichester: John Wiley and Sons Ltd. p 39–90.

Chen H-C, Huang Y-R, Hsu H-H, Lin C-S, Chen W-C, Lin C-M, Tsai Y-H. 2010. Determination of histamine and biogenic amines in fish cubes (*Tetrapturus angustirostris*) implicated in a food-borne poisoning. Food Control 21:13–8.

Cheng C, Chen J, Fang C, Xia Y, Shan Y, Liu Y, Fang W. 2013. *Listeria monocytogenes aguA1*, but not *aguA2*, encodes a functional agmatine deiminase: biochemical characterization of its catalytic properties and roles in acid tolerance. J Biol Chem 288:26606–15.

Chipeva V, Mehandjiyska L, Dumanova E. 1995. The polyamine cell composition as a chemotaxonomic marker in lactic acid bacteria identification. J Cult Collect 1:28–33.

Chong CY, Abu Bakar F, Russly AR, Jamilah B, Mahyudin NA. 2011. The effects of food processing on biogenic amines formation. Int Food Res J 18:867–76.

Claeys WL, Cardoen S, Daube G, De Block J, Dewettinck K, Dierick K, Herman L. 2013. Raw or heated cow milk consumption: review of risks and benefits. Food Control 31:251–62.

Commission Regulation. 2005. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJEU Lett L 338:1–26.

Costa MP, Balthazar CF, Rodrigues BL, Lazaro CA, Silva AC, Cruz AG, Conte Junior CA. 2015. Determination of biogenic amines by high-performance liquid chromatography (HPLC-DAD) in probiotic cow's and goat's fermented milks and acceptance. Food Sci Nutr 3:172–8.

Coton E, Mulder N, Coton M, Pochet S, Trip H, Lolkema JS. 2010a. Origin of the putrescine-producing ability of the coagulase-negative bacterium *Staphylococcus epidermidis* 2015B. Appl Environ Microbiol 76:5570–6.

Coton M, Delbes-Paus C, Irlinger F, Desmasures N, Le Fleche A, Stahl V, Coton E. 2012. Diversity and assessment of potential risk factors of Gram-negative isolates associated with French cheeses. Food Microbiol 29:88–98.

Coton M, Romano A, Spano G, Ziegler K, Vetrana C, Desmarais C, Coton E. 2010b. Occurrence of biogenic amine-forming lactic acid bacteria in wine and cider. Food Microbiol 27:1078–85.

Crow VL, Thomas TD. 1982. Arginine metabolism in lactic streptococci. J Bacteriol 150:1024–32.

Cunin R, Glansdorff N, Pierard A, Stalon V. 1986. Biosynthesis and metabolism of arginine in bacteria. Microbiol Rev 50:314–52.

Custódio FB, Tavares É, Glória MBA. 2007. Extraction of bioactive amines from grated Parmesan cheese using acid, alkaline and organic solvents. J Food Comp Anal 20:280–8.

Dapkevicius MLNE, Nout MJR, Rombouts FM, Houben JH, Wymenga W. 2000. Biogenic amine formation and degradation by potential fish silage starter microorganisms. Int J Food Microbiol 57:107–14.

Davis RH. 1986. Compartmental and regulatory mechanisms in the arginine pathways of *Neurospora crassa* and *Saccharomyces cerevisiae*. Microbiol Rev 50:280–313.

Davis RH, Lawless MB, Port LA. 1970. Arginaseless Neurospora: genetics, physiology, and polyamine synthesis. J Bacteriol 102:299–305.

de las Rivas B, Marcobal A, Munoz R. 2007. Gene organization of the ornithine decarboxylase-encoding region in *Morganella morganii*. J Appl Microbiol 102:1551–60.

de Las Rivas B, Ruiz-Capillas C, Carrascosa AV, Curiel JA, Jimenez-Colmenero F, Munoz R. 2008. Biogenic amine production by Gram-positive bacteria isolated from Spanish dry-cured "chorizo" sausage treated with high pressure and kept in chilled storage. Meat Sci 80:272–7.

del Rio B, Ladero V, Redruello B, Linares DM, Fernandez M, Martin MC, Alvarez MA. 2015a. Lactose-mediated carbon catabolite repression of putrescine production in dairy *Lactococcus lactis* is strain dependent. Food Microbiol 48:163–70.

del Rio B, Linares DM, Ladero V, Redruello B, Fernandez M, Martin MC, Alvarez MA. 2015b. Putrescine production via the agmatine deiminase pathway increases the growth of *Lactococcus lactis* and causes the alkalinization of the culture medium. Appl Microbiol Biotechnol 99:897–905.

Divol B, Tonon T, Morichon S, Gindreau E, Lonvaud-Funel A. 2003. Molecular characterization of *Oenococcus oeni* genes encoding proteins involved in arginine transport. J Appl Microbiol 94:738–46.

Driessen AJ, Smid EJ, Konings WN. 1988. Transport of diamines by *Enterococcus faecalis* is mediated by an agmatine-putrescine antiporter. J Bacteriol 170:4522–7.

Durlu-Ozkaya F. 2002. Biogenic amine content of some Turkish cheeses. J Food Process Preserv 26:259–65.

Edwards ST, Sandine WE. 1981. Public health significance of amines in cheese. J Dairy Sci 64:2431–8.

EFSA. 2011. Scientific opinion on risk based control of biogenic amine formation in fermented foods. EFSA J 9/2393:1–93. Available from: http://www.efsa.europa.eu/en/search/doc/2393.pdf. Accessed 2015 April 25.

Eliassen KA, Reistad R, Risøen U, Rønning HF. 2002. Dietary polyamines. Food Chem 78:273–80.

FAO/WHO. 2014. Joint FAO/WHO Expert Meeting on the public health risks of histamine and other biogenic amines from fish and fishery products. Rome, Italy.

Fernandez M, Linares DM, del Rio B, Ladero V, Alvarez MA. 2007. HPLC quantification of biogenic amines in cheeses: correlation with PCR-detection of tyramine-producing microorganisms. J Dairy Res 74:276–82.

Fernández M, Zúñiga M. 2006. Amino acid catabolic pathways of lactic acid bacteria. Cr Rev Microbiol 32:155–83.

Fernandez-Garcia E, Tomillo J, Nunez M. 2000. Formation of biogenic amines in raw milk Hispanico cheese manufactured with proteinases and different levels of starter culture. J Food Prot 63:1551–5.

Food and Drug Administration. 2011. Fish and fishery products hazards and controls guidance. 4th ed. Washington, DC: FDA, Center for Food Safety and Applied Nutrition.

Forouhar F, Lew S, Seetharaman J, Xiao R, Acton TB, Montelione GT, Tong L. 2010. Structures of bacterial biosynthetic arginine decarboxylases. Acta Crystallogr Sect F Struct Biol Cryst Commun 66:1562–6.

Forzale F, Giorgi M, Pedonese F, Nuvoloni R, D'Ascenzi C, Rindi S. 2011. Contenuto di amine biogene nel "Pecorino del parco di Migliarino–San Rossore." Associazione Italiana Veterinari Igienisti 1:149–53.

Galgano F, Caruso M, Condelli N, Favati F. 2012. Focused review: Agmatine in fermented foods. Front Microbiol 3:199. doi: 10.3389/fmicb.2012.00199. Available from:http://journal.frontiersin.org/article/10.3389/fmicb.2012.00199/pdf. Accessed 2014 April 7.

Garcia-Ruiz A, Gonzalez-Rompinelli EM, Bartolome B, Moreno-Arribas MV. 2011. Potential of wine-associated lactic acid bacteria to degrade biogenic amines. Int J Food Microbiol 148:115–20.

Gardan R, Rapoport G, Debarbouille M. 1997. Role of the transcriptional activator RocR in the arginine-degradation pathway of *Bacillus subtilis*. Mol Microbiol 24:825–37.

Gerner EW, Meyskens FL, Jr. 2004. Polyamines and cancer: old molecules, new understanding. Nat Rev Cancer 4:781–92.

Gerosa S, Skoet J. 2013. Milk availability: current production and demand and medium-term outlook. In: Muehlhoff E, Bennett A, McMahon D, editors. Milk and dairy products in human nutrition. Rome, Italy: Food and Agricultural Organisation of the United nations (FAO). p 11–40.

Gleeson D, O'Connell A, Jordan K. 2013. Review of potential sources and control of thermoduric bacteria in bulk-tank milk. Irish J Agr Food Res 52:217–27.

Gloria MBA, Saraiva PR, Rigueira JCS, Brandão SCC. 2011. Bioactive amines changes in raw and sterilised milk inoculated with *Pseudomonas fluorescens* stored at different temperatures. Int J Dairy Technol 64:45–51.

Gonzaga VE, Lescano AG, Huaman AA, Salmon-Mulanovich G, Blazes DL. 2009. Histamine levels in fish from markets in Lima, Peru. J Food Prot 72:1112–5.

Green SM, Eisenstein E, McPhie P, Hensley P. 1990. The purification and characterization of arginase from *Saccharomyces cerevisiae*. J Biol Chem 265:1601–7.

Griswold AR, Chen YY, Burne RA. 2004. Analysis of an agmatine deiminase gene cluster in *Streptococcus mutans* UA159. J Bacteriol 186:1902–4.

Griswold AR, Jameson-Lee M, Burne RA. 2006. Regulation and physiologic significance of the agmatine deiminase system of *Streptococcus mutans* UA159. J Bacteriol 188:834–41.

Hamana K, Akiba T, Uchino F, Matsuzaki S. 1989. Distribution of spermine in bacilli and lactic acid bacteria. Can J Microbiol 35:450–5.

Hanfrey CC, Pearson BM, Hazeldine S, Lee J, Gaskin DJ, Woster PM, Michael AJ. 2011. Alternative spermidine biosynthetic route is critical for growth of *Campylobacter jejuni* and is the dominant polyamine pathway in human gut microbiota. J Biol Chem 286:43301–12.

Herrera F, Martin V, Carrera P, Garcia-Santos G, Rodriguez-Blanco J, Rodriguez C, Antolin I. 2006. Tryptamine induces cell death with ultrastructural features of autophagy in neurons and glia: possible relevance for neurodegenerative disorders. Anat Rec A Discov Mol Cell Evol Biol 288:1026–30.

Herrero-Fresno A, Martinez N, Sanchez-Llana E, Diaz M, Fernandez M, Martin MC, Alvarez MA. 2012. *Lactobacillus casei* strains isolated from cheese reduce biogenic amine accumulation in an experimental model. Int J Food Microbiol 157:297–304.

Hill B, Smythe B, Lindsay D, Shepherd J. 2012. Microbiology of raw milk in New Zealand. Int J Food Microbiol 157:305–8.

Hobley L, Kim SH, Maezato Y, Wyllie S, Fairlamb AH, Stanley-Wall NR, Michael AJ. 2014. Norspermidine is not a self-produced trigger for biofilm disassembly. Cell 156:844–54.

Hosoya R, Hamana K, Niitsu M, Itoh T. 2004. Polyamine analysis for chemotaxonomy of thermophilic eubacteria: polyamine distribution profiles within the orders Aquificales, Thermotogales, Thermodesulfobacteriales, Thermales, Thermoanaerobacteriales, Clostridiales, and Bacillales. J Gen Appl Microbiol 50:271–87.

Hu Y, Xia W, Liu X. 2007. Changes in biogenic amines in fermented silver carp sausages inoculated with mixed starter cultures. Food Chem 104:188–95.

Ichise YK, Kosuge T, Uwate M, Nakae T, Maseda H. 2015. Complete genome sequence of *Pseudomonas aeruginosa* strain 8380, isolated from the human gut. Genome Announc 3:e00520–15. doi: 10.1128/genomeA.00520-15. Available from:http://www.ncbi.nlm.nih.gov/pubmed/25999558. Accessed 2016 April 7.

Igarashi K, Kashiwagi K. 2010. Modulation of cellular function by polyamines. Int J Biochem Cell Biol 42:39–51.

Ivanova N, Sorokin A, Anderson I, Galleron N, Candelon B, Kapatral V, Kyrpides N. 2003. Genome sequence of *Bacillus cereus* and comparative analysis with *Bacillus anthracis*. Nature 423:87–91.

Joosten HM, Northolt MD. 1987. Conditions allowing the formation of biogenic amines in cheese 2. Decarboxylative properties of some non-starter bacteria. Neth Milk Dairy J 41:259–80.

Kaur B, Kaur R. 2012. Statistical screening of media components for the production of arginine deiminase by *Weissella confusa* GR7. Int J Food Ferm Technol 2:71–9.

Kaur B, Kaur R. 2015. Isolation, identification and genetic organization of the ADI operon in *Enterococcus faecium* GR7. Ann Microbiol 65:1427–37.

Komprda T, Burdychova R, Dohnal V, Cwikova O, Sladkova P, Dvorackova H. 2008a. Tyramine production in Dutch-type semi-hard cheese from 2 different producers. Food Microbiol 25:219–27.

Komprda T, Dohnal V, Závodníková R. 2008b. Contents of some biologically active amines in a Czech blue-vein cheese. Czech J Food Sc 26:428–40.

Korn A, Da Prada M, Raffesberg W, Allen S, Gasic S. 1988a. Tyramine pressor effect in man: studies with moclobemide, a novel, reversible monoamine oxidase inhibitor. J Neural Transm Suppl 26:57–71.

Korn A, Da Prada M, Raffesberg W, Gasic S, Eichler HG. 1988b. Effect of moclobemide, a new reversible monoamine oxidase inhibitor, on absorption, and pressor effect of tyramine. J Cardiovasc Pharmacol 11:17–23.

Koutsoumanis A, Tassou C, Nychas GJE. 2010. Biogenic amines in foods. In: Juneja VK, Sofos JN, editors. Pathogens and toxins in foods: challenges and interventions. Washington: ASM Press. p 248–74.

Kusano T, Berberich T, Tateda C, Takahashi Y. 2008. Polyamines: essential factors for growth and survival. Planta 228:367–81.

La Gioia F, Rizzotti L, Rossi F, Gardini F, Tabanelli G, Torriani S. 2011. Identification of a tyrosine decarboxylase gene (*tdcA*) in *Streptococcus thermophilus* 1TT45 and analysis of its expression and tyramine production in milk. Appl Environ Microbiol 77:1140–4.

Ladero V, Calles-Enriquez M, Fernandez M, Alvarez MA. 2010. Toxicological effects of dietary biogenic amines. Cur Nutr Food Sci 6:145–56.

Ladero V, Cañedo E, Pérez M, Martín MC, Fernández M, Alvarez MA. 2012a. Multiplex qPCR for the detection and quantification of putrescine-producing lactic acid bacteria in dairy products. Food Control 27:307–13.

Ladero V, Fernandez M, Calles-Enriquez M, Sanchez-Llana E, Canedo E, Martin MC, Alvarez MA. 2012b. Is the production of the biogenic amines tyramine and putrescine a species-level trait in enterococci? Food Microbiol 30:132–8.

Ladero V, Rattray FP, Mayo B, Martin MC, Fernandez M, Alvarez MA. 2011a. Sequencing and transcriptional analysis of the biosynthesis gene cluster of putrescine-producing *Lactococcus lactis*. Appl Environ Microbiol 77:6409–18.

Ladero V, Sánchez-Llana E, Fernández M, Alvarez MA. 2011b. Survival of biogenic amine-producing dairy LAB strains at pasteurisation conditions. Int J Food Sci Technol 46:516–21.

Landete JM, Arena ME, Pardo I, Manca de Nadra MC, Ferrer S. 2010. The role of two families of bacterial enzymes in putrescine synthesis from agmatine via agmatine deiminase. Int Microbiol 13:169–77.

Lee J, Sperandio V, Frantz DE, Longgood J, Camilli A, Phillips MA, Michael AJ. 2009. An alternative polyamine biosynthetic pathway is widespread in bacteria and essential for biofilm formation in Vibrio cholerae. J Biol Chem 284:9899–907.

Leuschner RG, Hammes WP. 1998a. Tyramine degradation by micrococci during ripening of fermented sausage. Meat Sci 49:289–96.

Leuschner RGK, Hammes WP. 1998b. Degradation of histamine and tyramine by *Brevibacterium linens* during surface ripening of Munster cheese. J Food Prot 61:874–8.

Linares DM, del Rio B, Ladero V, Martinez N, Fernandez M, Martin MC, Alvarez MA. 2012. Factors influencing biogenic amines accumulation in dairy products. Front Microbiol 3:180.

Linares DM, del Rio B, Ladero V, Redruello B, Martin MC, Fernandez M, Alvarez MA. 2013. The putrescine biosynthesis pathway in *Lactococcus lactis* is transcriptionally regulated by carbon catabolic repression, mediated by CcpA. Int J Food Microbiol 165:43–50.

Linares DM, del Rio B, Redruello B, Ladero V, Martin MC, de Jong A, Alvarez MA. 2015. AguR, a transmembrane transcription activator of the putrescine biosynthesis operon in *Lactococcus lactis*, acts in response to the agmatine concentration. Appl Environ Microbiol 81:6145–57.

Linares DM, Martin MC, Ladero V, Alvarez MA, Fernandez M. 2011. Biogenic amines in dairy products. Crit Rev Food Sci Nutr 51:691–703.

Liu S, Pritchard GG, Hardman MJ, Pilone GJ. 1995. Occurrence of arginine deiminase pathway enzymes in arginine catabolism by wine lactic acid bacteria. Appl Environ Microbiol 61:310–6.

Liu Y, Zeng L, Burne RA. 2009. AguR is required for induction of the *Streptococcus mutans* agmatine deiminase system by low pH and agmatine. Appl Environ Microbiol 75:2629–37.

Lorencová E, Buňková L, Matoulková D, Dráb V, Pleva P, Kubáň V, Buňka F. 2012. Production of biogenic amines by lactic acid bacteria and bifidobacteria isolated from dairy products and beer. Int J Food Sci Technol 47:2086–91.

Lu CD. 2006. Pathways and regulation of bacterial arginine metabolism and perspectives for obtaining arginine overproducing strains. Appl Microbiol Biotechnol 70:261–72.

Lu S, Ji H, Wang Q, Li B, Li K, Xu C, Jiang C. 2015. The effects of starter cultures and plant extracts on the biogenic amine accumulation in traditional Chinese smoked horsemeat sausages. Food Control 50:869–75.

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 153:2221–30.

Lyte M. 2004. The biogenic amine tyramine modulates the adherence of *Escherichia coli* O157:H7 to intestinal mucosa. J Food Prot 67:878–83.

Mackey JK, Jr., Beck RW. 1968. Activities of arginine dihydrolase and phosphatase in *Streptococcus faecalis* and *Streptococcus faecium*. Appl Microbiol 16:1543–7.

Maghnouj A, de Sousa Cabral TF, Stalon V, Vander Wauven C. 1998. The arcABDC gene cluster, encoding the arginine deiminase pathway of *Bacillus licheniformis*, and its activation by the arginine repressor argR. J Bacteriol 180:6468–75.

Mah J-H, Hwang H-J. 2009. Inhibition of biogenic amine formation in a salted and fermented anchovy by *Staphylococcus xylosus* as a protective culture. Food Control 20:796–801.

Maifreni M, Frigo F, Bartolomeoli I, Innocente N, Biasutti M, Marino M. 2013. Identification of the *Enterobacteriaceae* in Montasio cheese and assessment of their amino acid decarboxylase activity. J Dairy Res 80:122–7.

Marcobal A, De las Rivas B, Landete JM, Tabera L, Munoz R. 2012. Tyramine and phenylethylamine biosynthesis by food bacteria. Crit Rev Food Sci Nutr 52:448–67.

Marcobal A, de las Rivas B, Moreno-Arribas MV, Munoz R. 2006a. Evidence for horizontal gene transfer as origin of putrescine production in *Oenococcus oeni* RM83. Appl Environ Microbiol 72:7954–8.

Marcobal A, de las Rivas B, Munoz R. 2006b. First genetic characterization of a bacterial beta-phenylethylamine biosynthetic enzyme in *Enterococcus faecium* RM58. FEMS Microbiol Lett 258:144–9.

Marcos A. 1993. Water activity in cheese in relation to composition, stability and safety. In: Fox PF, editor. Cheese: chemistry, physics and microbiology. London: Chapman & Hall, p 439–69.

Marino M, Maifreni M, Moret S, Rondinini G. 2000. The capacity of *Enterobacteriaceae* species to produce biogenic amines in cheese. Letters in Applied Microbiology 31:169–73.

Martuscelli M, Gardini F, Torriani S, Mastrocola D, Serio A, Chaves-Lopez C, Suzzi G. 2005. Production of biogenic amines during the ripening of Pecorino Abruzzese cheese. Int Dairy J 15:571–8.

Mascaro N, Stocchi R, Ricciutelli M, Cammertoni N, Renzi F, Cecchini S, Rea S, Bacac-iapfoi-ifd F, 49–53. 2010. Biogenic amine content and chemical and physical features of italian formaggio di Fossa. Associazione Italiana Veterinari Igienisti 8:49–53.

McCabe BJ. 1986. Dietary tyramine and other pressor amines in MAOI regimens: a review. J Am Diet Assoc 86:1059–64.

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 Comp Anal 19:S58–65.

Medina MA, Urdiales JL, Rodriguez-Caso C, Ramirez FJ, Sanchez-Jimenez F. 2003. Biogenic amines and polyamines: similar biochemistry for different physiological missions and biomedical applications. Crit Rev Biochem Mol Biol 38:23–59.

Min JS, Lee SO, Jang A, Lee M, Kim Y. 2004. Quantitative analysis of biogenic amines in raw and processed foods of animal origin on Korean domestic market. Asian-Aust J Anim Sci 17:1764–8.

Moinard C, Cynober L, de Bandt JP. 2005. Polyamines: metabolism and implications in human diseases. Clin Nutr 24:184–97.

Molenaar D, Bosscher JS, ten Brink B, Driessen AJ, Konings WN. 1993. Generation of a proton motive force by histidine decarboxylation and electrogenic histidine/histamine antiport in *Lactobacillus buchneri*. J Bacteriol 175:2864–70.

Moreno-Arribas V, Lonvaud-Funel A. 2001. Purification and characterization of tyrosine decarboxylase of *Lactobacillus brevis* IOEB 9809 isolated from wine. FEMS Microbiol Lett 195:103–7.

Murphy P. 2015. Control of thermoduric bacteria in raw milk supplies. Glanbia. Available from: https://agrilink.ie/MilkNews2007/4241 Thermoduric Bacteria.pdf. Accessed 2015 Nov 21.

Naila A, Flint S, Fletcher G, Bremer P, Meerdink G. 2010. Control of biogenic amines in food—existing and emerging approaches. J Food Sci 75:R139–R50.

Naila A, Flint S, Fletcher GC, Bremer PJ, Meerdink G. 2012. Histamine degradation by diamine oxidase, *Lactobacillus* and *Vergibacillus halodonitrificans* Nai18. J Food Process Technol 3:1–4.

Nakada Y, Jiang Y, Nishijyo T, Itoh Y, Lu CD. 2001. Molecular characterization and regulation of the aguBA operon, responsible for agmatine utilization in *Pseudomonas aeruginosa* PAO1. J Bacteriol 183:6517–24.

Nannelli F, Claisse O, Gindreau E, de Revel G, Lonvaud-Funel A, Lucas PM. 2008. Determination of lactic acid bacteria producing biogenic amines in wine by quantitative PCR methods. Lett Appl Microbiol 47: 594–9.

Narang D, Kerr PM, Lunn SE, Beaudry R, Sigurdson J, Lalies MD, Plane F. 2014. Modulation of resistance artery tone by the trace amine beta-phenylethylamine: dual indirect sympathomimetic and alpha1-adrenoceptor blocking actions. J Pharmacol Exp Ther 351:164–71.

Narang D, Tomlinson S, Holt A, Mousseau DD, Baker GB. 2011. Trace amines and their relevance to psychiatry and neurology: a brief overview. Bull Clin Psychopharmacol 21:73–9.

Nieto-Arribas P, Poveda JM, Seseña S, Palop L, Cabezas L. 2009. Technological characterization of *Lactobacillus* isolates from traditional Manchego cheese for potential use as adjunct starter cultures. Food Control 20:1092–8.

Nout MJR. 1994. Fermented foods and food safety. Food Res Int 27:291–8.

Novella-Rodríguez S, Veciana-Nogués MT, Izquierdo-Pulido M, Vidal-Carou MC. 2003. Distribution of biogenic amines and polyamines in cheese. J Food Sci 68:750–6.

Novella-Rodriguez S, Veciana-Nogues MT, Roig-Sagues AX, Trujillo-Mesa AJ, Vidal-Carou MC. 2002. Influence of starter and nonstarter on the formation of biogenic amine in goat cheese during ripening. J Dairy Sci 85:2471–8.

Novella-Rodriguez S, Veciana-Nogues MT, Roig-Sagues AX, Trujillo-Mesa AJ, Vidal-Carou MC. 2004. Evaluation of biogenic amines and microbial counts throughout the ripening of goat cheeses from pasteurized and raw milk. J Dairy Res 71:245–52.

Novella-Rodríguez S, Veciana-Nogués MT, Trujillo-Mesa AJ, Vidal-Carou MC. 2002. Profile of biogenic amines in goat cheese made from pasteurized and pressurized milks. J Food Sci 67:2940–4.

O'Brien NM, O'Connor T, O'Callaghan J, Dobson ADW. 2004. Toxins in cheese. In: Fox PF, McSweeney PLH, Cogan TM, Timothy P, Guinee TP, editors. Cheese: chemistry, physics and microbiology. 3rd ed. London: Elsevier. p 561–81.

Ohji S, Yamazoe A, Hosoyama A, Tsuchikane K, Ezaki T, Fujita N. 2014. The complete genome sequence of *Pseudomonas putida* NBRC 14164T confirms high intraspecies variation. Genome Announc 2:e00029–14. doi: 10.1128/genomeA.00029-14. Available from:http://www.ncbi.nlm.nih.gov/pubmed/24526630. Accessed 2016 April 7.

Ozdestan O, Uren A. 2010. Biogenic amine content of kefir: a fermented dairy product. Eur Food Res Technol 231:101–7.

Paik Jung HY, Bjeldanes LF. 1979. Effects of cadaverine on histamine transport and metabolism in isolated gut sections of the guinea-pig. Food Cosmet Toxicol 17:629–32.

Panagiotidis CA, Blackburn S, Low KB, Canellakis ES. 1987. Biosynthesis of polyamines in ornithine decarboxylase, arginine decarboxylase, and agmatine ureohydrolase deletion mutants of *Escherichia coli* strain K-12. Proc Natl Acad Sci USA 84:4423–7.

Patat A, Berlin I, Durrieu G, Armand P, Fitoussi S, Molinier P, Caille P. 1995. Pressor effect of oral tyramine during treatment with befloxatone, a new reversible monoamine oxidase—a inhibitor, in healthy subjects. J Clin Pharmacol 35:633–43.

Pattono D, Grassi MA, Civera TI. 2008. Production of biogenic amines by some enterobacteriaceae strains isolated from dairy products. Ital J Food Sci 20:411–7.

Paulsen JE, Reistad R, Eliassen KA, Sjaastad OV, Alexander J. 1997. Dietary polyamines promote the growth of azoxymethane-induced aberrant crypt foci in rat colon. Carcinogenesis 18:1871–5.

Pegg AE, Michael AJ. 2010. Spermine synthase. Cell Mol Life Sci 67: 113–21.

Pegg AE, Shantz LM, Coleman CS. 1995. Ornithine decarboxylase as a target for chemoprevention. J Cell Biochem Suppl 22:132–8.

Pereira CI, San Romao MV, Lolkema JS, Crespo MT. 2009. *Weissella halotolerans* W22 combines arginine deiminase and ornithine decarboxylation pathways and converts arginine to putrescine. J Appl Microbiol 107:1894–902.

Prozesky OW, Grabow WO, van der Merwe S, Coetzee JN. 1973. Arginine gene clusters in the *Proteus*-Providence group. J Gen Microbiol 77:237–40.

Rabie MA, Siliha H, el-Saidy S, el-Badawy A-A, Malcata FX. 2010. Effects of γ-irradiation upon biogenic amine formation in Egyptian ripened sausages during storage. Innov Food Sci Emerg Technol 11:661–5.

Rabie MA, Toliba AO. 2013. Effect of irradiation and storage on biogenic amine contents in ripened Egyptian smoked cooked sausage. J Food Sci Technol 50:1165–71.

Roberts PB. 2014. Food irradiation is safe: half a century of studies. Radiat Phys Chem 105:78–82.

Rauscher-Gabernig E, Gabernig R, Brueller W, Grossgut R, Bauer F, Paulsen P. 2012. Dietary exposure assessment of putrescine and cadaverine and derivation of tolerable levels in selected foods consumed in Austria. Eur Food Res Technol 235:209–20.

Rauscher-Gabernig E, Grossgut R, Bauer F, Paulsen P. 2009. Assessment of alimentary histamine exposure of consumers in Austria and development of tolerable levels in typical foods. Food Control 20:423–9.

Ravanis S, Lewis MJ. 1995. Observations on the effect of raw milk quality on the keeping quality of pasteurized milk. Lett Appl Microbiol 20:164–7.

Raynaud S, Perrin R, Cocaign-Bousquet M, Loubiere P. 2005. Metabolic and transcriptomic adaptation of *Lactococcus lactis* subsp. *lactis* Biovar *diacetylactis* in response to autoacidification and temperature downshift in skim milk. Appl Environ Microbiol 71:8016–23.

Rimaux T, Riviere A, Illeghems K, Weckx S, De Vuyst L, Leroy F. 2012. Expression of the arginine deiminase pathway genes in *Lactobacillus sakei* is strain dependent and is affected by the environmental pH. Appl Environ Microbiol 78:4874–83.

Rodriguez MBR, Carneiro C, Feijó MB, Júnior CAC, Mano SB. 2014. Bioactive amines: aspects of quality and safety in food. Food Nutr Sci 5:138–46.

Romano A, Ladero V, Alvarez MA, Lucas PM. 2014. Putrescine production via the ornithine decarboxylation pathway improves the acid stress survival of *Lactobacillus brevis* and is part of a horizontally transferred acid resistance locus. Int J Food Microbiol 175:14–9.

Romano A, Trip H, Lolkema JS, Lucas PM. 2013. Three-component lysine/ornithine decarboxylation system in *Lactobacillus saerimneri* 30a. J Bacteriol 195:1249–54.

Romano A, Trip H, Lonvaud-Funel A, Lolkema JS, Lucas PM. 2012. Evidence of 2 functionally distinct ornithine decarboxylation systems in lactic acid bacteria. Appl Environ Microbiol 78:1953–61.

Ruiz-Capillas C, Jimenez-Colmenero F. 2004. Biogenic amines in meat and meat products. Crit Rev Food Sci Nutr 44:489–99.

Ruiz-Capillas C, Moral A. 2004. Free amino acids and biogenic amines in red and white muscle of tuna stored in controlled atmospheres. Amino Acids 26:125–32.

Ryan S, Begley M, Gahan CG, Hill C. 2009. Molecular characterization of the arginine deiminase system in *Listeria monocytogenes*: regulation and role in acid tolerance. Environ Microbiol 11:432–45.

Schirone M, Tofalo R, Visciano P, Corsetti A, Suzzi G. 2012. Biogenic amines in Italian Pecorino cheese. Frontiers in Microbiology 3:171. doi: 10.3389/fmicb.2012.00171. Available from:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3347038/pdf/fmicb-03-00171.pdf. Accessed 2016 April 8.

Schneller R, Good P, Jenny M. 1997. Influence of pasteurised milk, raw milk, and different ripening cultures on biogenic amine concentrations in semi-soft cheeses during ripening. Z Lebensm Unters Forsch 204: 265–72.

Schwartz B, Hittelman A, Daneshvar L, Basu HS, Marton LJ, Feuerstein BG. 1995. A new model for disruption of the ornithine decarboxylase gene, SPE1, in *Saccharomyces cerevisiae* exhibits growth arrest and genetic instability at the MAT locus. Biochem J 312(Pt 1):83–90.

Seiler N. 1990. Polyamine metabolism. Digestion 46 Suppl 2:319–30.

Seiler N, Sarhan S, Grauffel C, Jones R, Knodgen B, Moulinoux JP. 1990. Endogenous and exogenous polyamines in support of tumor growth. Cancer Res 50:5077–83.

Sekowska A, Bertin P, Danchin A. 1998. Characterization of polyamine synthesis pathway in *Bacillus subtilis* 168. Mol Microbiol 29:851–8.

Shah P, Swiatlo E. 2008. A multifaceted role for polyamines in bacterial pathogens. Mol Microbiol 68:4–16.

Shalaby AR. 1996. Significance of biogenic amines to food safety and human health. Food Res Intl 29:675–90.

Shalaby AR. 2000. Changes in biogenic amines in mature and germinating legume seeds and their behavior during cooking. Nahrung 44:23–7.

Shalaby AR, Anwar MM, Sallam EM, Emam WH. 2016. Quality and safety of irradiated food regarding biogenic amines: ras cheese. Int J Food Sci Technol 51:1048–54.

Silla Santos MH. 1996. Biogenic amines: their importance in foods. Int J Food Microbiol 29:213–31.

Simon JP, Stalon V. 1982. Enzymes of agmatine degradation and the control of their synthesis in *Streptococcus faecalis*. J Bacteriol 152:676–81.

Simon JP, Wargnies B, Stalon V. 1982. Control of enzyme synthesis in the arginine deiminase pathway of *Streptococcus faecalis*. J Bacteriol 150:1085–90.

Smith TA. 1971. The occurrence, metabolism and functions of amines in plants. Biol Rev 46:201–41.

Spanjer MC, van Roode BASW. 1991. Towards a regulatory limit for biogenic amines in fish, cheese and sauerkraut. De Ware(n)-Chemicus 21:139–67.

Spano G, Massa S, Arena M, de Nadra M. 2007. Arginine metabolism in wine *Lactobacillus plantarum*: in vitro activities of the enzymes arginine deiminase (ADI) and ornithine transcarbamilase (OTCase). Ann Microbiol 57:67–70.

Spano G, Russo P, Lonvaud-Funel A, Lucas P, Alexandre H, Grandvalet C, Lolkema JS. 2010. Biogenic amines in fermented foods. Eur J Clin Nutr 64 Suppl 3:S95–100.

Špička J, Kalač P, Bover-Cid S, Křížek M. 2002. Application of lactic acid bacteria starter cultures for decreasing the biogenic amine levels in sauerkraut. Eur Food Res Technol 215:509–14.

Stratton JE, Hutkins RW, Taylor SL. 1991. Biogenic amines in cheese and other fermented foods: a review. J Food Prot 54:460–70.

Suarez C, Espariz M, Blancato VS, Magni C. 2013. Expression of the agmatine deiminase pathway in *Enterococcus faecalis* is activated by the AguR regulator and repressed by CcpA and PTS(Man) systems. PLoS One 8:e76170.

Tabor CW, Tabor H. 1985. Polyamines in microorganisms. Microbiol Rev 49:81–99.

Tabor H, Tabor CW. 1972. Biosynthesis and metabolism of 1,4-diaminobutane, spermidine, spermine, and related amines. Adv Enzymol Relat Areas Mol Biol 36:203–68.

Taylor SL, Keefe TJ, Windham ES, Howell JF. 1982. Outbreak of histamine poisoning associated with consumption of Swiss cheese. J Food Prot 45:455–7.

Ten Brink B, Damink C, Joosten HM, Huis in 't Veld JH. 1990. Occurrence and formation of biologically active amines in foods. Int J Food Microbiol 11:73–84.

Tsakalidou E. 2011. Microbial flora. In: Nollet LML, Toldra, editors. Safety analysis of foods of animal origin Part III: Milk and dairy foods. Boca Raton: CRC. p 781–98.

Tsoi HW, Tse H. 2011. *Staphylococcus lugdunensis* is the likely origin of the ornithine decarboxylase operon in *Staphylococcus epidermidis* 2015B. Appl Environ Microbiol 77:392–3.

Valsamaki K, Michaelidou A, Polychroniadou A. 2000. Biogenic amine production in Feta cheese. Food Chem 71:259–66.

Valyasevi R, Rolle RS. 2002. An overview of small-scale food fermentation technologies in developing countries with special reference to Thailand: scope for their improvement. Int J Food Microbiol 75:231–9.

van Schothorst M, Zwietering MH, Ross T, Buchanan RL, Cole MB. 2009. Relating microbiological criteria to food safety objectives and performance objectives. Food Control 20:967–79.

Verbeke R, Peeters G, Massart-Leen AM, Cocquyt G. 1968. Incorporation of DL-(2-14C) ornithine and DL-(5-14C) arginine in milk constituents by the isolated lactating sheep udder. Biochem J 106:719–24.

Vrancken G, Rimaux T, Weckx S, De Vuyst L, Leroy F. 2009. Environmental pH determines citrulline and ornithine release through the arginine deiminase pathway in *Lactobacillus fermentum* IMDO 130101. Int J Food Microbiol 135:216–22.

Wallace HM, Caslake R. 2001. Polyamines and colon cancer. Eur J Gastroenterol Hepatol 13:1033–9.

Wegmann U, Overweg K, Horn N, Goesmann A, Narbad A, Gasson MJ, Shearman C. 2009. Complete genome sequence of *Lactobacillus johnsonii* FI9785, a competitive exclusion agent against pathogens in poultry. J Bacteriol 191:7142–3.

Wei F, Xu X, Zhou G, Zhao G, Li C, Zhang Y, Qi J. 2009. Irradiated chinese Rugao ham: Changes in volatile N-nitrosamine, biogenic amine and residual nitrite during ripening and post-ripening. Meat Sci 81:451–5.

Williams BB, Van Benschoten AH, Cimermancic P, Donia MS, Zimmermann M, Taketani M, Fischbach MA. 2014. Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. Cell Host Microbe 16:495–503.

Wohrl S, Hemmer W, Focke M, Rappersberger K, Jarisch R. 2004. Histamine intolerance-like symptoms in healthy volunteers after oral provocation with liquid histamine. Allergy Asthma Proc 25:305–11.

Wunderlichová L, Buňková L, Koutný M, Jančová P, Buňka F. 2014. Formation, degradation, and detoxification of putrescine by foodborne bacteria: a review. Comp Rev Food Sci Food Safety 13:1012–30.

Xu Y, Xia W, Yang F, Kim JM, Nie X. 2010. Effect of fermentation temperature on the microbial and physicochemical properties of silver carp sausages inoculated with *Pediococcus pentosaceus*. Food Chem 118: 512–8.

Yu JJ, Park KB, Kim SG, Oh SH. 2013. Expression, purification, and biochemical properties of arginase from *Bacillus subtilis* 168. J Microbiol 51:222–8.

Zimmer R. 1990. Relationship between tyramine potentiation and monoamine oxidase (MAO) inhibition: comparison between moclobemide and other MAO inhibitors. Acta Psychiatr Scand Suppl 360:81–3.

Zimmer R, Puech AJ, Philipp F, Korn A. 1990. Interaction between orally administered tyramine and moclobemide. Acta Psychiatr Scand Suppl 360:78–80.