

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Glutamate Dehydrogenases: Enzymology, Physiological Role and Biotechnological Relevance

Eduardo Santero, Ana B. Hervás, Ines Canosa and Fernando Govantes

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/47767>

1. Introduction

Glutamate dehydrogenase (GDH) is present in all domains of life and is one of the most extensively studied enzymes at the biochemical and structural levels. These enzymes are generally reversible and catalyse either the reductive amination of 2-oxoglutarate (2-OG) to yield glutamate using NAD(P) as a cofactor, or the oxidative deamination of glutamate [1] (Fig. 1). Because of the reaction it catalyses, the main role of GDH is glutamate catabolism and ammonium assimilation. However, other physiological roles for GDH have been described in some organisms, as we will see below.

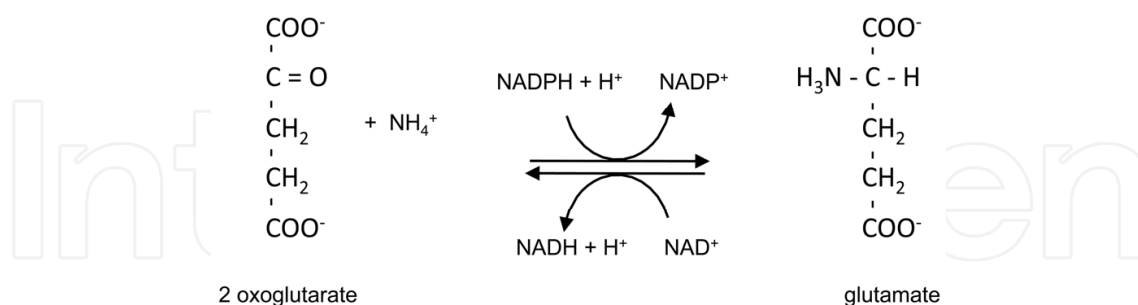


Figure 1. Reaction catalysed by glutamate dehydrogenase

The synthesis of both glutamate and glutamine are key steps in the cell metabolism in all organisms, because they represent the only means of incorporating inorganic nitrogen into carbon backbones. Inorganic nitrogen is assimilated in the form of ammonium, which is incorporated as an amino group to glutamate or an amido group to glutamine. These amino acids in turn act as amino group donors for the synthesis of most nitrogen-containing compounds in the cell. In particular, the amino group of glutamate is used in the synthesis

of purines, pyrimidines, amino sugars, histidine, tryptophan, asparagine, NAD and *p*-aminobenzoate. Therefore glutamate is a key element in the nitrogen flow, as it plays a role of nitrogen donor and acceptor.

Glutamate can be synthesized by two alternative routes: one involves catalysis of GDH in the aminating direction, but ammonium assimilation is also possible by the participation of two enzymes: glutamine synthetase (GS), and glutamate synthase, also named glutamine oxoglutarate aminotransferase or GOGAT (Figure 2). The disadvantage of this pathway is its extra energy requirement. Although GDH catalyses the reductive amination of 2-OG, it is noteworthy that because of its overall high K_m for ammonium, this reaction can only be used for the synthesis of glutamate when the ammonium concentration is high (>1 mM). When the ammonium concentration is lower, ammonia is incorporated to glutamate mainly via the GS-GOGAT pathway. Generally, GDH activity is not necessary for cell growth, since most organisms can synthesize glutamate from glutamine and 2-OG using GOGAT. In fact, some bacteria naturally lack GDH and are neither glutamate auxotrophs nor affected in nitrogen assimilation. While the amination reaction provides nitrogen required for many biosynthetic pathways, the oxidative deamination reaction of GDH provides carbon to the tricarboxylic acid cycle (TCA) by conversion of L-glutamate to 2-OG and probably contributes to balancing the glutamine to glutamate ratio.

Plants and microorganisms can utilise several inorganic nitrogen sources with different oxidation states such as N_2 (by nitrogen-fixing bacteria and archaea), nitrate or nitrite, by reducing them to ammonium, which is subsequently assimilated. After formation of glutamate, the α -amino group can be transferred to a wide variety of 2-oxo acceptors to give rise to amino acids. Also, the α -amino group can be transferred again to glutamate, when 2-oxoglutarate and other amino acids are available. These reactions are carried out by the reversible activity of aminotransferases (EC 2.6.1.x) (Figure 2). Plants and microorganisms can synthesize all carbon skeletons for their amino acids and incorporate the amino group to them by transamination using glutamine and glutamate as nitrogen donors. Incorporation of ammonium in animals also occurs through the GDH and GS/GOGAT pathways. However, higher organisms are unable to reduce oxidized forms of nitrogen to ammonium, to synthesize the structures of some branched or aromatic amino acids such as tryptophan or phenylalanine, or to incorporate sulphur into covalently bonded structures. They are, therefore, totally dependent on other organisms to convert oxidized forms of nitrogen into forms available for the organism, as well as to provide some essential amino acids (Figure 2). These are supplied in the diet or are provided by bacteria from the intestinal tract.

In plants and microorganisms, the physiological roles of GDH include nitrogen assimilation [2], glutamate catabolism [1, 3], but also osmotic balance [4] and tolerance to high temperatures [5, 6]. In vertebrates, multiple biochemical pathways involve glutamate, which is also used as a neurotransmitter. The imbalance of the GDH activity may lead to disturbances of clinical relevance for humans [7]. Free ammonia is highly toxic to organisms that excrete urea as the main nitrogenous waste such as mammals, fish and adult amphibians, leading to inhibition of brain respiration and an excess ketone body formation from acetyl-CoA in the liver. To prevent these deleterious effects, GDH and some of the other enzymes

that yield amino groups into the urea cycle are localized in the mitochondria. In the liver, glutamate is the source of excess ammonium release, and the concentration of glutamate modulates the rate of ammonia detoxification into urea. In pancreatic β -cells, the GDH is involved in insulin homeostasis, and oxidation of glutamate mediates amino acid-stimulated insulin secretion [8]. In the central nervous system, glutamate serves as a neurotransmitter and also as the precursor of the inhibitory neurotransmitter γ -aminobutyric acid (GABA), as well as glutamine, a potential mediator of hyperammonemic neurotoxicity [7]. Also, excessive glutamate signalling can lead to excitotoxicity, a phenomenon where over-activation of glutamate receptors initiates neuronal death [9]. The clinical importance of glutamate metabolism in β -pancreatic cells has been highlighted by the recent discovery of a dominantly expressed defect in glutamate metabolism, the hyperinsulinism/hyperammonemia syndrome (HHS). HHS was one of the first diseases that clearly linked GDH regulation to insulin and ammonia homeostasis [10]. Affected children suffer from recurrent hypoglycemia due to inappropriate secretion of insulin [10-12]. This syndrome is caused by the loss of the human glutamate dehydrogenase allosteric regulation (see below).

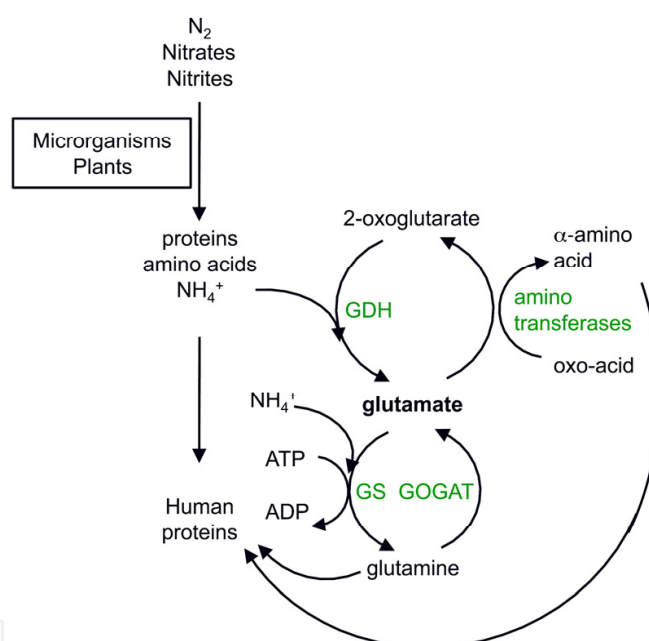


Figure 2. Flow of nitrogen in the biosphere. Molecular nitrogen, nitrites and nitrates are reduced to ammonium and assimilated by microorganisms and plants, whilst higher eukaryotes assimilate these nitrogenated compounds as protein in their diets.

2. Classification, evolution and structure of GDHs

Several GDH classifications have been done according to their size, oligomerisation state, coenzyme specificity or organism, among others. According to their cofactor specificity, there are three basic types of GDH: those that are cofactor specific for NAD (EC 1.4.1.2), those that are specific for NADP (EC 1.4.1.4) and those that can use either cofactor (EC 1.4.1.3) (dual coenzyme-specific GDHs). Lower eukaryotes and prokaryotes usually have GDHs that only function with one coenzyme whilst the enzymes that have dual coenzyme

specificity are commonly found in higher eukaryotes. However, some dual-GDHs, have also been described in prokaryotes [5, 13-15]. Glutamate dehydrogenases from non-vertebrate animals differ from the GDHs of vertebrates in that they are mono-coenzyme specific and are not regulated by nucleotides [16]. In higher plants GDH is ubiquitous and also very abundant. A number of isozymes are usually present in a single species, some of them being inducible, which correlate with their abundance depending on environmental or nutritional conditions [17]. GDHs have also been characterized from a number of eukaryotic microorganisms with different coenzyme specificity such as fungi, (NAD⁺ or NADP⁺) [18], algae (NAD⁺, NADP⁺ or dual), protozoa (NAD⁺ or NADP⁺) and also different intracellular localizations (i.e. cytoplasmic, mitochondrial or in the chloroplasts) [19].

According to the molecular weight of the monomer, three groups of GDHs can be distinguished: GDH50s (MW around 50 KDa), GDH115s (MW around 115 KDa) and GDH180s (MW around 180 KDa). All NADP and dual-GDHs reported so far belong to the GDH50 group, whereas there are representatives of NAD-GDHs in all of these groups. GDH115s have been found only in lower eukaryotes [20-22], whilst the largest GDHs are present only in bacteria. GDH180s were first identified in actinomycetes [23], but recently they have been also described in other Gram positive and Gram negative bacteria (see table 1). Most GDHs reported so far are homo-oligomeric enzymes, but they differ in the number of monomers that compose them. The majority of GDHs have a hexameric structure, as is the case of vertebrate GDHs, but tetrameric and even dimeric enzymes have also been found (see table 1 and references therein). Particularly, the most recently discovered family of prokaryotic GDH180s, have representatives of either hexameric [23, 24], tetrameric [25, 26] and dimeric [27] enzymes. In addition, a couple of GDH50s composed of two different subunits in the form of a hetero-hexamer have been reported [28, 29].

Analysis of the distribution pattern of *gdh* genes from all available sequenced genomes in the three domains of life reveal that all classes of *gdh* have been found in eubacteria and archaea and all but the large GDH have been found in eukaryotes. Both NAD⁺- and NADP⁺-dependent forms of GDH have been reported in higher plants, located in mitochondria and chloroplasts, respectively. The GDH enzyme is abundant in several plant organs, and its isoenzymatic profile can be influenced by dark stress, natural senescence or fruit ripening [30]. Genes coding for GDH seem to be absent in some archaeal genomes as well as in some of the smaller eubacterial and eukaryotic genomes. Among the organisms that do encode GDH, several genes coding for GDH may be found in the same genome. However, just one or two classes are represented, no genome has yet been shown to encode all classes.

Organism	cofactor	MW enzyme (KDa)	MW subunit (KDa)	subunit number	K _m NH ₄ (mM)	K _m 2-OG (mM)	K _m glu (mM)	Ref.
Archaea								
<i>Archaeoglobus fulgidus</i>	NADP	263	47	6	4	0.5	3.9	[31]
<i>Halobacterium halobium</i>	NAD				450	20.2	4	[32]

Organism	cofactor	MW enzyme (KDa)	MW subunit (KDa)	subunit number	K_m NH ₄ (mM)	K_m 2-OG (mM)	K_m glu (mM)	Ref.
<i>Halobacterium halobium</i>	NADP							[33]
<i>Thermococcus strain AN1</i>	NADP	204	47	4	15.5	1.7	9.12	[19]
<i>Pyrococcus furiosus</i>	NAD/NADP	270-290	48	6	6, 27 ^b	0.33	0.6	[34]
<i>Thermococcus profundus</i>	NADP	263	43	6	1.6, 22 ^b	0.2, 0.87 ^b	6.8	[6]
Eubacteria								
Gram negative								
<i>Capnocytophaga ochraea</i>	NAD				3.33	1.44	2.44	[35]
<i>Escherichia coli B/r</i>	NADP	300	50	6	1.1	0.64		[36]
<i>Escherichia coli PA340</i>	NADP				2.5	0.2	2.3	[37]
<i>Janthinobacterium lividum</i>	NAD	1065	170	6			7.1	[24]
<i>P. aeruginosa</i>	NAD		180	4	15	1.6		[25, 38]
<i>P. aeruginosa</i>	NADP	110			7	1		[38, 39]
<i>Psychrobacter sp TAD1</i>	NAD	290	160	2	24.6	2.36	28.6	[27]
<i>Psychrobacter sp TAD1</i>	NADP	290	47	6	4	ND	67.4	[37]
<i>Salmonella enterica</i>	NADP				0.29	4	50	[40]
<i>Thermus thermophilus</i>	NAD	289	46.5, 48 ^a	6				[29, 41]
<i>Thiobacillus novellus</i>	NADP	130	50-55	2?	7.5	7.4	35.5	[42]
<i>Thiobacillus novellus</i>	NAD	120	50-55	2?	7.4, 0.5 ^d	6.7, 0.67 ^d	11.8, 13.3 ^d	[43]
Gram positive								
<i>Bacillus macerans</i>	NADP				2.2	0.38		[44]
<i>Bacillus polymyxa</i>	NADP				2.9	1.4		[45]
<i>Bacillus subtilis</i>	NAD			6				[46, 47]
<i>Clostridium symbiosum</i>	NAD	282	49	6				[48, 49]
<i>Corynebacterium glutamicum</i>	NADP		49	6?				[50]
<i>Mycobacterium smegmatis</i>	NAD		180	4?				[26, 51]
<i>Mycobacterium smegmatis</i>	NADP	245.5	40	6	33	5	62.5	[52]
<i>Lactobacillus fermentum</i>	NADP	300	50	6	6.76	5.6	79	[53]

Organism	cofactor	MW enzyme (KDa)	MW subunit (KDa)	subunit number	K _m NH ₄ (mM)	K _m 2-OG (mM)	K _m glu (mM)	Ref.
<i>Peptostreptococcus asacharolyticus</i>	NAD	266	49	6	18.4	0.82	6	[54]
<i>Streptomyces clavuligerus</i>	NAD	1100	179	6				[23]
<i>Streptomyces fradiae</i>	NADP	200	49	4	30.8	1.54	28.6	[55]

^a NAD⁺-GDH of *Thermus thermophilus* is a heterohexamer composed by two types of subunits: GdhA (46, 5 KDa) and GdhB (48 KDa)

^b The K_m depends on the substrate concentration

^c The kinetic constants determined for each cofactor in enzymes with dual cofactor specificity are separated by slashes

^d The kinetic constants of NAD⁺-GDH of *T. novellus* are different depending on the presence of AMP

Table 1. Some characteristics of selected prokaryotic GDHs

The distribution of *gdh* genes does not show any strong pattern that correlate with the phylogeny [56]. It was believed for some time that NAD- and NADP-GDHs were originated via single gene duplication [57], but as genomes are sequenced and more *gdh* genes are identified this hypothesis has been ruled out. The analysis of phylogenetic distribution patterns of the *gdh* gene families provides strong support for numerous horizontal gene transfer events involving prokaryotes, as well as microbial eukaryotes. Differential gene loss, on the other hand, does not seem to have played an important role in the evolution of *gdh* genes in any of the three domains of life. Sequence comparisons for GDHs from a diverse range of sources show that the hexameric enzymes are similar whatever their coenzyme specificity [58, 59]. On the other hand, the tetrameric enzymes are less well understood because of a lower number of characterized tetrameric GDHs. Organisms bearing a tetrameric GDH, which have catabolic roles, also possesses a genetically distinct hexameric NADP-linked enzyme with a biosynthetic role. Mammalian GDHs represent a clear deviation from its ancestral forms, since they have the so-called antenna, a 48 amino acid insertion near the carboxy terminus, although it is not clear when this feature evolved. Sequenced genomes from *Ciliates* show that their GDHs present a smaller *antenna* from that of mammals, although other members of the Protista, such as trypanosomes, have GDH almost identical to the bacterial forms. *Ciliates* are an evolutionary missing link in the GDH evolution [60]

The structure of GDH of many eukaryotic and prokaryotic organisms has been considerably studied and characterized since the beginning of the 50s. As mentioned above, Most GDHs studied so far are homopolymers consisting of two to six subunits of molecular weight 40,000 to 60,000 (fungal NAD-specific GDHs and bacterial large GDH are exceptions). Most of the characterized GDHs are hexameric and the most common structure found is two trimers of subunits stacked directly on top of each other [61-63]. Some GDHs such as that from bovine liver, which is the best-characterized enzyme [1], may have higher order multimeric structures. This enzyme, which is a hexamer in solution, aggregates to form a high molecular weight species and this polymerization is promoted by a high concentration

of enzyme, by high ionic strength and also by allosteric ligands or cofactors [64]. Since the local concentration of GDH in some tissues is very high, aggregation might be a regulatory mechanism of the activity *in vivo*.

Eukaryotic and prokaryotic GDHs share relatively high conservation in their primary and secondary structures [61] and the crystal structures of the bacterial [59, 65, 66] and mammalian forms [61, 63] of GDH confirm that the general architecture and the locations of the catalytically important residues have remained unchanged throughout evolution. Each subunit in this multimeric enzyme is organised into two domains separated by a deep cleft. One domain directs the self-assembly of the molecule into a hexameric oligomer with 32 symmetry. The other domain is structurally similar to the classical pyridine nucleotide-binding domain but with the direction of one of the β -strands reversed. Upon glutamate binding, the enzyme can adopt different conformations by flexing about the cleft between its two domains. NAD⁺ binds in an extended conformation with the nicotinamide moiety buried deep in the cleft between the two domains [59, 61, 63, 65, 66]. The bottom domains of each trimer make wide contacts with each other, while the NAD⁺-binding domains bearing the nucleotide-binding motif are poised at the top of the structure.

The largest structural difference between mammalian and bacterial GDH is the *antenna*, which has a helix-loop-helix conformation. The *antenna* ascends from the NAD⁺-binding domain surface via a long, 23-residue helix and then descends back with a random coil structure. The helices of the "antenna" domains in each subunit of the trimer wrap around each other with a right-handed twist to form the core of the antenna protrusion. Extensive contacts between "antennae" may represent hexamer interactions in solution and, perhaps, with other enzymes within the mitochondrial matrix [61]. The fact that *antennae* are only found in the forms of GDH that are allosterically regulated by numerous ligands leads to the interpretation that it plays a major part in this regulation. In contrast to the extensive allosteric homotropic and heterotropic regulation observed in mammalian GDH (see below), bacterial forms of GDH are relatively unregulated.

3. GDH enzymology and physiological role

As a reversible enzyme, GDH has the potential for catalysing the reaction in the biosynthetic, aminating direction, or in the catabolic, deaminating direction. The actual physiological reaction of each GDH depends on several factors, as the kinetic constants of the enzyme for its different substrates or the environment where the cell is developed may widely vary. In general, NADP⁺-GDHs usually operate in the biosynthetic direction, that is, synthesizing glutamate by the assimilation of ammonia into 2-OG [6, 31, 39, 40, 45, 67, 68], whereas NAD⁺-GDHs have primarily a catabolic function, yielding ammonia and 2-oxoglutarate from the oxidative catabolism of glutamate [23, 24, 39, 68] (Table 1). Sometimes, both enzymes are present in the same organism, and play a different physiological role due to their different kinetic properties or their different time or place of expression [27, 51, 69, 70]. *Pseudomonas aeruginosa* and presumably other members of the genus *Pseudomonas* have a NADP⁺-specific and a NAD⁺-specific GDH, and it has been

hypothesized than the latter acts specifically in arginine catabolism by converting glutamate, a product of the ammonia-producing arginine succinyl transferase (AST) pathway, into 2-OG, since it is allosterically modulated by arginine (positively) and citrate (negatively) [25]. Similarly, the only active GDH from *Bacillus subtilis* (RocG, NAD⁺-dependent) appears to be involved in arginine and proline catabolism [46]. On the other hand, despite the catabolic function assigned to NAD⁺-GDHs, the existence of an NAD⁺-specific GDH with an unusual biosynthetic role has been reported in the oral bacterium *Capnocytophaga ochraea* [35]. In this case, it was found that only the NAD⁺-GDH ammonium assimilating activity could be detected in cell free extracts, probably due to the high concentration of ammonium and ammonium precursors that can be found in the gingival crevicular fluid. Interestingly, GDHs have been shown to play a substantial and even predominant role in nitrogen assimilation in conditions of N₂ fixation in the Gram positive bacteria *Bacillus macerans* and *Bacillus polymyxa* [44, 45]. Nitrogen fixation only occurs under extreme nitrogen-limiting conditions, when nitrogen from other sources is very scarce. In these conditions nitrogen is always assimilated using the GS/GOGAT pathway, since the K_m of GS for ammonium is much lower than that of GDH. This is not the case in *B. macerans* and *B. polymyxa*, as in these organisms the GOGAT activity is much lower than GDH activity in nitrogen-fixing cells. A NAD⁺-GDH involved in glutamate fermentation has also been described in the anaerobic Gram-positive bacteria *Peptostreptococcus asaccharolyticus* [54]. In this organism, GDH is the first enzyme of the glutamate fermentation via the hydroxyglutarate pathway, and can represent as much as 10% of total protein when grown on glutamate. Very high levels of GDH production has also been reported in some hyperthermophilic archaea like *Pyrococcus furiosus* or some *Thermococcus* strains [5, 6, 71]. These preferentially biosynthetic enzymes represent an exceptionally high percentage of total soluble protein of the cell, in some cases up to 20%, which suggests an important role of these enzymes in these organisms at an extremely high temperature for life.

Determination of the GDH enzymatic structure has allowed the elucidation of the mechanisms for allosteric regulation and negative cooperativity. The activity of glutamate dehydrogenase in animals is allosterically regulated by purine nucleoside phosphates and other metabolic intermediates. In brief, GTP and ATP are allosteric inhibitors whereas GDP and ADP are allosteric activators. Hence, a lowering of the energy charge accelerates the oxidation of amino acids. Their *in vivo* regulation may be dependent on the metabolic status of the cell, or in the tissue they are located. The intracellular compartmentalization of the cofactors, and the GDH itself, may also drive the reaction in one or the other direction. In vertebrate cells, GDH appears to be localized primarily in the mitochondrial matrix [1]. The effects of nucleosides on mammalian GDH are complex. For the bovine liver GDH, four binding sites per subunit have been described, being the active site (site I), the adenine nucleotide regulatory site (site II), the guanine nucleotide regulatory site (site III) and the reduced coenzyme regulatory site (site IV). Some substrate and effectors bind just the active site (glutamate, oxoglutarate, ammonia, NADP⁺) while others are able to bind two different sites (NAD⁺, ADP, NAD(P)H) [19]

One characteristic of microbial GDHs is the absence of the *antenna* structure that functions as a heterotropic allosteric site. In agreement with this, the vast majority of microbial GDHs do not appear to have this level of complexity in GDH modulation by purine nucleoside phosphates. However, some microbial GDHs also show homotropic and even heterotropic allosteric control, especially those from the GDH180 family. GDHs from *Psychrobacter* sp. TAD1, *Streptomyces clavuligerus* or *Pseudomonas aeruginosa* show positive cooperativity of substrate binding, a common feature associated with the complex regulation in vertebrate GDHs, but unusual in bacteria [23, 25, 37]. Furthermore, the Gram-positive bacterium *Clostridium symbiosum* displays an apparent negative cooperativity and inhibitory effect of the enzyme cofactor in certain conditions of pH and concentration [49]. On the other hand, heterotropic control, either positive or negative, has been found in an increasingly number of microorganisms. Accordingly, some aminoacids such as L-aspartate or L-arginine are positive allosteric effectors of NAD⁺-GDH from the psychrophilic bacterium *Janthinobacterium lividum* and *P.aeruginosa* [24, 25], while nucleotides such as ATP or AMP modulate NADP⁺-GDH from *Salmonella enterica* sv. Typhimurium and NAD⁺-GDH from *S. clavuligerus* and *Thiobacillus novellus* [23, 40, 43]. In the latter case, AMP has been found to be actually an essential activator for *S. clavuligerus* GDH activity. Conversely, some microbial GDHs have allosteric inhibitors, such as TCA cycle intermediates in the archaeon *Halobacterium halobium*, in *Salmonella enterica* sv Typhimurium, and in *P. aeruginosa* [25, 40, 72], or nucleotides such as ADP in the NAD⁺-GDH of *Capnocytophaga ochraea* [35]. Finally, the NAD⁺-GDH from the actinomycete *Mycobacterium smegmatis* is modulated by the small protein kinase GarA [26].

4. Regulation of bacterial GDH gene expression

The diverse roles of bacterial GDH in different organisms provide for a variety of regulatory mechanisms. Here we show a few examples for selected bacteria in which transcriptional regulation of GDH genes has been characterized.

4.1. Regulation of GDH synthesis in the enterobacteria

Transcriptional regulation of the *gdhA* gene, encoding NADP-GDH, was first described in the diazotrophic enterobacterium *Klebsiella pneumoniae*, and then in *Escherichia coli* [73]. Transcription of *gdhA* is repressed in both enteric bacteria under nitrogen limitation by the general nitrogen control system (Figure 3,A). This is consistent with the fact that low affinity for ammonium limits the use of GDH for glutamate synthesis at ammonium concentrations below 1 mM. The enterobacterial general nitrogen control (Ntr) system is a very well characterized signal transduction and regulatory network encompassing seven elements: the alternative σ factor σ^{54} , encoded by *rpoN*, the uridylyl transferase-uridylyl removing enzyme GlnD, two PII signal transduction proteins, GlnB and GlnK, the two-component system NtrB-NtrC and the LysR-type transcriptional regulator Nac. Nitrogen status is signalled by the intracellular pools of glutamine (indicative of nitrogen sufficiency), and 2-OG (indicative of nitrogen limitation). These signals are perceived by the PII proteins by means of their

reversible GlnD-dependent uridylylation in response to decreased glutamine levels and by allosteric modulation via 2-oxoglutarate binding. Interaction with PII proteins in either their uridylylated or deuridylylated states is responsible for posttranslational regulation of the activities of a variety of proteins involved in nitrogen metabolism, including glutamine synthetase, nitrogenase and the sensor kinase/phosphatase NtrB. Deuridylylated GlnB promotes NtrB-dependent dephosphorylation of NtrC under nitrogen excess [2, 74]. In nitrogen-limiting conditions, phosphorylated NtrC (NtrC-P) activates multiple σ^{54} -dependent promoters, controlling the expression of over one hundred genes related in *E. coli* [75]. NtrC-regulated genes encode multiple functions related to nitrogen metabolism, including transport and utilization pathways for diverse nitrogen sources, the nitrogen fixation sensor-regulator pair NifL-NifA (in the diazotrophic *Klebsiella pneumoniae*), and the nitrogen regulator Nac. Transcription of Nac is initiated from an NtrC-activated σ^{54} -dependent promoter and autorepressed [76, 77]. Nac in turn activates and represses a set of genes whose products are mostly related to nitrogen metabolism. Nac-activated genes include those involved in the catabolism of histidine, proline, urea and alanine, among others [78-80]. Notably, Nac represses the genes coding for the two enzymes that synthesize glutamate, *gdhA*, encoding GDH, and *gltAB*, encoding GOGAT [81]. Unlike most LysR-type transcriptional regulators, the activity of Nac is not modulated by the presence of a small ligand, and the protein is synthesized in its active form [79]. Thus, the nitrogen limitation dependency of Nac regulation exclusively reflects the increase in concentration under nitrogen limitation due to its transcriptional regulation. Nac is not present in many bacteria containing the Ntr system, and is conspicuously absent in the closely related and well-characterized *S. enterica* [82]. As the need for two different regulators (NtrC and Nac) has been questioned, Nac has been proposed to act as an "adaptor" that integrates genes transcribed from σ^{70} -dependent promoters (which cannot be directly regulated by the activator of σ^{54} -dependent promoters NtrC) into the general nitrogen control network [83].

Transcription of the *gdhA* gene is repressed by Nac under nitrogen limitation in both *E. coli* and *K. pneumoniae* (in contrast, *gdhA* expression is not nitrogen-regulated in *S. enterica*). Nac exhibits two modes of transcriptional repression of the *gdhA* promoter. "Weak" repression involves Nac binding as a dimer to the promoter region in a single site located at -100 to -75 relative to the transcriptional start site [84]. "Strong" repression is observed when a Nac tetramer is simultaneously bound to the aforementioned site, centered at -89, and a downstream site centered at +47. The proposed regulatory mechanism for "strong" repression involves Nac bending DNA, looping out the intervening region, and forming a repressor loop reminiscent of those formed by LacI and GalR at the *lac* and *gal* promoters, respectively [79].

In addition to Nac-mediated repression, *gdhA* is subjected to positive regulation by a second LysR-type transcriptional regulator, ArgP. ArgP is an activator whose activity is antagonized by lysine. ArgP interacts with the *gdhA* promoter region at a single site between -100 and -50. Lysine inhibits interaction of ArgP with this site, thus preventing activation [85]. Activation is also prevented by Nac interaction with the overlapping binding site centered at -89, as both regulatory proteins bind in a mutually exclusive fashion. Thus

"weak" repression is in fact the result of the antagonist action of Nac on ArgP-mediated activation [82, 85]. The role of lysine as a signal can be rationalized if one considers that glutamate must serve two roles in the cell, as both an amino group donor for over 80% of the nitrogenous compounds in the cell, and as a counterion to K^+ in osmotic pressure homeostasis. In order to sense the amount of glutamate being used for biosynthesis, the pools of one or more amino group acceptor may be used instead of glutamate itself. Lysine is likely one of those nitrogenous compounds that serve as surrogates for glutamate in signaling the extent of glutamate overflow from osmotic pressure homeostasis into the biosynthetic pathways for other nitrogenous compounds [85]. Consistently, *gdhAp* activity is decreased in rich media containing amino acids [81], and lysine levels may be one of several signals that mediate the feedback regulation of *gdhAp*. However, the identity of other compounds that may fulfill this role is as of yet unknown.

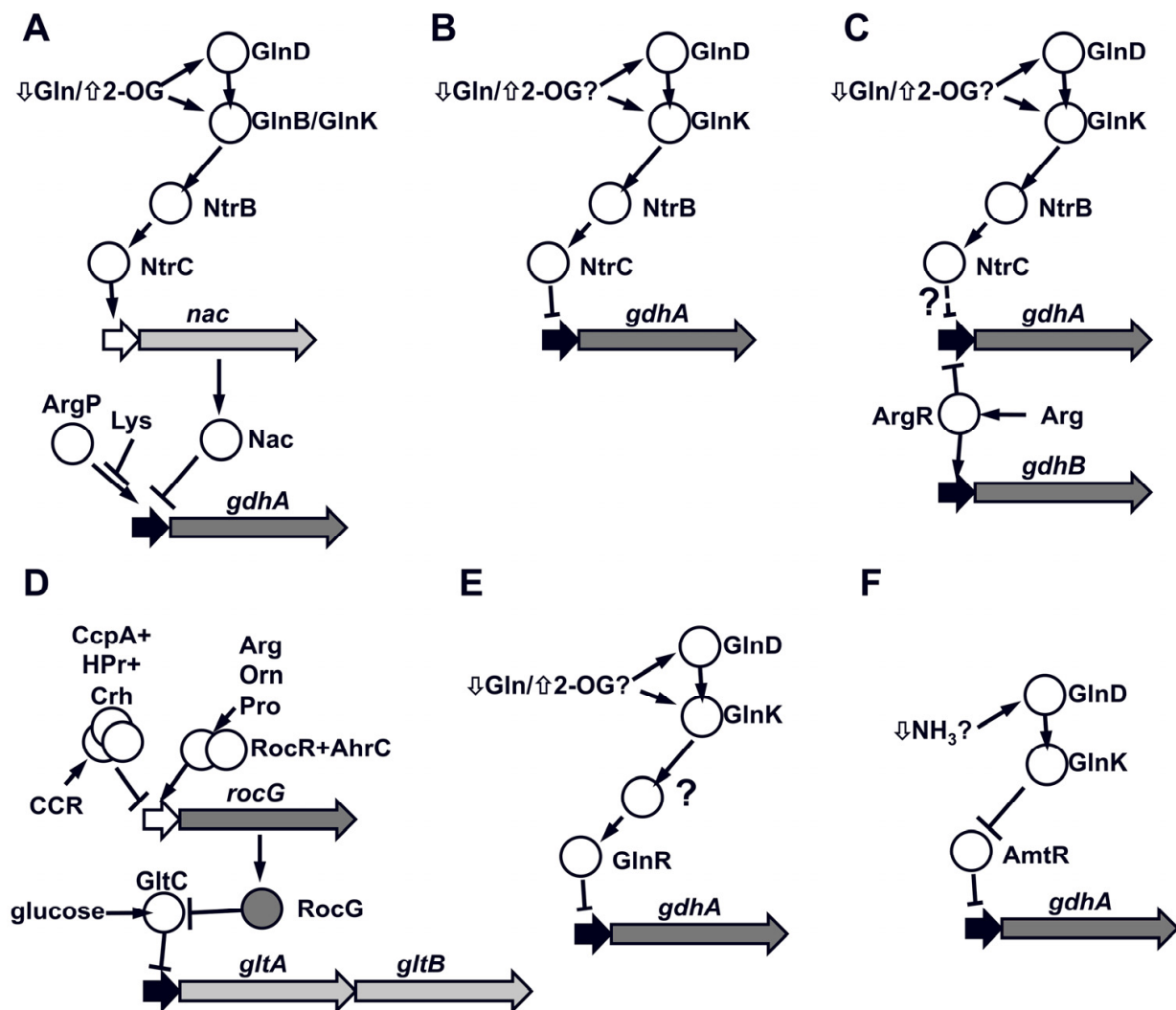


Figure 3. Regulatory circuits for bacterial glutamate dehydrogenase genes. The cartoons represent the known regulatory circuits for *K. pneumoniae* (A), *P. putida* (B), *P. aeruginosa* (C), *B. subtilis* (D), *S. coelicolor* (E) and *C. glutamicum* (E). Sigma 54- or SigL-dependent promoters are displayed as white arrows. Other promoter types are displayed as black arrows. Question marks represent aspects that are not well characterized. CCR: carbon catabolite repression.

4.2. Regulation of GDH synthesis in pseudomonads

Little is known about the transcriptional regulation of GDH synthesis in most Gram-negative bacteria. However, the regulatory mechanisms behind *gdhA* regulation were recently revealed in the soil bacterium *Pseudomonas putida* [86]. Similarly to its enteric counterparts, *P. putida* represses *gdhA* expression during nitrogen-limited growth in a Ntr system-dependent fashion (Figure 3,B). *P. putida* harbors a simplified general nitrogen control system that lacks the major PII protein, GlnB, and the transcriptional regulator Nac. In this scheme, the only PII protein, GlnK, controls the phosphorylation/dephosphorylation balance of NtrC in response to nitrogen availability using mechanisms likely similar to those found in the enterics [87]. In the absence of Nac, NtrC has been shown to directly regulate some of the functions that are controlled by Nac in the enterics, including activation of *codB* and *ureD*, encoding cytosine deaminase and urease respectively [88], and repression of the GDH gene *gdhA* [86]. NtrC binds the σ^{70} -dependent *gdhAp* promoter region cooperatively at four different sites, centered at positions -118, -95, -21 and +12 relative to the transcriptional start. While simultaneous occupancy of all four sites yields the maximal levels of repression, only the promoter-proximal I and II sites were found to be absolutely required for repression. A mechanism based on a repressor loop involving four NtrC dimers has been proposed for negative regulation of the *gdhAp* promoter in *P. putida*. Although repression is partially sensitive to the phosphorylation state of NtrC *in vitro*, *in vivo* evidence indicated that phosphorylation of NtrC is not required for efficient repression of the *gdhAp* promoter. Since NtrC synthesis is itself nitrogen regulated *via* the *glnAp* promoter, the increase in repressor concentration under nitrogen limitation appears to be the major determinant of nitrogen-regulated expression of *gdhA* in *P. putida* [86].

Pseudomonas aeruginosa encodes two GDH enzymes, a NAD-dependent GDH encoded by *gdhB*, primarily involved in glutamate deamination to 2-oxoglutarate, and a NADP-dependent GDH, encoded by *gdhA*, primarily involved in ammonium assimilation. The catabolic enzyme is linked to the AST pathway for aerobic utilization of arginine as a carbon source. Glutamate is the end product of the AST pathway, and NAD-GDH serves the purpose of connecting arginine catabolism to the central metabolism *via* 2-oxoglutarate. Transcription of *gdhB* is activated by the arginine regulatory protein ArgR in response to the presence of arginine, which is also an allosteric modulator of the enzyme activity (Figure 3,C). An ArgR binding site is found immediately upstream from the -35 box of the *gdhB* promoter. Induction of the expression of *gdhB* and activation of the encoded dehydrogenase by arginine serve to direct the flow of glutamate into the TCA cycle [25]. Conversely, transcription of *gdhA* is repressed by ArgR in the presence of arginine. Such repression could serve to minimize the operation of an energy-consuming futile cycle involving the simultaneous function of *gdhA* and *gdhB* when *P. aeruginosa* uses arginine as a carbon source. Repression of *gdhA* expression is exerted from a single ArgR binding site centered at position -41, and a simple steric hindrance mechanism has been proposed at this promoter [89]. Downregulation of *gdhA* expression by the Ntr system under nitrogen limitation has also been reported [90], but the factors and mechanisms involved are uncharacterized. Since *P. aeruginosa* also lacks Nac, direct repression by NtrC similar to that observed in *P. putida* [86], may also occur in this organism.

4.3. Regulation of GDH synthesis in *Bacillus subtilis*

The *Bacillus subtilis* genome contains two genes encoding GDHs, *rocG* and *gudB*. While RocG is an enzymatically active GDH, GudB is inactive, due to a duplication of three amino acid residues at its active center. Decryptification of *gudB* in a *rocG* background is achieved by high-frequency acquisition of a suppressor mutation consisting of the precise deletion of part of the 9-bp direct repeat that prevents activity [46, 47]. Both RocG and decryptified GudB are primarily catabolic dehydrogenases, and *de novo* glutamate synthesis in *B. subtilis* is performed exclusively by GOGAT.

Similarly to *P. aeruginosa* GdhB, RocG is not nitrogen-regulated and is linked to arginine and proline catabolism, as its expression is induced by arginine, ornithine and, to a lesser extent, proline. Transcription from the *rocG* promoter depends on the product of the *Bacillus subtilis* *sigL* gene, an ortholog of the alternative σ factor σ^{54} [91], and is activated by the UAS binding protein RocR in response to the presence of arginine, ornithine, or proline with the assistance of the arginine-dependent activator AhrC (Figure 3,D). SigL, RocR and AhrC also control transcription of the two operons, *rocABC* and *rocDEF*, involved in arginine conversion into glutamate. In that regard, RocG-dependent deamination of glutamate to 2-oxoglutarate can be viewed as the final step in the use of arginine, ornithine, and proline as carbon or nitrogen, providing rapidly metabolizable carbon- or nitrogen-containing compounds for biosynthesis [92]. Interestingly, inducibility of RocG synthesis by arginine precludes growth on glutamate as the sole carbon source.

The most salient feature of the *rocGp* SigL-dependent promoter is the absence of an upstream activator sequence (UAS) for RocR. Instead, the UAS present at the *rocAp* promoter, located immediately downstream from the *rocG* coding sequence, is the *cis*-acting element essential for RocR-dependent activation of *rocGp*. This sequence has been shown to be active when placed upstream or downstream and as far as 15 kb away from the target promoter [93]. According to the general model of σ^{54} -dependent promoter activation, RocR bound to its target sequence activates transcription by interacting with promoter-bound σ^{54} -RNA polymerase by a mechanism that involves looping out of the intervening DNA sequences. The AhrC protein is also required for activation of *rocG* (as well as *rocABC* and *rocDEF*), and it apparently modulates the activity of RocR by means of protein-protein contacts [94].

Unlike the arginine utilization operons *rocABC* and *rocDEF*, *rocG* transcription is subjected to carbon catabolite repression in the presence of glucose. Such repression is mediated by the regulatory protein CcpA. A *cre* (catabolite responsive element) site is located at positions +39 and +51, and is required for CcpA binding and *rocGp* repression. CcpA binding at this location prevents transcription from the *rocGp* promoter, but also acts as a roadblock for low-level readthrough transcription from an upstream promoter, which is relevant in the absence of both glucose and the cognate inducers [92]. As in other CcpA-regulated genes, CcpA-mediated repression requires the assistance of the accessory proteins HPr and Crh.

A final feature of the *B. subtilis* GDH RocG worth mentioning is its role in the regulation of the assimilatory glutamate synthase operon, *gltAB*. RocG belongs to a group of proteins

designated "trigger enzymes", which have catalytic as well as regulatory functions. RocG interacts with the LysR-type transcriptional regulator GltC, which is the cognate activator of the *gltAB* operon. RocG-GltC interaction results in inactivation of the latter, which in turn prevents activation of the assimilatory glutamate synthase in conditions in which glutamate is already being synthesized from arginine-related amino acids or proline. This mechanism allows tight control of glutamate metabolism by the availability of carbon and nitrogen sources [95-97].

4.4. Regulation of GDH synthesis in other Gram-positives

The *Streptomyces coelicolor* gene *gdhA*, encoding a NADPH-dependent GDH, is negatively regulated under ammonium limitation by GlnR (Figure 3,E). GlnR is an OmpR-like transcriptional factor, which is the master regulator of nitrogen metabolism in *S. coelicolor*. This response includes activation of *glnA*, *glnII* genes, encoding two glutamine synthetases, and repression of *gdhA*, among others [98]. The GlnR regulon appears to be conserved in *Mycobacterium* and other actinomycetes. The nitrogen signal is transduced by a variation of the enterobacterial Ntr signal transduction system, including the PII protein GlnK and GlnD, which catalyzes adenylation and deadenylation of GlnK in response to the nitrogen status. GlnR activity is presumably regulated by phosphorylation, as it displays the conserved phosphorylatable aspartate residue present in many response regulators, but the identity of its sensor kinase is currently unknown [99]. GlnR exerts repression of the *gdhA* promoter region by binding at a conserved site centered at position -73, but the underlying mechanisms of repression are not yet understood [98]. Interestingly, synthesis of the regulator GlnR is repressed by the response regulator PhoP under phosphate limitation, thus providing a link between phosphorus and nitrogen metabolism in *S. coelicolor* [100].

AmtR is a repressor of the TetR family, which acts as the global nitrogen regulator in the industrially relevant *Corynebacterium glutamicum* [Burkowsky, 2003]. Because of the high basal intracellular concentrations of glutamate and glutamine (up to 200 mM and up to 50 mM, respectively), *C. glutamicum* does not use these amino acid pools to sense nitrogen availability. Instead, ammonium is probably used to modulate the activity of the adenylyl transferase GlnD [101]. Adenylylated GlnK interacts with AmtR to release the repressor from its target promoters under nitrogen limitation. AmtR represses the expression of at least 35 genes, including *glnA*, encoding glutamine synthetase, *gltBD*, encoding glutamate synthase and the *amtB-glnK-glnD* operon, encoding a high-affinity ammonium transporter and the signal transduction proteins, GlnK and GlnD. GDH activity is high and relatively constant in different growth conditions [50]. Transcription from the *gdhAp* promoter is repressed 2-fold by AmtR (Figure 3,F) under nitrogen excess and interaction of AmtR with the *gdhAp* promoter region has been detected [102]. Intriguing as it may be, upregulation of GDH under nitrogen limitation does not appear to be physiologically relevant, as sufficient activity is present under the high ammonium concentration conditions in which GDH contributes to ammonium assimilation. Other transcription factors (FarR, WhiH and OxyR) have been documented to bind the *gdhAp* promoter region, but the relevance of these interactions is so far unknown [102].

5. Involvement of GDH in biotechnological processes

In addition to the different physiological roles of GDHs from different bacteria in nitrogen assimilation, amino acid catabolism, osmotic balance or tolerance to high temperatures, GDH catalysis is crucial for a number of biotechnological processes. These include industrial production of glutamate by *C. glutamicum* and related species, which involves catalysis in the aminating direction, and production of aromas by lactic acid bacteria during cheeses ripening, which involves catalysis in the opposite direction.

5.1. Production of L-Glutamate by *Corynebacterium glutamicum*

C. glutamicum is a facultatively anaerobic, nonpathogenic, non-motile, biotin-auxotrophic, Gram-positive soil bacterium that was isolated more than 50 years ago in a screen for bacteria that excrete glutamate. Since then, derivative strains of this bacterium and related species have been isolated as glutamate producers, and industrial processes for biological glutamate production have been developed. Annual L-glutamate production is estimated to be over 2 million tons, with a continuously increasing demand (3% per year), above all in developing countries. L-glutamate is mainly used in food as a flavour enhancer.

In addition to its importance in industrial biotechnology, *C. glutamicum* has gained interest as a model organism for the *Corynebacterineae*, an industrially relevant suborder of the actinomycetes. Because of the academic and industrial interest, its physiology and metabolism have been deeply characterised, three research groups have independently determined the genomic sequence of *C. glutamicum* strains [103-105], and global analysis techniques such as proteomics, transcriptomics, metabolomics and metabolic flux analysis have been used to obtain a holistic view of the glutamate production process.

C. glutamicum exponentially growing cells do not accumulate glutamate. However, glutamate production and excretion can be easily induced by an astonishing variety of treatments, which allow accumulation of glutamate in the culture medium to a concentration as high as 80 g L⁻¹. These include biotin limitation [106], which was the first identified condition for glutamate overproduction, addition of fatty acid ester surfactants such as Tween 40 (polyoxyethylene sorbitan monopalmitate) or Tween 60 (polyoxyethylene sorbitan monoestearate) [107], addition of certain β -lactam antibiotics such as penicillin [108], use of glycerol-auxotrophic or fatty acid-auxotrophic strains [109], use of temperature-sensitive strains cultured at higher temperature [110], or addition of ethambutol, an inhibitor of cell wall arabinogalactan synthesis [111]. In spite of the apparent diversity of treatments to induce glutamate production and secretion, all of them have features in common and there is a unified view that glutamate production is triggered by environmental conditions that produce damage of the cell surface structures [112-114].

Since *C. glutamicum* is a biotin auxotroph, supplementation of a defined medium with a limiting concentration of biotin in batch cultures results in reduced total biomass and concomitant glutamate production. Biotin is a co-factor of acetyl-CoA carboxylase, the first

enzyme for fatty acid biosynthesis, and a reduction of this enzyme activity leads to changes in the fatty acid composition of the membrane. In support of this view, a mutation in *dtsR1*, which codes for the beta subunit of acetyl/propionyl-CoA carboxylase, requires fatty acids for growth and, when limited, overproduces glutamate even when grown with an excess of biotin [109]. On the contrary, amplification of its gene dosage resulted in reduction of glutamate production induced by biotin limitation, detergents or penicillin [115]. Glycerol limitation in mutants unable to produce it provokes similar membrane alterations due to the limitation of membrane lipid precursors. Besides the ubiquitous cell membrane, members of the genus *Corynebacterium*, together with *Mycobacteria* and *Nocardia*, have a special cell envelope structure, which consists of a second lipid layer containing mycolic acids, which has a highly ordered structure and plays an important role in determining solute fluxes [116]. Interestingly, mycolic acids of the outer lipid layer are covalently linked to an arabinogalactan layer, which in turn is covalently linked to the underlying peptidoglycan of the cell wall. This structure may thus be envisioned as one large macromolecule, the mycolyl-arabinogalactan-peptidoglycan complex. Treatments with ethambutol or penicillin clearly affect the structure of the cell envelope. The triggering effect of Tween 40 or Tween 60 (but not Tween 20 or Tween 80) may be due to alterations of the mycolic layer structure [112] although it may also affect the activity of acetyl-CoA carboxylase [113], thus leading to cell envelope alterations.

It was thought for some time that all these treatments affecting cell envelope structure increased the membrane permeability, which would in turn allow leakage of glutamate. However, it is obvious that a specific carrier-mediated export is required, as the increase in the membrane permeability is specific for glutamate, not for other solutes, and glutamate is still exported against a concentration gradient. The glutamate transporter was identified by characterising mutants that produced and accumulated glutamate. All these mutants had substitution mutations or even an insertion mutation in the NCgl1221 gene, which codes for a product showing homology to mechanosensitive channels such as the *E. coli* *yggB* gene product [113]. The mutant alleles appear to code for a constitutively opened glutamate channel. Exchange of the wild-type allele for these mutant alleles led to glutamate overproduction and excretion without any inducing treatment, and rendered cells resistant to the L-glutamate analog 4-fluoroglutamate. Overexpressing wild type NCgl1221 did not result in constitutive glutamate excretion but led to increased glutamate production and excretion after the induction treatments. On the contrary, a deletion mutant lacking NCgl1221 could not excrete glutamate [113]. Therefore, opening the glutamate channel appears to be essential for glutamate production and export. In addition, this is the first response of *C. glutamicum* to membrane tension alterations, which triggers all other metabolic adaptations leading to efficient glutamate production. The NCgl1221 (*yggB*) product has 4 transmembrane segments, is located in the cytoplasmic membrane [117] and has been recently shown to work as a mechanosensitive channel able to increase the cell survival rate of *Bacillus subtilis* after osmotic down-shock [118].

Although *C. glutamicum* has other potential ammonium assimilating enzymes such as alanine dehydrogenase or diaminopimelate dehydrogenase, their contribution to

ammonium assimilation is very limited, according to the *in vivo* flux analyses [119]. *C. glutamicum* and related glutamate-producing species have GDH, GS and GOGAT. Thus, *C. glutamicum* assimilates ammonium, the preferred carbon source of most bacteria, either via GS/GOGAT or via GDH. As in many other bacteria, GS/GOGAT is the main ammonium assimilation pathway when its concentration is limiting, whilst GDH assimilate most of the ammonium when it is present in concentrations above 5 mM [120]. Under glutamate production conditions, which implies high ammonium concentration, the *gltB* and *gltD* genes encoding the GOGAT subunits, are fully repressed by ammonium and no GOGAT activity is detected [119, 121]. Therefore, the main glutamate producing enzyme in these conditions is GDH.

Production and excretion of large quantities of glutamate require glutamate export to the culture medium but also modification of metabolic fluxes to produce high amounts of glutamate. Metabolic flux analysis under exponential growth vs. glutamate producing conditions [119, 122-125], indicated significant changes in catabolic pathways (Figure 4). Under non-growing, glutamate-producing conditions, glucose catabolism either via glycolysis or the pentose phosphate pathway was clearly reduced. Additionally, there was a significant redistribution of the fluxes at the 2-OG branch point between the TCA cycle and the glutamate biosynthetic pathway, that reduced the flux towards succinyl CoA formation about 2/3, with a concomitant increase in the reductive aminating reaction catalysed by GDH. The increase of the metabolic flux towards glutamate is not due to an increase in the 2-OG-producing isocitrate dehydrogenase (ICDH) or GDH activities because they remain constant [125]. In fact, increasing either ICDH or GDH activity by overexpressing their coding genes, did not affect glutamate production [123].

The factor with greatest impact on glutamate production is a reduction of the 2-oxoglutarate dehydrogenase complex (ODHC) activity [123, 125]. During exponential growth, although sufficient GDH specific activity was observed, the flux catalyzed by GDH was very small because the K_m value of GDH for 2-OG is much higher (approximately 50-fold higher) than that of ODHC. Once the ODHC specific activity was decreased after the triggering signals, 2-OG accumulated, and consequently, glutamate was overproduced by GDH. In spite of the initial report claiming that an *odhA* deletion mutant, which has no ODHC activity, overproduced and excreted glutamate [126], there are a number of reports on *odhA* mutants with contradictory results. It appears that those *OdhA* mutants that overproduced glutamate had an additional mutation in *Ncgl1221 (yggB)* [113], which clearly indicates that reduction or elimination of ODHC activity is necessary but not sufficient for glutamate overproduction.

Obviously, GDH activity requires the presence of sufficient concentration of its substrates. Paradoxically, most of the NADPH required for anabolic reactions is generated at the pentose phosphate pathway, which is reduced under these conditions. However, it appears that the reaction catalysed by ICDH provides sufficient NADPH to fulfill the demands of the GDH reaction. Similarly, glutamate production and excretion requires a continuous supply of carbon. This is achieved by an increase in the anaplerotic reactions that produce

oxaloacetate. In *C. glutamicum* two reactions can yield oxaloacetate. One is the standard anaplerotic reaction catalysed by the phosphoenol pyruvate carboxylase (PEPc). The other is pyruvate carboxylase (Pc), which catalyses oxaloacetate production from pyruvate. Metabolic flux of PEPc remains constant in both conditions whilst the Pc flux, undetectable during exponential growth, is clearly increased in the glutamate-producing conditions upon addition of Tween-40 [124]. This suggests a relevant role of Pc in providing sufficient oxaloacetate for glutamate production. Despite this evidence, pyruvate carboxylase cannot be relevant for glutamate production when induced by biotin limitation, as biotin is also a prosthetic group of Pc. As expected, *pyc* disruptants lacking Pc can produce and excrete glutamate as the wild type strain [127], indicating that PEPc may be sufficient to provide the required carbon under this particular inducing regime.

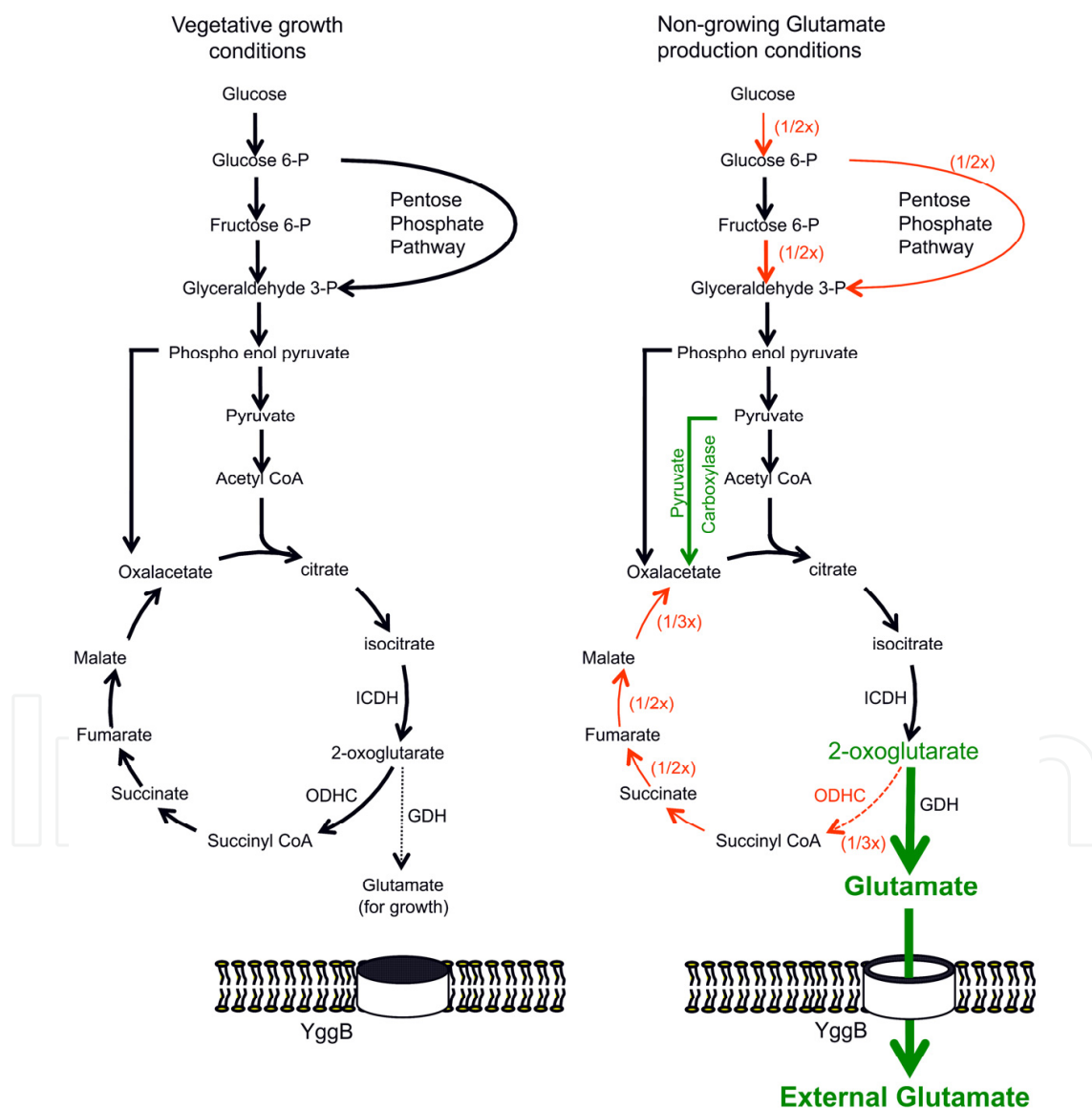


Figure 4. Metabolic fluxes from glucose to glutamate under vegetative growth or non-growing glutamate-producing conditions. Red arrows show metabolic fluxes reduced during glutamate production. Green arrows show those reactions that appear or are increased under the same condition.

How is ODHC activity decreased during glutamate production? ODHC is a complex composed of three different subunits: OdhA (E1), SucB (E2) and LpdA (E3). Many TCA cycle enzymes are regulated at transcriptional or posttranscriptional levels [128]. Activity of this complex is controlled by OdhI, a small protein (15,4 KDa) that binds OdhA (the E1 subunit). Induced glutamate excretion is virtually abolished in a OdhI deletion mutant, thus indicating that inhibition of ODHC activity through OdhI is critical for glutamate overproduction [129]. The OdhI function is regulated by phosphorylation by PknG, which phosphorylates and inactivates OdhI [130], and dephosphorylation by Ppp. PknG deletion mutants showed higher glutamate production when induced by some treatments [129], which is consistent with its role in controlling ODHC activity.

The main question is why do *C. glutamicum* cells excrete large amounts of L-glutamate when exposed to the mentioned induction treatments? Certainly, it does not appear that it is a response to metabolic changes since the inducing treatments do not imply changes in nutritional status. Since these treatments all affect cell surface structures and may therefore alter membrane tension, L-glutamate production may be the response to this membrane stress, as glutamate might function as a compatible solute to prevent cells from bursting. Thus, L-glutamate production by *C. glutamicum* may be a mechanism of adaptation to environmental changes affecting cell surface structures that starts by opening a glutamate export mechanosensitive channel, which in turn somehow triggers the metabolic adaptations (reduction of pentose phosphate pathway, reduction of ODHC activity, increase of the anaplerotic reactions) required to produce and excrete high amounts of glutamate. A similar role of L-glutamate in osmoprotection was also described for *E. coli* [131], and confirmed by the osmosensitive phenotype provoked by mutations unable to activate *gdhA* in a GOGAT deficient background during osmotic upshift [4]. However, there are no reports on metabolic adaptations leading to glutamate production and excretion upon osmotic shock in this bacterium.

5.2. Aroma and flavour production by Lactic Acid Bacteria (LAB)

Accelerating or diversifying flavour development in cheese is of major economical interest since final flavour of cheeses partly determines the consumer's choice. Flavour formation occurs during cheese ripening, and is associated to non-starter lactic acid bacteria (NSLAB), adventitious microflora that occur in the milk or appear later during cheese manufacturing. After characterisation, some strains have been selected as flavour-producing adjuncts and shown that cheeses with these adjuncts are richer in free amino acids and have enhanced flavour intensity [132]. However, flavour development is a time consuming and expensive process that is still not well mastered, and selection of strains for cheese ripening is still an empirical process based on cheese trials with different strains and sensory analyses, that has had varying success [133].

Amino acid catabolism, particularly that of aromatic amino acids, branched chain amino acids, and methionine, is a major player in flavour formation in cheese, especially in cheeses containing only LAB. Amino acid conversion to aroma compounds proceeds by two

different pathways: (i) elimination reactions catalysed by amino acid lyases that produce different alcohols, and (ii) transamination reactions leading to different 2-oxoacids. The latter is the major pathway in LAB. The resulting 2-oxoacids are not responsible for flavour production but are then transformed to aldehydes, alcohols, carboxylic acids, hydroxy acids or methanethiol by additional steps that may be catalysed by the 2-oxoacid-producing LAB or by the inoculated starter LABs [134, 135]. Figure 5 shows the transaminations and further reactions leading to aroma compounds. It appears that the different flavours produced by different LAB strains depend on the proportion of the different 2-oxoacid produced and, therefore, on the relative aminotransferases activities [136].

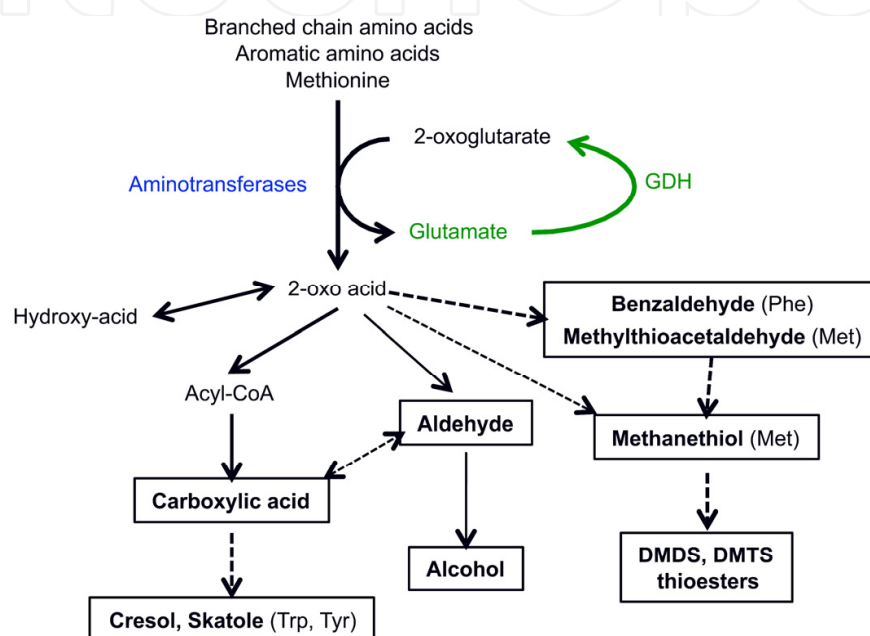


Figure 5. Main amino acid catabolic pathways in LAB leading to aroma compounds (boxed). Doted arrows represent chemical reactions.

Although lactococci have high aminotransferase activity, only very low and slow amino acid degradation occurs. Cheeses are rich in amino acids and peptides but the concentration of 2-OG is low. Supplementation of several types of cheeses with 2-OG led to a decrease in the levels of amino acids important for aroma development, which indicated that catabolism of these amino acids had been enhanced [137, 138], and sensory analyses indicated that addition of 2-OG resulted in changes in creamy character, aroma intensity and fruity notes. These results clearly established that 2-OG availability is the limiting factor for the transamination reactions that convert aminoacids to aroma compounds and prompted scientist to look for sources of 2-OG.

Occurrence of GDH in starter and non-starter LAB is heterogeneous, as just a few LAB strains show GDH activity. Some strains of the starter *Lactococcus lactis* have GDH activity but it is coded in a plasmid (pGDH442) rather than in its chromosome [139]. However, these GDH⁺ strains are glutamate auxotrophs and cannot synthesize glutamate because of 2-OG limitation. The oxidative TCA cycle in these strains is interrupted at the isocitrate dehydrogenase step [140], thus blocking the major source of 2-OG for most bacteria. Because

of this, GDH can only be used for glutamate catabolism, and this reaction constitutes the major source of 2-OG in these strains. As shown in Fig. 5, the GDH role in aroma development by LAB in cheeses is to catalyse the oxidative deamination of glutamate in order to replenish the 2-OG consumed by the transamination reactions. The importance of GDH in aroma production was initially shown by cloning and heterologous expression of the GDH gene from *Peptostreptococcus asaccharolyticus* into a GDH⁻ strain of *L. lactis*, which resulted in an increase of amino acids degradation and, more importantly, an increase in the proportion of carboxylic acids, which are important aroma compounds [141]. This result is the proof of concept that GDH can substitute the exogenous 2-OG. Other reports describing the effect of GDH on aroma production have followed, including the natural transfer by conjugation of the *gdh* gene coded in the pGDH442 plasmid among LAB strains [142]. The relevance of this is that the resulting transconjugants are not considered genetically modified organisms and can be used in the food industry as starters or adjuncts.

An evident correlation between GDH activity and aroma production has been established for both mesophilic and thermophilic LAB [143, 144]. Because of this, the presence of GDH activity has been proposed as a criterion to select flavour-producing LAB strains for cheese ripening. The use of different LAB strains with different aminotransferases specificities together with the use of GDH to enhance the transamination reactions may represent an effective way of intensifying and diversifying cheese aromas.

Author details

Eduardo Santero*, Ines Canosa and Fernando Govantes

Centro Andaluz de Biología del Desarrollo, CSIC-Universidad Pablo de Olavide-Junta de Andalucía, Seville, Spain

Ana B. Hervás

Department of Microbiology, Faculty for Biology. University of Freiburg, Freiburg, Germany

Acknowledgement

We are very grateful to Nuria Pérez and Guadalupe Martín-Cabello for their technical help and all members of the laboratory for their insights and suggestions. Work in the authors' laboratories is supported by the Spanish Ministry of Economy and Competitiveness, grants BIO2008-01805, BIO2010-17853, BIO2011-24003 and CSD2007-00005, and by the Andalusian government, grants P05-CVI-131 and P07-CVI-2518.

6. References

- [1] Smith EL, Austen, BM, Blumenthal, KM, Nyc, JF (1975) Glutamate dehydrogenases. In: Boyer PD, editor. *The Enzymes*: Academic Press. p. 293–367.

* Corresponding Author

- [2] Reitzer L (2003) Nitrogen assimilation and global regulation in *Escherichia coli*. Annu Rev Microbiol. 57:155-176.
- [3] Tanous C, Chambellon E, Sepulchre AM, Yvon M (2005) The gene encoding the glutamate dehydrogenase in *Lactococcus lactis* is part of a remnant Tn3 transposon carried by a large plasmid. J Bacteriol. 187:5019-5022.
- [4] Nandineni MR, Laishram RS, Gowrishankar J (2004) Osmosensitivity associated with insertions in *argP* (*iciA*) or *glnE* in glutamate synthase-deficient mutants of *Escherichia coli*. J Bacteriol. 186:6391-6399.
- [5] Consalvi V, Chiaraluce R, Politi L, Vaccaro R, De Rosa M, Scandurra R (1991) Extremely thermostable glutamate dehydrogenase from the hyperthermophilic archaeobacterium *Pyrococcus furiosus*. Eur J Biochem. 202:1189-1196.
- [6] Kobayashi T, Higuchi S, Kimura K, Kudo T, Horikoshi K (1995) Properties of glutamate dehydrogenase and its involvement in alanine production in a hyperthermophilic archaeon, *Thermococcus profundus*. J Biochem. 118:587-592.
- [7] Kelly A, Stanley CA (2001) Disorders of glutamate metabolism. Ment Retard Dev Disabil Res Rev. 7:287-295.
- [8] Smith TJ, Stanley CA. Glutamate dehydrogenase. In: D'Mello JPF, editor. Amino Acids in Human Nutrition and Health Wallingford, Oxfordshire, UK: CAB International; 2012. p. 1-19.
- [9] Aarts MM, Tymianski M (2004) Molecular mechanisms underlying specificity of excitotoxic signaling in neurons. Curr Mol Med. 4:137-147.
- [10] Stanley CA, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ, *et al.* (1998) Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. N Engl J Med. 338:1352-1357.
- [11] MacMullen C, Fang J, Hsu BY, Kelly A, de Lonlay-Debeney P, Saudubray JM, *et al.* (2001) Hyperinsulinism/hyperammonemia syndrome in children with regulatory mutations in the inhibitory guanosine triphosphate-binding domain of glutamate dehydrogenase. J Clin Endocrinol Metab. 86:1782-1787.
- [12] Stanley CA, Fang J, Kutyna K, Hsu BY, Ming JE, Glaser B, *et al.* (2000) Molecular basis and characterization of the hyperinsulinism/hyperammonemia syndrome: predominance of mutations in exons 11 and 12 of the glutamate dehydrogenase gene. HI/HA Contributing Investigators. Diabetes. 49:667-673.
- [13] Yarrison G, Young DW, Choules GL (1972) Glutamate dehydrogenase from *Mycoplasma laidlawii*. J Bacteriol. 110:494-503.
- [14] Maulik P, Ghosh S (1986) NADPH/NADH-dependent cold-labile glutamate dehydrogenase in *Azospirillum brasilense*. Purification and properties. Eur J Biochem. 155:595-602.
- [15] Consalvi V, Chiaraluce R, Politi L, Pasquo A, De Rosa M, Scandurra R (1993) Glutamate dehydrogenase from the thermoacidophilic archaeobacterium *Sulfolobus solfataricus*: studies on thermal and guanidine-dependent inactivation. Biochim Biophys Acta. 1202:207-215.
- [16] Hoffmann RJ, Bishop SH, Sassaman C (1978) Glutamate dehydrogenase from coelenterates is NADP specific. J Exp Zool. 203:165-170.
- [17] Srivastava HS, Singh, R. P. (1987) Role and regulation of L-glutamate dehydrogenase activity in higher plants. Phytochemistry. 26:597-610.

- [18] Le John HB (1971) Enzyme regulation, lysine pathways and cell wall structures as indicators of major lines of evolution in fungi. *Nature*, . 231:164-168.
- [19] Hudson RC, Daniel RM (1993) L-glutamate dehydrogenases: distribution, properties and mechanism. *Comp Biochem Physiol B*. 106:767-792.
- [20] Hemmings BA (1980) Purification and properties of the phospho and dephospho forms of yeast NAD-dependent glutamate dehydrogenase. *J Biol Chem*. 255:7925-7932.
- [21] Uno I, Matsumoto K, Adachi K, Ishikawa T (1984) Regulation of NAD-dependent glutamate dehydrogenase by protein kinases in *Saccharomyces cerevisiae*. *J Biol Chem*. 259:1288-1293.
- [22] Veronese FM, Nyc JF, Degani Y, Brown DM, Smith EL (1974) Nicotinamide adenine dinucleotide-specific glutamate dehydrogenase of *Neurospora*. I. Purification and molecular properties. *J Biol Chem*. 249:7922-7928.
- [23] Minambres B, Olivera ER, Jensen RA, Luengo JM (2000) A new class of glutamate dehydrogenases (GDH). Biochemical and genetic characterization of the first member, the AMP-requiring NAD-specific GDH of *Streptomyces clavuligerus*. *J Biol Chem*. 275:39529-39542.
- [24] Kawakami R, Sakuraba H, Ohshima T (2007) Gene cloning and characterization of the very large NAD-dependent l-glutamate dehydrogenase from the psychrophile *Janthinobacterium lividum*, isolated from cold soil. *J Bacteriol*. 189:5626-5633.
- [25] Lu CD, Abdelal AT (2001) The *gdhB* gene of *Pseudomonas aeruginosa* encodes an arginine-inducible NAD(+)-dependent glutamate dehydrogenase which is subject to allosteric regulation. *J Bacteriol*. 183:490-499.
- [26] O'Hare HM, Duran R, Cervenansky C, Bellinzoni M, Wehenkel AM, Pritsch O, *et al.* (2008) Regulation of glutamate metabolism by protein kinases in mycobacteria. *Mol Microbiol*. 70:1408-1423.
- [27] Camardella L, Di Fraia R, Antignani A, Ciardiello MA, di Prisco G, Coleman JK, *et al.* (2002) The Antarctic Psychrobacter sp. TAD1 has two cold-active glutamate dehydrogenases with different cofactor specificities. Characterisation of the NAD+-dependent enzyme. *Comp Biochem Physiol A Mol Integr Physiol*. 131:559-567.
- [28] Prunkard DE, Bascomb NF, Molin WT, Schmidt RR (1986) Effect of Different Carbon Sources on the Ammonium Induction of Different Forms of NADP-Specific Glutamate Dehydrogenase in *Chlorella sorokiniana* Cells Cultured in the Light and Dark. *Plant Physiol*. 81:413-422.
- [29] Tomita T, Miyazaki T, Miyazaki J, Kuzuyama T, Nishiyama M (2010) Hetero-oligomeric glutamate dehydrogenase from *Thermus thermophilus*. *Microbiology*. 156:3801-3813.
- [30] Lam HM, Coschigano KT, Oliveira IC, Melo-Oliveira R, Coruzzi GM (1996) The Molecular-Genetics of Nitrogen Assimilation into Amino Acids in Higher Plants. *Annu Rev Plant Physiol Plant Mol Biol*. 47:569-593.
- [31] Aalen N, Steen IH, Birkeland NK, Lien T (1997) Purification and properties of an extremely thermostable NADP+-specific glutamate dehydrogenase from *Archaeoglobus fulgidus*. *Arch Microbiol*. 168:536-539.
- [32] Bonete MJ, Camacho, M. L., Cadenas, E. (1986) Purification and some properties of NAD+-dependent glutamate dehydrogenase from *Halobacterium halobium*. *Int. J. Biochem*. 18:785-789.

- [33] Bonete MJ, Camacho, M. L., Cadenas, E. (1987) A new glutamate dehydrogenase from *Halobacterium halobium* with different coenzyme specificity. *Int. J. Biochem.* 19:1149-1155.
- [34] Hudson RC, Ruttersmith LD, Daniel RM (1993) Glutamate dehydrogenase from the extremely thermophilic archaeobacterial isolate AN1. *Biochim Biophys Acta.* 1202:244-250.
- [35] Grantham WC, Brown AT (1983) Ammonia utilization by a proposed bacterial pathogen in human periodontal disease, *Capnocytophaga ochracea*. *Arch Oral Biol.* 28:327-338.
- [36] Sakamoto N, Kotre AM, Savageau MA (1975) Glutamate dehydrogenase from *Escherichia coli*: purification and properties. *J Bacteriol.* 124:775-783.
- [37] Di Fraia R, Wilquet V, Ciardiello MA, Carratore V, Antignani A, Camardella L, *et al.* (2000) NADP+-dependent glutamate dehydrogenase in the Antarctic psychrotolerant bacterium *Psychrobacter* sp. TAD1. Characterization, protein and DNA sequence, and relationship to other glutamate dehydrogenases. *Eur J Biochem.* 267:121-131.
- [38] Janssen DB, op den Camp HJ, Leenen PJ, van der Drift C (1980) The enzymes of the ammonia assimilation in *Pseudomonas aeruginosa*. *Arch Microbiol.* 124:197-203.
- [39] Brown CM, Macdonald-Brown DS, Stanley SO (1973) The mechanisms of nitrogen assimilation in pseudomonads. *Antonie Van Leeuwenhoek.* 39:89-98.
- [40] Coulton JW, Kapoor M (1973) Studies on the kinetics and regulation of glutamate dehydrogenase of *Salmonella typhimurium*. *Can J Microbiol.* 19:439-450.
- [41] Ruiz JL, Ferrer, J., Camacho, M., Bonete, M. J. (1998) NAD-specific glutamate dehydrogenase from *Thermus thermophilus* HB8: purification and enzymatic properties. *FEMS Microbiology Letters.* 159:15-20.
- [42] LeJohn HB, Suzuki, I. and J. A. Wrights (1968) Glutamate Dehydrogenases of *Thiobacillus novellus*, kinetic properties and a possible control mechanism. *J Biol Chem.* 243:118-128.
- [43] Kanamori K, Weiss RL, Roberts JD (1987) Role of glutamate dehydrogenase in ammonia assimilation in nitrogen-fixing *Bacillus macerans*. *J Bacteriol.* 169:4692-4695.
- [44] Kanamori K, Weiss RL, Roberts JD (1987) Ammonia assimilation in *Bacillus polymyxa*. 15N NMR and enzymatic studies. *J Biol Chem.* 262:11038-11045.
- [45] Belitsky BR, Sonenshein AL (1998) Role and regulation of *Bacillus subtilis* glutamate dehydrogenase genes. *J Bacteriol.* 180:6298-6305.
- [46] Gunka K, Tholen S, Gerwig J, Herzberg C, Stulke J, Commichau FM (2012) A high-frequency mutation in *Bacillus subtilis*: requirements for the decryptification of the gudB glutamate dehydrogenase gene. *J Bacteriol.* 194:1036-1044.
- [47] Rice DW, Baker PJ, Farrants GW, Hornby DP (1987) The crystal structure of glutamate dehydrogenase from *Clostridium symbiosum* at 0.6 nm resolution. *Biochem J.* 242:789-795.
- [48] Hamza MA, Engel PC (2008) Homotropic allosteric control in clostridial glutamate dehydrogenase: different mechanisms for glutamate and NAD+? *FEBS Lett.* 582:1816-1820.
- [49] Bormann ER, Eikmanns BJ, Sahm H (1992) Molecular analysis of the *Corynebacterium glutamicum* gdh gene encoding glutamate dehydrogenase. *Mol Microbiol.* 6:317-326.
- [50] Harper CJ, Hayward D, Kidd M, Wiid I, van Helden P (2010) Glutamate dehydrogenase and glutamine synthetase are regulated in response to nitrogen availability in *Mycobacterium smegmatis*. *BMC Microbiol.* 10:138.

- [51] Sarada KV, Rao NA, Venkitasubramanian TA (1980) Isolation and characterisation of glutamate dehydrogenase from *Mycobacterium smegmatis* CDC 46. *Biochim Biophys Acta*. 615:299-308.
- [52] Misono H, Goto, N., Nagasaki, S. (1985) Purification, Crystallization and Properties of NADP⁺ -Specific Glutamate Dehydrogenase from *Lactobacillus fermentum*. *Agric. Biol Chem*. 49:117-123.
- [53] Hornby DP, Engel PC (1984) Characterization of *Peptostreptococcus asaccharolyticus* glutamate dehydrogenase purified by dye-ligand chromatography. *J Gen Microbiol*. 130:2385-2394.
- [54] Miñambres B, Olivera ER, Jensen RA, Luengo JM (2000) A new class of glutamate dehydrogenases (GDH). Biochemical and genetic characterization of the first member, the AMP-requiring NAD-specific GDH of *Streptomyces clavuligerus*. *J Biol Chem*. 275:39529-39542.
- [55] Vancurova I, Vancura A, Volc J, Kopecky J, Neuzil J, Basarova G, *et al.* (1989) Purification and properties of NADP-dependent glutamate dehydrogenase from *Streptomyces fradiae*. *J Gen Microbiol*. 135:3311-3318.
- [56] Andersson JO, Roger AJ (2003) Evolution of glutamate dehydrogenase genes: evidence for lateral gene transfer within and between prokaryotes and eukaryotes. *BMC Evol Biol*. 3:14.
- [57] Benachenhou-Lahfa N, Forterre P, Labedan B (1993) Evolution of glutamate dehydrogenase genes: evidence for two paralogous protein families and unusual branching patterns of the archaebacteria in the universal tree of life. *J Mol Evol*. 36:335-346.
- [58] Lilley KS, Baker PJ, Britton KL, Stillman TJ, Brown PE, Moir AJ, *et al.* (1991) The partial amino acid sequence of the NAD(+)-dependent glutamate dehydrogenase of *Clostridium symbiosum*: implications for the evolution and structural basis of coenzyme specificity. *Biochim Biophys Acta*. 1080:191-197.
- [59] Baker PJ, Britton KL, Engel PC, Farrants GW, Lilley KS, Rice DW, *et al.* (1992) Subunit assembly and active site location in the structure of glutamate dehydrogenase. *Proteins*. 12:75-86.
- [60] Smith TJ, Stanley CA (2008) Untangling the glutamate dehydrogenase allosteric nightmare. *Trends Biochem Sci*. 33:557-564.
- [61] Peterson PE, Smith TJ (1999) The structure of bovine glutamate dehydrogenase provides insights into the mechanism of allostery. *Structure*. 7:769-782.
- [62] Smith TJ, Peterson PE, Schmidt T, Fang J, Stanley CA (2001) Structures of bovine glutamate dehydrogenase complexes elucidate the mechanism of purine regulation. *J Mol Biol*. 307:707-720.
- [63] Smith TJ, Schmidt T, Fang J, Wu J, Siuzdak G, Stanley CA (2002) The structure of apo human glutamate dehydrogenase details subunit communication and allostery. *J Mol Biol*. 318:765-777.
- [64] Bitensky MW, Yielding KL, Tomkins GM (1965) The Effect of Allosteric Modifiers on the Rate of Denaturation of Glutamate Dehydrogenase. *J Biol Chem*. 240:1077-1082.
- [65] Yip KS, Stillman TJ, Britton KL, Artymiuk PJ, Baker PJ, Sedelnikova SE, *et al.* (1995) The structure of *Pyrococcus furiosus* glutamate dehydrogenase reveals a key role for ion-pair networks in maintaining enzyme stability at extreme temperatures. *Structure*. 3:1147-1158.

- [66] Stillman TJ, Baker PJ, Britton KL, Rice DW (1993) Conformational flexibility in glutamate dehydrogenase. Role of water in substrate recognition and catalysis. *J Mol Biol.* 234:1131-1139.
- [67] Noor S, Puneekar NS (2005) Allosteric NADP-glutamate dehydrogenase from aspergilli: purification, characterization and implications for metabolic regulation at the carbon-nitrogen interface. *Microbiology.* 151:1409-1419.
- [68] Joseph AA, Wixon RL (1970) Ammonia incorporation in *Hydrogenomonas eutropha*. *Biochim Biophys Acta.* 201:295-299.
- [69] Sanwal BD, Lata M (1961) The occurrence of two different glutamic dehydrogenases in *Neurospora*. *Can J Microbiol.* 7:319-328.
- [70] Lawit SJ, Miller PW, Dunn WI, Mirabile JS, Schmidt RR (2003) Heterologous expression of cDNAs encoding *Chlorella sorokiniana* NADP-specific glutamate dehydrogenase wild-type and mutant subunits in *Escherichia coli* cells and comparison of kinetic and thermal stability properties of their homohexamers. *Plant Mol Biol.* 52:605-616.
- [71] LeJohn HB, McCrea BE (1968) Evidence for two species of glutamate dehydrogenases in *Thiobacillus novellus*. *J Bacteriol.* 95:87-94.
- [72] Bonete MJ, Perez-Pomares F, Ferrer J, Camacho ML (1996) NAD-glutamate dehydrogenase from *Halobacterium halobium*: inhibition and activation by TCA intermediates and amino acids. *Biochim Biophys Acta.* 1289:14-24.
- [73] Bender RA (2010) A NAC for regulating metabolism: the nitrogen assimilation control protein (NAC) from *Klebsiella pneumoniae*. *J Bacteriol.* 192:4801-4811.
- [74] Leigh JA, Dodsworth JA (2007) Nitrogen Regulation in Bacteria and Archaea. *Annu Rev Microbiol.*
- [75] Zimmer DP, Soupene E, Lee HL, Wendisch VF, Khodursky AB, Peter BJ, *et al.* (2000) Nitrogen regulatory protein C-controlled genes of *Escherichia coli*: scavenging as a defense against nitrogen limitation. *Proc Natl Acad Sci U S A.* 97:14674-14679.
- [76] Feng J, Goss TJ, Bender RA, Ninfa AJ (1995) Activation of transcription initiation from the nac promoter of *Klebsiella aerogenes*. *J Bacteriol.* 177:5523-5534.
- [77] Feng J, Goss TJ, Bender RA, Ninfa AJ (1995) Repression of the *Klebsiella aerogenes* nac promoter. *J Bacteriol.* 177:5535-5538.
- [78] Frisch RL, Bender RA (2010) An Expanded Role for the Nitrogen Assimilation Control protein (NAC) in the Response of *Klebsiella pneumoniae* to Nitrogen Stress. *J Bacteriol.* Published online ahead of print.
- [79] Goss TJ, Bender RA (1995) The nitrogen assimilation control protein, NAC, is a DNA binding transcription activator in *Klebsiella aerogenes*. *J Bacteriol.* 177:3546-3555.
- [80] Macaluso A, Best EA, Bender RA (1990) Role of the nac gene product in the nitrogen regulation of some NTR-regulated operons of *Klebsiella aerogenes*. *J Bacteriol.* 172:7249-7255.
- [81] Bender RA, Snyder PM, Bueno R, Quinto M, Magasanik B (1983) Nitrogen regulation system of *Klebsiella aerogenes*: the nac gene. *J Bacteriol.* 156:444-446.
- [82] Goss TJ, Janes BK, Bender RA (2002) Repression of glutamate dehydrogenase formation in *Klebsiella aerogenes* requires two binding sites for the nitrogen assimilation control protein, NAC. *J Bacteriol.* 184:6966-6975.
- [83] Bender RA (1991) The role of the NAC protein in the nitrogen regulation of *Klebsiella aerogenes*. *Mol Microbiol.* 5:2575-2580.

- [84] Rosario CJ, Bender RA (2005) Importance of tetramer formation by the nitrogen assimilation control protein for strong repression of glutamate dehydrogenase formation in *Klebsiella pneumoniae*. J Bacteriol. 187:8291-8299.
- [85] Goss TJ (2008) The ArgP protein stimulates the *Klebsiella pneumoniae* *gdhA* promoter in a lysine-sensitive manner. J Bacteriol. 190:4351-4359.
- [86] Hervás AB, Canosa I, Santero E (2010) Regulation of glutamate dehydrogenase expression in *Pseudomonas putida* results from its direct repression by NtrC under nitrogen-limiting conditions. Mol Microbiol. 78:305-319.
- [87] García-González V, Jiménez-Fernández A, Hervás AB, Canosa I, Santero E, Govantes F (2009) Distinct roles for NtrC and GlnK in nitrogen regulation of the *Pseudomonas* sp. strain ADP cyanuric acid utilization operon. FEMS Microbiol Lett. 300:222-229.
- [88] Hervás AB, Canosa I, Little R, Dixon R, Santero E (2009) NtrC-dependent regulatory network for nitrogen assimilation in *Pseudomonas putida*. J Bacteriol. 191:6123-6135.
- [89] Hashim S, Kwon DH, Abdelal A, Lu CD (2004) The arginine regulatory protein mediates repression by arginine of the operons encoding glutamate synthase and anabolic glutamate dehydrogenase in *Pseudomonas aeruginosa*. J Bacteriol. 186:3848-3854.
- [90] Li W, Lu CD (2007) Regulation of carbon and nitrogen utilization by CbrAB and NtrBC two-component systems in *Pseudomonas aeruginosa*. J Bacteriol. 189:5413-5420.
- [91] Debarbouille M, Martin-Verstraete I, Kunst F, Rapoport G (1991) The *Bacillus subtilis* sigL gene encodes an equivalent of sigma 54 from gram-negative bacteria. Proc Natl Acad Sci U S A. 88:9092-9096.
- [92] Belitsky BR, Kim HJ, Sonenshein AL (2004) CcpA-dependent regulation of *Bacillus subtilis* glutamate dehydrogenase gene expression. J Bacteriol. 186:3392-3398.
- [93] Belitsky BR, Sonenshein AL (1999) An enhancer element located downstream of the major glutamate dehydrogenase gene of *Bacillus subtilis*. Proc Natl Acad Sci U S A. 96:10290-10295.
- [94] Gardan R, Rapoport G, Debarbouille M (1997) Role of the transcriptional activator RocR in the arginine-degradation pathway of *Bacillus subtilis*. Mol Microbiol. 24:825-837.
- [95] Commichau FM, Herzberg C, Tripal P, Valerius O, Stulke J (2007) A regulatory protein-protein interaction governs glutamate biosynthesis in *Bacillus subtilis*: the glutamate dehydrogenase RocG moonlights in controlling the transcription factor GltC. Mol Microbiol. 65:642-654.
- [96] Gunka K, Newman JA, Commichau FM, Herzberg C, Rodrigues C, Hewitt L, et al. (2010) Functional dissection of a trigger enzyme: mutations of the *Bacillus subtilis* glutamate dehydrogenase RocG that affect differentially its catalytic activity and regulatory properties. J Mol Biol. 400:815-827.
- [97] Picossi S, Belitsky BR, Sonenshein AL (2007) Molecular mechanism of the regulation of *Bacillus subtilis* *gltAB* expression by GltC. J Mol Biol. 365:1298-1313.
- [98] Tiffert Y, Supra P, Wurm R, Wohlleben W, Wagner R, Reuther J (2008) The *Streptomyces coelicolor* GlnR regulon: identification of new GlnR targets and evidence for a central role of GlnR in nitrogen metabolism in actinomycetes. Mol Microbiol. 67:861-880.
- [99] Wray LV, Jr., Fisher SH (1993) The *Streptomyces coelicolor* *glnR* gene encodes a protein similar to other bacterial response regulators. Gene. 130:145-150.
- [100] Rodríguez-García A, Sola-Landa A, Apel K, Santos-Beneit F, Martín JF (2009) Phosphate control over nitrogen metabolism in *Streptomyces coelicolor*: direct and

- indirect negative control of *glnR*, *glnA*, *glnII* and *amtB* expression by the response regulator PhoP. *Nucleic Acids Res.* 37:3230-3242.
- [101] Nolden L, Ngouoto-Nkili CE, Bendt AK, Kramer R, Burkovski A (2001) Sensing nitrogen limitation in *Corynebacterium glutamicum*: the role of *glnK* and *glnD*. *Mol Microbiol.* 42:1281-1295.
- [102] Hanssler E, Muller T, Palumbo K, Patek M, Brocker M, Kramer R, *et al.* (2009) A game with many players: control of *gdh* transcription in *Corynebacterium glutamicum*. *J Biotechnol.* 142:114-122.
- [103] Ikeda M, Nakagawa S (2003) The *Corynebacterium glutamicum* genome: features and impacts on biotechnological processes. *Appl Microbiol Biotechnol.* 62:99-109.
- [104] Kalinowski J, Bathe B, Bartels D, Bischoff N, Bott M, Burkovski A, *et al.* (2003) The complete *Corynebacterium glutamicum* ATCC 13032 genome sequence and its impact on the production of L-aspartate-derived amino acids and vitamins. *J Biotechnol.* 104:5-25.
- [105] Yukawa H, Omumasaba CA, Nonaka H, Kos P, Okai N, Suzuki N, *et al.* (2007) Comparative analysis of the *Corynebacterium glutamicum* group and complete genome sequence of strain R. *Microbiology.* 153:1042-1058.
- [106] Shiio I, Otsuka SI, Takahashi M (1962) Effect of biotin on the bacterial formation of glutamic acid. I. Glutamate formation and cellular permeability of amino acids. *J Biochem.* 51:56-62.
- [107] Duperray F, Jezequel D, Ghazi A, Letellier L, Shechter E (1992) Excretion of glutamate from *Corynebacterium glutamicum* triggered by amine surfactants. *Biochim Biophys Acta.* 1103:250-258.
- [108] Nunheimer TD, Birnbaum J, Ihnen ED, Demain AL (1970) Product inhibition of the fermentative formation of glutamic acid. *Appl Microbiol.* 20:215-217.
- [109] Kimura E, Abe C, Kawahara Y, Nakamatsu T, Tokuda H (1997) A *dtsR* gene-disrupted mutant of *Brevibacterium lactofermentum* requires fatty acids for growth and efficiently produces L-glutamate in the presence of an excess of biotin. *Biochem Biophys Res Commun.* 234:157-161.
- [110] Delaunay S, Gourdon P, Lapujade P, Mailly E, Oriol E, Engasser JM, *et al.* (1999) An improved temperature triggered process for glutamate production with *Corynebacterium glutamicum*. *Enzyme Microb Technol.* 25:762-768.
- [111] Radmacher E, Stansen KC, Besra GS, Alderwick LJ, Maughan WN, Hollweg G, *et al.* (2005) Ethambutol, a cell wall inhibitor of *Mycobacterium tuberculosis*, elicits L-glutamate efflux of *Corynebacterium glutamicum*. *Microbiology.* 151:1359-1368.
- [112] Eggeling L, Krumbach K, Sahm H (2001) L-glutamate efflux with *Corynebacterium glutamicum*: why is penicillin treatment or Tween addition doing the same? *J Mol Microbiol Biotechnol.* 3:67-68.
- [113] Nakamura J, Hirano S, Ito H, Wachi M (2007) Mutations of the *Corynebacterium glutamicum* NCgl1221 gene, encoding a mechanosensitive channel homolog, induce L-glutamic acid production. *Appl Environ Microbiol.* 73:4491-4498.
- [114] Sano C (2009) History of glutamate production. *Am J Clin Nutr.* 90:728S-732S.
- [115] Kimura E (2002) Triggering mechanism of L-glutamate overproduction by DtsR1 in coryneform bacteria. *J Biosci Bioeng.* 94:545-551.
- [116] Belisle JT, Vissa VD, Sievert T, Takayama K, Brennan PJ, Besra GS (1997) Role of the major antigen of *Mycobacterium tuberculosis* in cell wall biogenesis. *Science.* 276:1420-1422.

- [117] Hashimoto K, Nakamura K, Kuroda T, Yabe I, Nakamatsu T, Kawasaki H (2010) The protein encoded by NCgl1221 in *Corynebacterium glutamicum* functions as a mechanosensitive channel. *Biosci Biotechnol Biochem.* 74:2546-2549.
- [118] Tesch M, de Graaf AA, Sahm H (1999) In vivo fluxes in the ammonium-assimilatory pathways in *Corynebacterium glutamicum* studied by ¹⁵N nuclear magnetic resonance. *Appl Environ Microbiol.* 65:1099-1109.
- [119] Tesch M, Eikmanns BJ, de Graaf AA, Sahm H (1998) Ammonia assimilation in *Corynebacterium glutamicum* and a glutamate dehydrogenase-deficient mutant. *Biotechnol. Lett.* 20:953-957.
- [120] Beckers G, Nolden L, Burkovski A (2001) Glutamate synthase of *Corynebacterium glutamicum* is not essential for glutamate synthesis and is regulated by the nitrogen status. *Microbiology.* 147:2961-2970.
- [121] Sahm H, Eggeling L, de Graaf AA (2000) Pathway analysis and metabolic engineering in *Corynebacterium glutamicum*. *Biol Chem.* 381:899-910.
- [122] Shimizu H, Tanaka H, Nakato A, Nagahisa K, Kimura E, Shioya S (2003) Effects of the changes in enzyme activities on metabolic flux redistribution around the 2-oxoglutarate branch in glutamate production by *Corynebacterium glutamicum*. *Bioprocess Biosyst Eng.* 25:291-298.
- [123] Shirai T, Fujimura K, Furusawa C, Nagahisa K, Shioya S, Shimizu H (2007) Study on roles of anaplerotic pathways in glutamate overproduction of *Corynebacterium glutamicum* by metabolic flux analysis. *Microb Cell Fact.* 6:19.
- [124] Shirai T, Nakato A, Izutani N, Nagahisa K, Shioya S, Kimura E, *et al.* (2005) Comparative study of flux redistribution of metabolic pathway in glutamate production by two coryneform bacteria. *Metab Eng.* 7:59-69.
- [125] Asakura Y, Kimura E, Usuda Y, Kawahara Y, Matsui K, Osumi T, *et al.* (2007) Altered metabolic flux due to deletion of *odhA* causes L-glutamate overproduction in *Corynebacterium glutamicum*. *Appl Environ Microbiol.* 73:1308-1319.
- [126] Sato H, Orishimo K, Shirai T, Hirasawa T, Nagahisa K, Shimizu H, *et al.* (2008) Distinct roles of two anaplerotic pathways in glutamate production induced by biotin limitation in *Corynebacterium glutamicum*. *J Biosci Bioeng.* 106:51-58.
- [127] Bott M (2007) Offering surprises: TCA cycle regulation in *Corynebacterium glutamicum*. *Trends Microbiol.* 15:417-425.
- [128] Schultz C, Niebisch A, Gebel L, Bott M (2007) Glutamate production by *Corynebacterium glutamicum*: dependence on the oxoglutarate dehydrogenase inhibitor protein OdhI and protein kinase PknG. *Appl Microbiol Biotechnol.* 76:691-700.
- [129] Niebisch A, Kabus A, Schultz C, Weil B, Bott M (2006) Corynebacterial protein kinase G controls 2-oxoglutarate dehydrogenase activity via the phosphorylation status of the OdhI protein. *J Biol Chem.* 281:12300-12307.
- [130] Csonka LN, Epstein W. Osmoregulation. In: Neidhardt C, Curtiss R, Ingraham JL, Lin ECC, Low KB, Magasanik B, *et al.*, editors. *Escherichia coli* and *Salmonella*: cellular and molecular biology,. 2nd ed. Washington, DC.: American Society for Microbiology Press,; 1996. p. 1210-1217.
- [131] Swearingen PA, O'Sullivan DJ, Warthesen JJ (2001) Isolation, characterization, and influence of native, nonstarter lactic acid bacteria on Cheddar cheese quality. *J Dairy Sci.* 84:50-59.

- [132] Crow V, Curry B, Hayes M (2001) The ecology of non-starter lactic acid bacteria (NSLAB) and their use as adjuncts in New Zealand Cheddar. *International Dairy Journal*. 11:275-283.
- [133] Yvon M, Rijnen L (2001) Cheese flavour formation by amino acid catabolism. *Int. Dairy J*. 11:185-201.
- [134] Curtin A, McSweeney P. Catabolism of Amino Acids in Cheese during Ripening. In: Patrick F. Fox PLHM, Timothy M. Cogan and Timothy P. Guinee, editor. *Cheese: Chemistry, Physics and Microbiology*. 3rd ed: Elsevier; 2004. p. 435-454.
- [135] Kieronczyk A, Skeie S, Langsruda T, Le Bars D, Yvon M (2004) The nature of aroma compounds produced in a cheese model by glutamate dehydrogenase positive *Lactobacillus* INF15D depends on its relative aminotransferase activities towards the different amino acids. *International Dairy J*. 14:227-235.
- [136] Banks JM, Yvon M, Gripon JC, de la Fuente MA, Brechany EY, Williams AG, *et al.* (2001) Enhancement of amino acid catabolism in Cheddar cheese using α -ketoglutarate: amino acid degradation in relation to volatile compounds and aroma character. *Int. Dairy J*. 11:215-243.
- [137] Shakeel-Ur-Rehman, Fox RE (2002) Effect of added α -ketoglutaric acid, pyruvic acid or pyridoxal phosphate on proteolysis and quality of Cheddar cheese. *Food Chem*. 76:21-26.
- [138] Tanous C, Chambellon E, Yvon M (2007) Sequence analysis of the mobilizable lactococcal plasmid pGdh442 encoding glutamate dehydrogenase activity. *Microbiology*. 153:1664-1675.
- [139] Morishita T, Yajima M (1995) Incomplete operation of biosynthetic and bioenergetic functions of the citric acid cycle in multiple auxotrophic lactobacilli. *Bios. Biotech. Biochem*. 59:251-255.
- [140] Rijnen L, Courtin P, Gripon JC, Yvon M (2000) Expression of a heterologous glutamate dehydrogenase gene in *Lactococcus lactis* highly improves the conversion of amino acids to aroma compounds. *Appl Environ Microbiol*. 66:1354-1359.
- [141] Tanous C, Chambellon E, Le Bars D, Delespaul G, Yvon M (2006) Glutamate dehydrogenase activity can be transmitted naturally to *Lactococcus lactis* strains to stimulate amino acid conversion to aroma compounds. *Appl Environ Microbiol*. 72:1402-1409.
- [142] Tanous C, Kieronczyk A, Helinck S, Chambellon E, Yvon M (2002) Glutamate dehydrogenase activity: a major criterion for the selection of flavour-producing lactic acid bacteria strains. *Antonie Van Leeuwenhoek*. 82:271-278.
- [143] Helinck S, Le Bars D, Moreau D, Yvon M (2004) Ability of thermophilic lactic acid bacteria to produce aroma compounds from amino acids. *Appl Environ Microbiol*. 70:3855-3861.