Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

REVIEW ARTICLE

# Role of glutamate metabolism in bacterial responses towards acid and other stresses

C. Feehily and K.A.G. Karatzas

Bacterial Stress Response Group, Department of Microbiology, School of Natural Sciences, National University of Ireland, Galway, Ireland

**Keywords**
acid resistance, glutamate decarboxylase, glutamate metabolism, *Listeria monocytogenes*.

**Correspondence**
Kimon-Andreas G. Karatzas, School of Food Biosciences, University of Reading, Reading RG6 6AD, UK. E-mail: k.karatzas@reading.ac.uk

2012/0437: received 8 March 2012, revised 15 August 2012 and accepted 16 August 2012

doi:10.1111/j.1365-2672.2012.05434.x

## Summary

Glutamate plays a central role in a wide range of metabolic processes in bacterial cells. This review focuses on the involvement of glutamate in bacterial stress responses. In particular, it reviews the role of glutamate metabolism in response against acid stress and other stresses. The glutamate decarboxylase (GAD) system has been implicated in acid tolerance in several bacterial genera. This system facilitates intracellular pH homoeostasis by consuming protons in a decarboxylation reaction that produces γ-aminobutyrate (GABA) from glutamate. An antiporter system is usually present to couple the uptake of glutamate to the efflux of GABA. Recent insights into the functioning of this system will be discussed. Finally, the intracellular fate of GABA will also be discussed. Many bacteria are capable of metabolizing GABA to succinate via the GABA shunt pathway. The role and regulation of this pathway will be addressed in the review.

## Introduction

Glutamate is an important molecule for all living organisms, which plays a role in various metabolic processes. It is a nonessential amino acid involved in protein synthesis and other fundamental processes such as glycolysis, gluconeogenesis and the citric acid cycle (Berg *et al.* 2007). It is also a key metabolite because it serves to link nitrogen and carbon metabolism (Berg *et al.* 2007). Catabolism of glutamate occurs mainly by the action of either glutamate dehydrogenase or glutamate decarboxylase (GAD) (Berg *et al.* 2007). The first enzyme, among other roles, is important for the assimilation of ammonia to amino acids, while the second is important for resistance mainly against acid but also other stresses (Berg *et al.* 2007). As glutamate metabolism in bacterial stress responses is at the core of this review, it will focus in the decarboxylation of glutamate to γ-aminobutyrate (GABA) and the subsequent catabolism of GABA through the GABA shunt.

The GAD is an enzyme that catalyses the decarboxylation of glutamate to GABA (O'Byrne and Karatzas 2008). The decarboxylation of glutamate is the first step of the GABA shunt pathway that leads to the production of succinate by the sequential actions of GABA-aminotransferase (GABA-AT) and succinic semialdehyde dehydrogenase (SSDH; Zhu *et al.* 2010). The GAD enzyme is present in a wide variety of organisms from all kingdoms of life. In mammals, glutamate is the main excitatory and GABA the main inhibitory neurotransmitter (Petroff 2002). The conversion of the one to the other by GAD plays an important role in various brain functions.

However, in plants and micro-organisms, the GAD system appears to play other roles (Maras *et al.* 1992). In plants, the GAD system is expressed in response to a variety of stress conditions such as temperature shock, hypoxia or increasing levels of $Ca^{2+}$ (Shelp *et al.* 1999). In micro-organisms, the system seems to be related mainly with resistance against acidic conditions, although a role involving other stresses has been seen also. The GAD system has been described as the most important mechanism of acid resistance in the highly acid tolerant bacterium *Escherichia coli* (Capitani *et al.* 2003; Foster 2004). Acid resistance would allow various foodborne pathogens or spoilage bacteria to grow on acidic foods. Furthermore, this property is also a virulence or probiotic factor, as it allows pathogens or probiotic organisms, respectively, to pass through the extremely acidic

conditions of the stomach barrier. However, a balance between acid resistance and other functions is essential as increased acid and stress resistance have been shown to impair colonization or virulence (Karatzas *et al.* 2005, 2007, 2008). The decarboxylation of glutamate consumes a proton, and therefore, micro-organisms take advantage of this property to remove protons from the intracellular milieu under acidic conditions (O'Byrne and Karatzas 2008). It operates in a cycle that starts with the import of an extracellular glutamate molecule ($Glt_e$) in exchange for an intracellular GABA ($GABA_i$) through the glutamate/GABA antiporter (O'Byrne and Karatzas 2008). These antiporters are usually a part of the GAD system in most micro-organisms (Table 1). Subsequently, the imported glutamate is decarboxylated by the GAD, with the incorporation of a proton in the α-carbon of this molecule in the place of the carboxyl group to form GABA (Karatzas *et al.* 2012; Fig. 1). The bond is highly stable and it cannot be subjected to ionization, preventing the release of the proton in the intracellular milieu. Subsequently, the GABA molecule that carries the removed proton is either exported by the antiporter as extracellular GABA ($GABA_e$) or remains inside the cell ($GABA_i$) as has been shown previously (Karatzas *et al.* 2010). The current model for the removal of protons by the GAD system has been debated, however, and other hypotheses have been put forward, which are analysed here. In bacteria, the GAD system is usually accompanied with one or two glutamate/GABA antiporters (Table 1). The antiporter increases the availability of glutamate to the GAD enzymes increasing the capacity of the system to remove protons and, therefore, the efficiency of the system.

The by-product of the GAD system is GABA, which is the substrate of the GABA shunt pathway. The GABA shunt pathway was first described during the study of guinea pig brain cells in 1970 (Balazs *et al.* 1970). This pathway involves the catabolism of GABA derived from the GAD system to succinate via a two step enzymatic process (Fig. 2). In the first step, a GABA-AT catalyses the reversible conversion of GABA to succinic semialdehyde (SSA), where the amino group of GABA is donated to an α-ketoglutarate molecule with the subsequent production of glutamate (Zhu *et al.* 2010). The second step is catalysed by an SSA dehydrogenase and converts the SSA to succinate (Zhu *et al.* 2010). Despite over forty years of study, the role of the GABA shunt pathway in bacteria has not been fully elucidated, although involvement in glutamate metabolism, anaplerosis and oxidative stress response has been suggested (de Carvalho *et al.* 2011). The pathway has been shown to play an important role in mammalian cells, including a mechanism for the recovery of four of the five carbons lost from the TCA cycle, although there is a discrepancy in the estimation of metabolic activity of this cycle. Cells lose approximately 8% of the energy they would normally generate by passage through the alternative TCA cycle reactions (Waagepetersen *et al.* 1999). The GABA shunt pathway may also be a way to generate energy from glutamate without the production of ammonia as the enzymes of the pathway are pyridoxal phosphate dependent (Waagepetersen *et al.* 1999).

## The glutamate decarboxylase system

The GAD system is present in various bacterial species (Table 1), and it has been shown to play a role in their acid resistance (Cotter *et al.* 2001; Capitani *et al.* 2003; Su *et al.* 2011). With the recent developments in

**Table 1** The variability in the architecture of the bacterial glutamate decarboxylase (GAD) system

| Species | Decarboxylases | Antiporters | Reference |
|---|---|---|---|
| *Escherichia coli* | GadA, GadB | GadC | Smith *et al.* (1992) |
| *Shigella flexneri* | GadA, GadB | GadC | Waterman and Small (2003a) |
| *Mycobacterium tuberculosis* | GadA | | Cole *et al.* (1998), Cotter *et al.* (2001) |
| *Listeria monocytogenes* | GadD1, GadD2, GadD3 | GadT1, GadT2 | Cotter *et al.* (2005), Karatzas *et al.* (2012) |
| *L. monocytogenes* ser. 4 and various strains | GadD2, GadD3 | GadT2 | Cotter *et al.* (2005), Karatzas *et al.* (2012) |
| *Lactobacillus reuteri* | GadB | GadC1, GadC2 | Su *et al.* (2011) |
| *Lactococcus lactis* | GadB | GadC | Su *et al.* (2011) |
| *Lactobacillus plantarum* | GadB | GadC | Su *et al.* (2011) |
| *Clostridium perfringens* | | GadC | |
| *Methanocaldococcus jannaschii** | GadB | N/A | Kezmarsky *et al.* (2005) |
| *Brucella abortus** | GadB | GadC | Roop *et al.* (2003) |
| Bifidobacterium *dentium* | GadB | GadC | Ventura *et al.* (2009) |
| *Bifidobacteria* (other species than *Bif. dentium*) | Not present | Not present | Ventura *et al.* (2009) |

N/A represents not available information regarding the presence of the gene in the organism.
*Presence of GAD based on bioinformatic evidence.

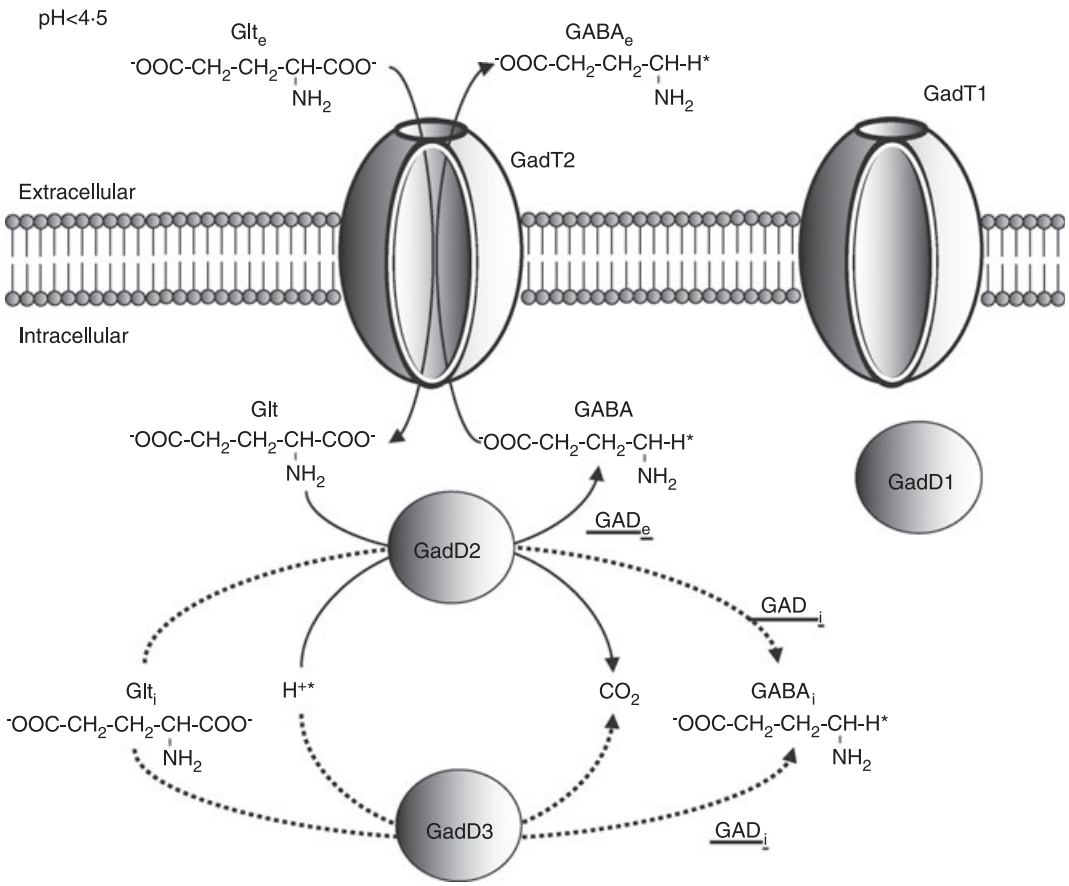


**Figure 1** Schematic representation of the function of the extracellular ($GAD_i$) and intracellular ($GAD_i$) GAD system in *Listeria monocytogenes* under severe acidic conditions (adopted from Karatzas *et al.* 2012). GadD2 and GadD3 convert $Glt_i$ to $GABA_i$ ($GAD_i$). GadD2 also processes $Glt_e$ imported by the GadT2 antiporter ($GAD_e$) which is transformed to GABA, which is then exported by GadT2. Under these conditions, GadD1 and GadT1 do not play a role in acid resistance (Cotter *et al.* 2005; Karatzas *et al.* 2012). GABA, γ-aminobutyrate; GAD, glutamate decarboxylase.

genomics, and the increasing availability of genomes of various organisms, it became clear that the GAD system seems to be present even in archaea like *Methanocaldococcus jannaschii* (Kezmarsky *et al.* 2005). Despite this wealth of information, it has not been possible to associate the presence of the GAD system with a specific kind of micro-organisms or an environmental niche. It has been suggested that the GAD system is important for survival in the low pH of the stomach, although *Salmonella* sp. (Foster 2004) and *Campylobacter* sp. (unpublished data) that are responsible for most of the cases of food-related diseases around the world (WHO 2012) do not possess a GAD system.

Furthermore, the architecture of the GAD system seems to be subject to high variability between species as some possess one, two or even three decarboxylases accompanied with none, one or two antiporters (Table 1). The case of *Mycobacterium tuberculosis* is quite interesting as it seems to possess a GAD that is not accompanied by an antiporter (Cole *et al.* 1998; Cotter *et al.* 2001). In addition, in other cases, there is variability within the same species. For example, *Listeria monocytogenes* normally possesses three decarboxylases (GadD1, D2, D3) and two antiporters (GadT1, T2), but serotype 4 and a few other strains do not have the GadD1 and GadT1 (Cotter *et al.* 2005; Karatzas *et al.* 2012). More interestingly, *Bifidobacterium dentium* possesses a GAD system, in contrast to all other *Bifidobacteria* spp. that do not seem to possess one, according to the genomes of this species that have been deciphered (Ventura *et al.* 2009).

The GAD enzyme, the heart of the GAD system, is a pyridoxal 5′-phosphate (PLP)-dependent enzyme with an acidic optimum pH (3·8–4·6) which forms a hexamer as demonstrated by its crystal structure (Capitani *et al.* 2003). At neutral pH, the enzyme (GadB) is localized exclusively in the cytoplasm, while at acidic conditions, it is recruited to the membrane where it is able to work synergistically with the glutamate/GABA antiporters.

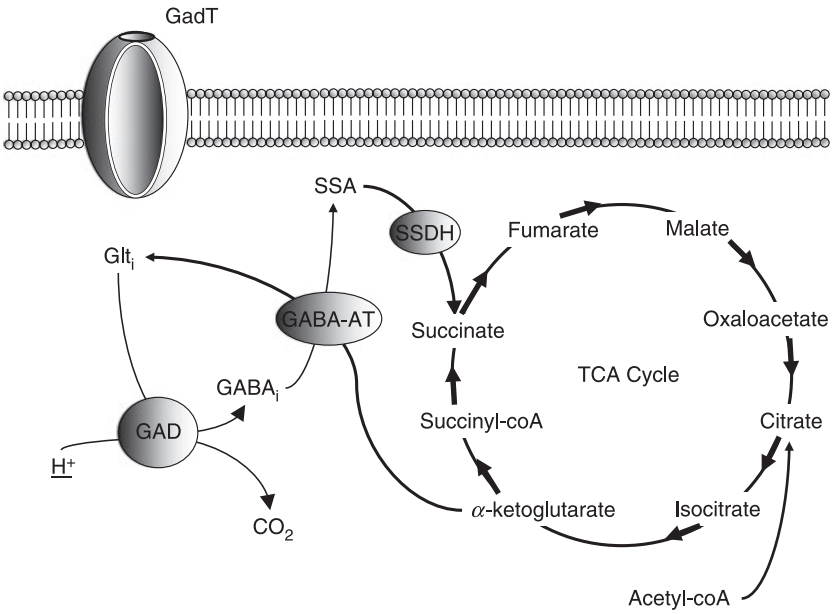


**Figure 2** Depiction of the possible catabolism of intracellular GABA through the GABA shunt. The $Glt_i$ is transformed to $GABA_i$ through glutamate decarboxylase under acidic conditions (left side of the vertical line). Subsequently, GABA transaminase (GABA-AT) could convert GABA to glutamate and in parallel α-ketoglutarate is converted to succinate semialdehyde (SSA). Subsequently, SSA could be transformed to succinate by the effect of succinate semialdehyde dehydrogenase (SSDH). GABA, γ-aminobutyrate.

Residues 13–57 of GadB act as hexamerization arms, while residues 3–15 assume different conformations in the neutral- and low-pH forms, playing a significant role in the acid pH-driven association of GadB to the membrane (Capitani *et al.* 2003). However, there is evidence showing that a GAD from *L. monocytogenes* (probably GadD3) deviates from this model, processing solely pools of $Glt_i$ and being unable to act synergistically with the antiporters (Karatzas *et al.* 2012).

### The function of the glutamate decarboxylase system

In various publications, it has been mentioned that the conversion of glutamate to GABA is beneficial for the cell because GABA is less acidic than glutamate (Tramonti *et al.* 2002; Lei *et al.* 2011) due to the fact that the pI of the former is close to 7·0 (neutral), while that of the latter is approximately 3·1 (acidic). However, how could this affect their buffering capacity? The glutamate and GABA have similar pKa values for the side chain carboxyl group (pKa = 4·0) and the α-amino group (pKa = 10; Foster 2004). The only difference lies in the fact that glutamate possesses an α-carboxyl group that has a pKa of 2·1. This suggests that a possible difference in the ability of these two compounds to remove protons would occur in a $pH_e$ <3 that is lethal for most bacteria. In addition, $pH_i$ values would never reach to such low levels. Furthermore, in contrast to GABA, glutamate incorporates protons at 2 < pH < 3 because of its additional α-carboxyl group suggesting that $Glt_e$ is more beneficial than equimolar levels of $GABA_e$. This is exemplified by a simple experiment where we constructed the titration curves of 100 mmol $l^{-1}$ aqueous solutions of monosodium glutamate and GABA for 10% HCl w/w (Fig. 3). It is clear that up to pH 3·0, both solutions have the same buffering capacity. But below pH 3·0, the glutamate solution has a higher buffering capacity as it requires more than triple the amount of HCl required compared to the GABA solution, to reduce the pH from 3·0 to 2·0 (Fig. 3). This suggests that it is more beneficial for the cells not to convert glutamate to GABA. However, it is evident that this conversion is beneficial because it occurs in a wide range of pH values and it incorporates one proton from the intracellular milieu in the molecule, in the place of the α-carboxyl group (Fig. 1).

### The futile proton hypothesis

Until now, there is little experimental work demonstrating the functionality of the commonly accepted model for GAD system function and the fate of the protons. In contrast, the futile proton hypothesis has been proposed by various investigators in the field (Castanie-Cornet *et al.* 1999; Storz and Hengge 2000; Tucker *et al.* 2002; Waterman and Small 2003b), which proposes that if the pKa of glutamate and GABA are taken in account, the

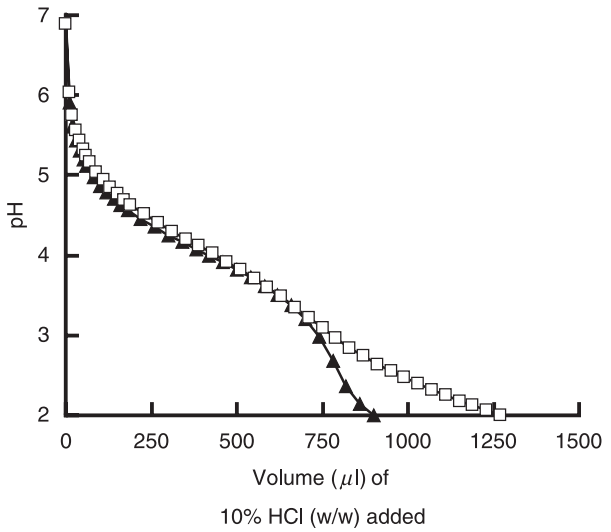


**Figure 3** Titration curves of 100 mmol $l^{-1}$ aqueous solutions of monosodium glutamate (MSG) and γ-aminobutyrate (GABA) for a solution of 10% HCl w/w. (▲) 100 mmol $l^{-1}$ GABA and (□) 100 mmol $l^{-1}$ MSG.

GAD system is unable to remove intracellular protons under severe acid stress and the acid resistance might be through an unknown mechanism. A careful investigation into the pKa of glutamate, GABA and the range of pH values intracellularly and extracellularly leads us in two different possibilities that are demonstrated in Fig. 4. The first scenario (A) refers to a mild pH stress where the extracellular pH is >4·5. In this case, $Glt_e$ is mainly deprotonated and as such is imported in the cell, where the decarboxylation of glutamate removes one intracellular proton ($H^+$*). Subsequently, the GABA carries out this proton, and thus, there is a benefit for the cell. However, in the second scenario (B) where the pH is very low (e.g. 3·5), the chain carboxyl group of glutamate is mainly protonated. This means that the antiporter imports the glutamate in a protonated state, and once intracellularly, this proton ($H^+$) is released in the intracellular milieu that counteracts the benefit from the proton consumed by the decarboxylation process. The latter scenario supports the futile proton hypothesis. However, the GAD system has an obvious increase in acid resistance probably through an unknown mechanism. It has been suggested that this mechanism is a pathway for endogenous glutamate generation or regeneration coupled with the GAD system (Castanie-Cornet *et al.* 1999; Tucker *et al.* 2002). The intracellularly generated glutamate might not introduce any protons in the intracellular milieu, and therefore, its decarboxylation will remove protons with a net gain for the cell. A possible candidate pathway that could generate intracellular glutamate ($Glt_i$) might involve a glutaminase that converts glutamine to glutamate (Castanie-Cornet *et al.* 1999; Tucker *et al.* 2002; Waterman and Small 2003b). Glutamine's only carboxyl group has a pKa of 2, and it would be deprotonated under normal acidic conditions (pH >2). Therefore, its import would not introduce any protons in the intracellular milieu, and the glutamate decarboxylation would result in a net loss of protons and a benefit for the cell. Another alternative pathway could be the GABA shunt that leads to the regeneration of intracellular glutamate by the action of GABA-AT (Fig. 2).

### The intracellular glutamate decarboxylase system ($GAD_i$)

All of the above suggest the conversion of intracellular glutamate to GABA by the GAD system. Such a process would also remove intracellular protons from the cell under acidic conditions. Some workers have previously demonstrated the presence of $GABA_i$ in *E. coli* (Castanie-Cornet *et al.* 1999), *Shigella flexneri* (Waterman and Small 2003b) and *L. monocytogenes* (Karatzas *et al.* 2010). However, the significance of this finding has been overlooked. Until now, all studies on the GAD system assume that the exported $GABA_e$ represents the total GAD activity. The antiporter works in such a way that, for every molecule of glutamate that is imported, one molecule of GABA is exported. Therefore, the GAD system through the utilization of $Glt_e$ should not be able to affect the levels of $Glt_i$ and $GABA_i$. This suggests that $GABA_i$ derives directly through the decarboxylation of $Glt_i$ under acidic conditions and that the GAD system utilizes $Glt_i$ (Fig. 1). This is supported by data showing that under acidic conditions, mechanisms that increase the levels of $Glt_i$ are upregulated. In *Streptococcus mutans*, it has been demonstrated that the import of glutamate through the GlnQHMP transporter increases acid resistance (Krastel *et al.* 2010), while in *L. monocytogenes*, the most highly acid-induced genes are coding for a putative glutamate synthase (*lmo1734*) and a putative ABC transporter of glutamine (*lmo1738* and *lmo1740*) a glutamate precursor (Satorhelyi 2005). Because the utilization of the $Glt_e$ and $Glt_i$ are two independent processes, we have previously proposed the division of the GAD system in extracellular ($GAD_e$) and intracellular ($GAD_i$; Karatzas *et al.* 2012). At first glance, $GAD_i$ does not seem to be efficient because of the limited availability of the $Glt_i$ substrate compared to the vast levels of $Glt_e$. However, $GAD_i$ plays a significant role in the acid resistance of *L. monocytogenes* removing in an average between strains, a quarter of the protons removed by the overall GAD system (Karatzas *et al.* 2012). Furthermore, EGD-e, the most studied strain of *L. monocytogenes*, appears to have a defective $GAD_e$ unable to export GABA, although it has an active $GAD_i$,

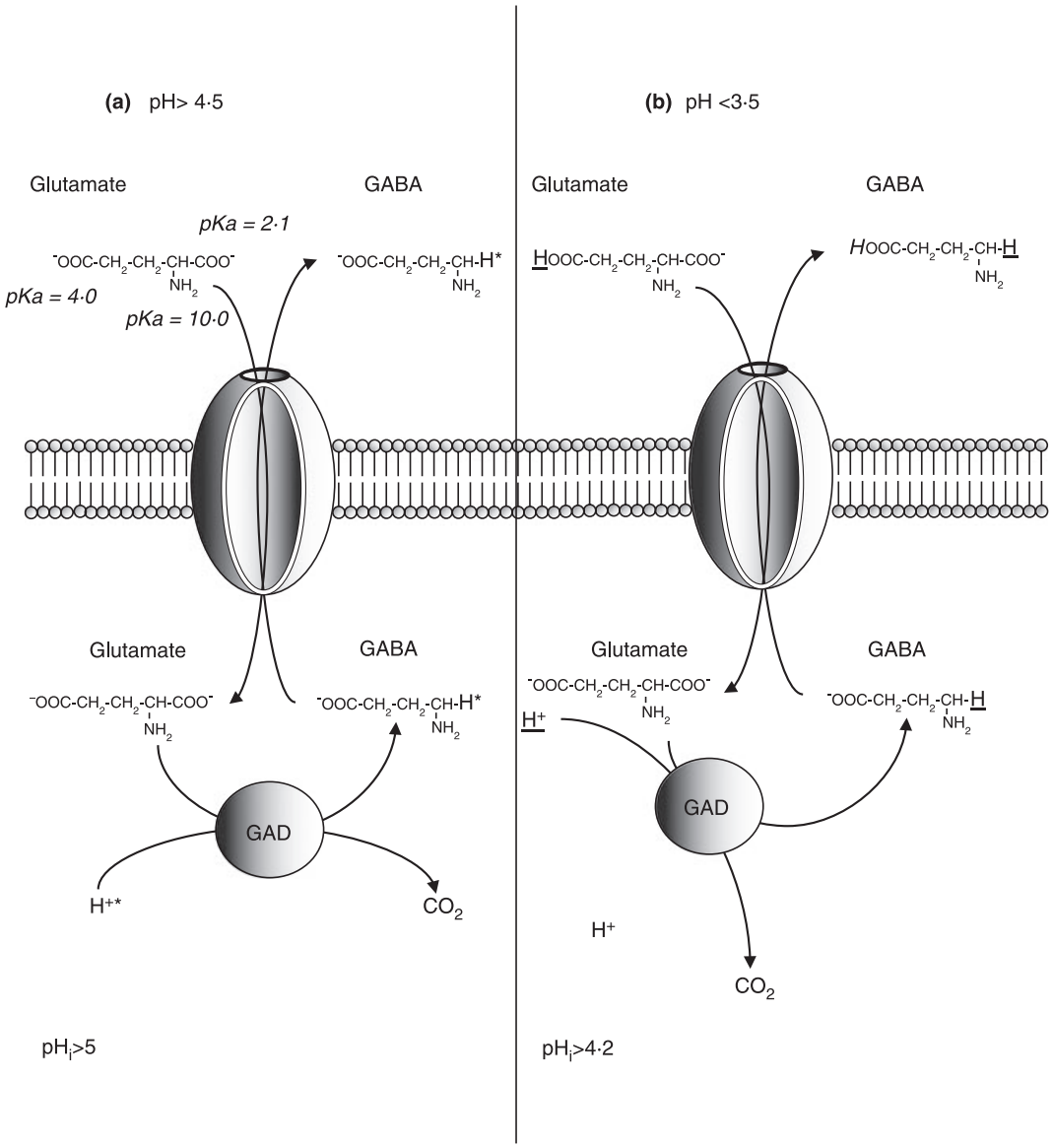


**Figure 4** Representation of two different scenarios on the fate of protons derived from glutamate and source of the protons removed by glutamate decarboxylation. Scenario (a) describes the fate of glutamate, γ-aminobutyrate (GABA) and protons under mild acidic conditions. In this case, both carboxyl groups of glutamate are deprotonated as their pKa is lower than the extracellular pH. In the case of scenario (b), the extracellular pH is very low (<3·5) in the lethal range for most bacteria. In this case, the side chain carboxyl group is mainly protonated (H) because its pKa is higher than the extracellular pH. In this protonated state, glutamate is imported in the cell. Subsequently, this proton ($H^+$) is released in the intracellular milieu, and it is consumed during the glutamate decarboxylation to GABA and thus exported out of the cell. In this case, no proton is removed from the intracellular milieu, and therefore, no benefit exists for the cell, as described by the futile proton hypothesis.

accumulating $GABA_i$ under acidic conditions (Karatzas *et al.* 2012). The effect of $GAD_i$ in acid resistance might also explain the function of the GAD system in *Myco. tuberculosis* that possesses a GadA but no glutamate/GABA antiporters (Cole *et al.* 1998; Cotter *et al.* 2001). The important role of the $GAD_i$ plays in acid resistance remains to be elucidated in other microorganisms.

### Regulation of the glutamate decarboxylase system

The regulation of the GAD system is complex and it has been studied extensively in *E. coli*. In this organism, RpoS activates *gadY*, whose small-RNA (sRNA) product stabilizes *gadX* (Sayed *et al.* 2007). This increases the GadX levels, which together with GadW binds and activates the *gadA* and *gadBC* promoters *in vitro* (Tramonti *et al.*

2006). However, *in vivo*, the latter process requires *gadE* (Gong *et al.* 2004), which is activated by GadX and GadW (Sayed *et al.* 2007), and in turn, activates *gadA* and *gadBC* (Tucker *et al.* 2003; Hommais *et al.* 2004; Weber *et al.* 2005). Similarly to the architecture of the GAD system, its regulation is subject to variation. In *L. monocytogenes*, all regulatory elements from *E. coli* (*gadE*, *gadY*, *gadX*, *gadW*) have not been identified, while upregulation of *gadT2D2* and *gadD3* but not *gadD1T1* is SigB-dependent and takes place during entrance in the stationary phase (Cotter *et al.* 2001; Wemekamp-Kamphuis *et al.* 2004; O'Byrne and Karatzas 2008). However, exposure of stationary phase cells of *L. monocytogenes* 10403S to acidic conditions upregulates *gadD1T1* and *gadD3*, but not *gadT2D2* operon. However, this is peculiar as this operon plays an important role under severe acidic conditions (Karatzas *et al.* 2010).

Furthermore, various compounds and conditions seem to activate the GAD system. Apart from acidic conditions, it has been demonstrated that sodium (Richard and Foster 2007) and polyamines (Jung and Kim 2003) lead to the upregulation of the GAD system in *E. coli*. In both *E. coli* (Blankenhorn *et al.* 1999) and *L. monocytogenes* (Jydegaard-Axelsen *et al.* 2004), hypoxia has been shown to upregulate the GAD system. Interestingly, the GAD system functionality is variable between different bacteria and probably depends on the environmental niches they are present. For example, although the GAD system of *E. coli* is functional in minimal media (Castanie-Cornet and Foster 2001), in *L. monocytogenes*, the system is nonfunctional despite the presence of glutamate (Karatzas *et al.* 2010). Variability exists also between strains of the same species as *L. monocytogenes* LO28 utilizes the GAD system in rich media like TSBY, but not in BHI although the 10403S strain utilizes the GAD system in both media efficiently (Karatzas *et al.* 2012).

## The γ-aminobutyrate shunt pathway

The conversion of glutamate to GABA by GAD is the first step of the GABA shunt pathway. However, in bacteria where most of the GABA is exported by the GAD antiporter, the GABA shunt deals with the steps that follow the generation of GABA. The accumulation of intracellular GABA under acidic conditions suggests that its removal should involve a catabolic pathway. This could be assumed as the glutamate/GABA antiporters are only able to remove quantities of GABA that are equimolar to those of glutamate they import, leaving the excess in the cell. Therefore, the first enzyme in the GABA shunt is a GABA-AT (Zhu *et al.* 2010). This involves the reversible conversion of GABA to SSA, where the amino group of GABA is donated to an α-ketoglutarate molecule. The by-product of this reaction is glutamate (Fig. 2). It has been shown that plants can use pyruvate and glyoxylate as alternative amino group acceptors (Bouché and Fromm 2004; Clark *et al.* 2009); however, bacteria appear to only have α-ketoglutarate-dependent GABA-AT activity, with the exception of some *Rhizobium* spp. (Prell *et al.* 2002). The sole reliance on α-ketoglutarate as the receiving oxoacid is also the case for GABA-ATs present in mammals (Shelp *et al.* 1999). The importance of this enzyme in bacteria is often highlighted by the presence of more than one gene coding a functional GABA-AT. Studies with *E. coli* and *Pseudomonas syringae* have identified the possession of up to three genes encoding functional GABA-ATs (Buell *et al.* 2003; Kurihara *et al.* 2010). In *E. coli*, it appears that induction of GabT, the primary GABA-AT, occurs at pH 9·0 (Stancik *et al.* 2002) suggesting that this pathway may be more active at alkaline pH conditions.

The second enzyme in the pathway is a SSDH (Zhu *et al.* 2010). This irreversible step involves the oxidation of SSA to succinate with the formation of $CO_2$. Similarly to GABA-AT, bacteria can possess up to three SSDH encoding genes (Buell *et al.* 2003). Each SSDH can possess different characteristics and cofactor specificities. For example, in *E. coli*, there are currently two described SSDH enzymes, GabD and YneI; the former is dependent on $NADP^+$, and YneI preferentially utilizes $NAD^+$ (Donnelly and Cooper 1981b; Fuhrer *et al.* 2007). YneI can, however, use NADP+, but the activity utilizing this cofactor is only 15% (Donnelly and Cooper 1981a). The presence of this varying $NAD(P)^+$ dependency has also been well described in several species of *Pseudomonas* (Jakoby and Scott 1959; Padmanabhan and Tchen 1969), as well as both $NADP^+$- and $NAD^+$-dependent SSDH activity shown in *Bacillus thuringiensis* (Zhu *et al.* 2010). Partially purified SSDH from plants shows an optimal alkaline pH of 9 (Shelp *et al.* 1999). This correlates well with the optimal pH for the GABA-AT if the two enzymes are to work together in a metabolic pathway.

Metabolism through the GABA shunt is an important source of nitrogen for bacteria. Arginine, ornithine, agmatine and putrescine are all used as nitrogen sources by *E. coli* (Schneider *et al.* 2002). Arginine, ornithine and agmatine are first converted to putrescine, and the pathway subsequently converts putrescine to GABA that is then catabolized via the GABA shunt. In the absence of either the *gabT* or *gabD* genes, GABA-AT and SSDH activity remain. This activity is induced through the use of putrescine as a sole nitrogen source (Kurihara *et al.* 2010). Furthermore, *puuE*, a gene forming an operon with other members of the putrescine metabolic pathway, was shown to act as a secondary GABA-AT (Fuhrer *et al.* 2007). It is interesting to note that none of the other

genes in the *puu* gene cluster encode an SSDH; however, YneI, which has been predicted as a secondary SSDH (Kurihara *et al.* 2010), is induced along with PuuE by putrescine (Zaboura and Halpern 1978). Despite this evidence for the role of the GABA shunt in nitrogen metabolism, *E. coli* cannot use GABA as a sole source of nitrogen (Belitsky and Sonenshein 2002). The reasons for this are unclear, as *E. coli* does possess a transporter capable of transporting GABA. *Bacillus subtilis* has also been shown to utilize GABA; however, unlike *E. coli*, *B. subtilis* cannot catabolize putrescine or generate GABA through GAD because of the absence of any GAD encoding genes (Dover and Halpern 1972). Therefore, it imports extracellular GABA via a GABA permease *gapP* (Ferson *et al.* 1996). This pathway can serve as the sole nitrogen source for *B. subtilis*. Prell *et al.* (2002) suggested that the pathway may be used in nitrogen metabolism for bacteroides, in particular *Rhizobium leguminosarum*. Some atmospheric nitrogen that is fixed to ammonium during its symbiotic relationship with legume nodules is retained by the bacteria. Results show that GabT is specifically induced by the bacteroid in the nodule state, indicating the enzyme may be involved in catabolizing the glutamate derived from ammonium (Prell *et al.* 2002).

The regulation of the GABA shunt pathway is a subject that little is known about. Much of the initial work investigating this pathway was performed in *E. coli*. It was observed that the pathway is regulated by a form of catabolite repression (Dover and Halpern 1972). Further work has since localized the genes to a region called the *gab* cluster, which contains the four genes *gabT*, *gabD*, *gabP* and *gabC* (Metzer *et al.* 1979; Bartsch *et al.* 1990). Transcriptional control of these genes is complex and involves the *csiD-ygaF-gabDTP* regulon. This operon is reported to be controlled by the three promoters *csiDp*, *gabDp1* and *gabDp2* (Metzner *et al.* 2004). The first two promoters are $\sigma^S$-dependent, while the third is likely to be induced under limited nitrogen availability by Nac/$\sigma^{70}$ (Metzner *et al.* 2004). More recent work with *B. thuringiensis* (Zhu *et al.* 2010) identified the role of a sigma factor, $\sigma^{54}$, in controlling the GABA shunt. This regulates a transcriptional activator, GabR, which itself is also autoregulated. The complete mechanism for regulation has not yet been elucidated in these bacteria; however, the *gab* cluster in *B. thuringiensis* is a good example of how difficult is to make comparisons and generalizations on GABA shunt regulation, even between species of the same genus. Both *gabD* and *gabT* form a GABA-inducible operon in *B. subtilis* (Belitsky and Sonenshein 2002); however, these two genes are individually regulated in *B. thuringiensis* by GabR (Zhu *et al.* 2010). The involvement of ATP as a noncompetitive inhibitor of *Arabidopsis thaliana* SSDH increases the ways by which the GABA shunt in bacteria could be regulated. This implies that the energy status of the cell itself could be involved in the regulation of the pathway (de Carvalho *et al.* 2011).

While much of the focus of the GABA shunt research has been on its role in carbon and nitrogen metabolism, there is ground to suggest that it plays a much broader role in bacteria and has a significant impact in terms of both survival under environmental stresses and towards their ability to cause disease. As discussed earlier, the GABA shunt can operate as an alternative pathway to certain steps of the TCA cycle, providing succinate. Many bacteria including *L. monocytogenes*, *Myco. tuberculosis* and *B. thuringiensis* lack a complete set of genes required for the TCA cycle (Glaser *et al.* 2001; Tian *et al.* 2005a, b). In fact it has been demonstrated that the SSDHs of *Myco. tuberculosis* along with an α-ketoglutarate decarboxylase can lead to the generation of succinate in a pathway which is required for normal growth of the bacteria (Metzner *et al.* 2004). While *Myco. tuberculosis* does not use the first stage of the GABA shunt, it does indicate that shunt-derived succinate could be of beneficial value to bacteria. Furthermore, the TCA cycle has been proposed as an important process in the formation of spores in *Bacillus* species (Rutberg and Hoch 1970); however, Aronson *et al.* (1975) describe that the GABA shunt can successfully complement the loss of α-ketoglutarate dehydrogenase in this process (Tian *et al.* 2005a).

As the *gab* gene cluster in *E. coli* is under the control the multistress response factor $\sigma^S$, it is thought that the GABA shunt may play a role in stress response (Metzner *et al.* 2004). The mechanism of its involvement is unclear; however, it has been suggested that the control of the GABA shunt may affect the glutamate levels that are seen to increase in response to osmotic stress (Metzner *et al.* 2004). This may well be the case as the first step in the pathway generates glutamate. Furthermore, a putative SSDH in *L. monocytogenes*, namely Lmo0913, has been shown to be induced in response to NaCl (Abram *et al.* 2008b) that would be required to remove the toxic SSA accumulated by the generation of glutamate in this manner. The GABA shunt may also play a role in acid tolerance. The antiport-independent accumulation of GABA in response to acid by *L. monocytogenes* (Karatzas *et al.* 2010) would necessitate the catabolism of this metabolite. In fact deletion of *lmo0913* in this bacterium results in an acid-sensitive strain (Abram *et al.* 2008a), highlighting the potential link between acid stress survival and the GABA shunt.

A further, yet understudied role for the pathway has been seen to link GABA metabolism and virulence. A mutation in GABA-AT has been reported to reduce bacterial virulence in the plant pathogen *Ps. syringae* (Tian

*et al.* 2005a). Another micro-organism, *Agrobacterium tumefaciens*, uses GABA in damaged plant tissues as signalling molecule that regulates virulence functions (Chevrot *et al.* 2006). Levels of GABA increase in wounded plant tissues and they signal the modulation of quorum sensing in the bacterium, thereby affecting its virulence on plants. A role for GABA metabolism has been outlined in plant fungal pathogens such as *Fusarium graminearum* and *Cladosporium fulvum* (Solomon and Oliver 2002; Carapito *et al.* 2008). These fungi alter the metabolism of the plant-host to provide the GABA as a nitrogen source. The GABA shunt is also involved in the formation of crystals and spores in *B. thuringiensis* (Zhu *et al.* 2010).

Overall, the GABA shunt pathway is a much more important metabolic route than the attention and knowledge of it would suggest. Apart from its role in glutamate cycling, it has been shown to play a role in both secondary carbon and nitrogen metabolism of bacteria. This simple two step pathway is quite variable among bacteria, with the number of genes encoding the pathway as well as the regulation of these genes varying greatly. This makes the establishment of a general model for the GABA shunt in bacteria difficult and necessitates the need for species-specific studies. The majority of our knowledge in this area has been attained through work with mammals, typically with an emphasis on novel drug discovery. The GABA shunt is, however, showing to be a much more important pathway in bacteria than it would appear.

## The role of glutamate metabolism in other stresses

It is well known that the GAD system and glutamate catabolism play a role in plants under hypoxia (Shelp *et al.* 1999). However, there is not so much evidence linking the GAD system and hypoxia in micro-organisms. In previous work, it has been demonstrated that anaerobic conditions trigger the transcription of the *gad* genes in *L. monocytogenes* (Jydegaard-Axelsen *et al.* 2004) and *E. coli* (Blankenhorn *et al.* 1999). Furthermore, the *gad* genes have been shown to play an important role in the survival and growth of *L. monocytogenes* in various modified atmosphere-packaged foods (Francis *et al.* 2007). This suggests that the GAD system might play a role in survival or growth under oxygen limitation. The role of the GAD system under anaerobic conditions might be linked with the acidification of the medium and the intracellular milieu that occurs during fermentation. This remains to be investigated. However, in *E. coli*, upregulation of GadA has also been reported anaerobically under alkaline conditions (pH 9; Blankenhorn *et al.* 1999). This might suggest that the GAD system plays an additional role apart from increasing the intracellular pH during fermentation. The production of GABA during fermentation has been studied extensively in lactic acid bacteria (Siragusa *et al.* 2007). The lactic acid produced during fermentation by the bacteria lowers the pH and thus protects the product from growth of pathogenic bacteria. The acidic conditions created lead the lactic acid bacteria to utilize the GAD system and export GABA. Interestingly, this property has lately gained interest as the GABA contained in foods has been shown to have beneficial health effects like the reduction in blood pressure (Inoue *et al.* 2003; Hayakawa *et al.* 2004; Gobbetti *et al.* 2010).

In *E. coli* O157:H7, it has been demonstrated that glutamate and arginine play an important role in the protection against oxidative stress (achieved through $H_2O_2$ or diamide) under acidic conditions (Bearson *et al.* 2009). Furthermore, the authors demonstrated that this protection required *gadC* and *adiA* for the glutamate- and arginine-dependent acid resistance systems, respectively (Bearson *et al.* 2009). In *Saccharomyces cerevisiae*, it has been shown that expression of a GAD homologue is required for normal oxidative stress tolerance (Coleman *et al.* 2001; Capitani *et al.* 2003). In addition, they showed that exposure to $H_2O_2$ increased the expression of SSDH, which catalyses GABA (Coleman *et al.* 2001). This suggests that the activity of the glutamate catabolic pathway (GAD system and GABA shunt) promotes resistance against oxidative stress. An explanation for the role of the GAD system in oxidative stress might be related to its effects on the intracellular pH. It is well known that oxidation reactions are affected by the pH, and therefore, the increase in pH achieved by the GAD system might change the route of oxidative reactions and processes (Coleman *et al.* 2001). It has also been suggested that the NADH or NADPH produced by succinate SSDH during the catabolism of GABA (formed by the GAD system) affects redox changes in the cell (Coleman *et al.* 2001).

Glutamate and GABA are two of the most prominent compatible solutes present in bacteria (Csonka 1989; Wood 2011). Compatible solutes are defined as intracellular organic solutes, which, at high concentration, allow 'conventional' enzymes to function efficiently (Brown 1990). They play diverse roles in various stresses protecting macromolecular structures helping to maintain metabolic functions under multiple stresses (Brown 1976) including solvent, chaotrope and hydrocarbon-induced stresses (Bhaganna *et al.* 2010), high temperature, freeze-thaw treatment or drying (Welsh 2000). Each individual compatible solute has physicochemical properties that confer protection in relation to each of the stress mechanisms. Under osmotic stress, cells accumulate potassium ions and increase glutamate de novo synthesis to counterbalance the high levels of potassium (Goude *et al.* 2004).

The high levels of potassium glutamate provide temporary protection (Gralla and Vargas 2006; Wood 2011). The potassium salts of glutamate are thought to be the primary intracellular ions that play a role in the stabilization of DNA–protein interactions (Wood 2011). However, high concentrations of these salts disturb cellular metabolism, and therefore, they are less preferred osmolytes for long-term protection against hyper-osmotic shock. The high internal concentration of potassium glutamate is apparently not retained under conditions of restored growth in hyper-osmotic conditions (Csonka 1989). However, the high levels of potassium glutamate act as an intracellular signal of osmotic stress inducing the uptake of osmoprotectants (Goude *et al.* 2004). It has been shown that levels of GABA increase in response to osmotic stress in a variety of bacteria, but this was rather because of uptake from the medium than synthesis (Measures 1975).

The GAD system of *Lactobacillus plantarum* has been shown to be upregulated in response to bile (Bron *et al.* 2006). Furthermore, other genes encoding proteins involved in glutamate metabolism are regulated by bile, namely glutamate dehydrogenase and glutamate tRNA-ligase (lp_1169 and lp_0609; Bron *et al.* 2006). However, the homologue of GadC (lp_2799) was not upregulated, which might be though due to sufficient levels of the antiporter protein to carry out the glutamate/GABA antiporter process (Bron *et al.* 2006). Furthermore, in *L. monocytogenes*, disruption of *gadT1* encoding for a glutamate/GABA antiporter resulted in sensitivity to bile (Begley *et al.* 2002). It is suggested that the GAD system may play a role in combating the low-pH stress arising from intracellular dissociation of bile salts (Begley *et al.* 2002).

The GAD system has also been shown to play a role in nisin resistance of *L. monocytogenes* (Begley *et al.* 2010). This is achieved through the GadD1 that has been shown to play a role in growth under mild acidic conditions. Mutants missing GadD1 were impaired in their ability to tolerate lethal and sublethal concentrations of the lantibiotic nisin (Begley *et al.* 2010). This decarboxylase seems to be important for nisin resistance of other Gram-positive bacteria including *Lactococcus lactis* subsp *lactis* that produces nisin. Interestingly, *Lactococcus lactis* subsp *cremoris* that has a nonfunctional GadB because of a frameshift mutation is unable to grow in the presence of nisin (Nomura *et al.* 2002; Begley *et al.* 2010).

## Conclusions

Overall, glutamate metabolism plays an important role in resistance to acid stress and multiple other stresses in well-studied bacteria like *E. coli* and *L. monocytogenes*. However, the great variability in the usage of this mechanism between different species and even strains of the same species suggests that more work needs to be performed to fully understand its contribution in stress resistance. Furthermore, as more genomes of microorganisms become available, it is revealed that this pathway affects the stress resistance of more microorganisms than previously thought. This can form the basis for further work in the understanding of the stress resistance of these organisms and reveal possible novel functions for glutamate metabolism.

## Acknowledgements

The authors are grateful to their colleagues and especially to Dr Conor O'Byrne in Microbiology at NUI Galway for helpful discussions and comments on the manuscript. The work was supported by a Science Foundation Ireland Starting Investigator Research Grant (SIRG; 09/SIRG/B1570) and a Marie Curie European Reintegration Grant (ERG 265154) awarded to K.A.G. Karatzas.

## References

Abram, F., Starr, E., Karatzas, K.A.G., Matlawska-Wasowska, K., Boyd, A., Wiedmann, M., Boor, K.J., Connally, D. *et al.* (2008a) Identification of components of the SigB regulon in *L. monocytogenes* that contribute to acid and salt tolerance. *Appl Environ Microbiol* **74**, 6848–6858.

Abram, F., Su, W.-L., Wiedmann, M., Boor, K.J., Coote, P., Botting, C., Karatzas, K.A.G. and O'Byrne, C.P. (2008b) Proteomic analyses of a *L. monocytogenes* mutant lacking *sigB* identify new components of the SigB regulon and highlight a role for SigB in the utilization of glycerol. *Appl Environ Microbiol* **74**, 594–604.

Aronson, J.N., Borris, D.P., Doerner, J.F. and Akers, E. (1975) γ-Aminobutyric acid pathway and modified tricarboxylic acid cycle activity during growth and sporulation of *Bacillus thuringiensis*. *Appl Microbiol* **30**, 489–492.

Balazs, R., Machiyama, Y., Hammond, B.J., Julian, T. and Richter, D. (1970) The operation of the γ-aminobutyrate bypath of the tricarboxylic acid cycle in brain tissue in vitro. *Biochem J* **116**, 445–461.

Bartsch, K., von Johnn-Marteville, A. and Schulz, A. (1990) Molecular analysis of two genes of the *E. coli gab* cluster: nucleotide sequence of the glutamate:succinic semialdehyde transaminase gene (*gabT*) and characterization of the succinic semialdehyde dehydrogenase gene (*gabD*). *J Bacteriol* **172**, 7035–7042.

Bearson, B.L., Lee, I.S. and Casey, T.A. (2009) *E. coli* O157 : H7 glutamate- and arginine-dependent acid-resistance systems protect against oxidative stress during extreme acid challenge. *Microbiology* **155**, 805–812.

Begley, M., Gahan, C.G.M. and Hill, C. (2002) Bile stress response in *L. monocytogenes* LO28: adaptation, cross-protection, and identification of genetic loci involved in bile resistance. *Appl Environ Microbiol* **68**, 6005–6012.

Begley, M., Cotter, P.D., Hill, C. and Ross, R.P. (2010) Glutamate decarboxylase-mediated nisin resistance in *L. monocytogenes*. *Appl Environ Microbiol* **76**, 6541–6546.

Belitsky, B.R. and Sonenshein, A.L. (2002) GabR, a member of a novel protein family, regulates the utilization of γ-aminobutyrate in *Bacillus subtilis*. *Mol Microbiol* **45**, 569–583.

Berg, J.M., Tymoczko, J.L. and Stryer, L. (2007) *Biochemistry*, 6th edn. New York, NY: Friedman & Co.

Bhaganna, P., Volkers, R.J.M., Bell, A.N.W., Kluge, K., Timson, D.J., McGrath, J.W., Ruijssenaars, H.J. and Hallsworth, J.E. (2010) Hydrophobic substances induce water stress in microbial cells. *Microb Biotechnol* **3**, 701–716.

Blankenhorn, D., Phillips, J. and Slonczewski, J.L. (1999) Acid- and base-induced proteins during aerobic and anaerobic growth of *E. coli* revealed by two-dimensional gel electrophoresis. *J Bacteriol* **181**, 2209–2216.

Bouché, N. and Fromm, H. (2004) GABA in plants: just a metabolite? *Trends Plant Sci* **9**, 110–115.

Bron, P.A., Molenaar, D., de Vos, W.M. and Kleerebezem, M. (2006) DNA micro-array-based identification of bile-responsive genes in *L. plantarum*. *J Appl Microbiol* **100**, 728–738.

Brown, A.D. (1976) Microbial water stress. *Bacteriol Rev* **40**, 803–846.

Brown, A.D. (1990) *Microbial Water Stress Physiology. Principles and Perspectives*. Chichester, UK: John Wiley & Sons Ltd.

Buell, C.R., Joardar, V., Lindeberg, M., Selengut, J., Paulsen, I. T., Gwinn, M.L., Dodson, R.J., Deboy, R.T. *et al.* (2003) The complete genome sequence of the *Arabidopsis* and tomato pathogen *P. syringae* pv. tomato DC3000. *PNAS*, **100**, 10181–10186.

Capitani, G., Biase, D.D., Aurizi, C., Gut, H., Bossa, F. and Grutter, M.G. (2003) Crystal structure and functional analysis of *E. coli* glutamate decarboxylase. *EMBO J* **22**, 4027–4037.

Carapito, R., Hatsch, D., Vorwerk, S., Petkovski, E., Jeltsch, J. M. and Phalip, V. (2008) Gene expression in *F. graminearum* grown on plant cell wall. *Fungal Genet Biol* **45**, 738–748.

de Carvalho, L.P.S., Ling, Y., Shen, C., Warren, J.D. and Rhee, K.Y. (2011) On the chemical mechanism of succinic semialdehyde dehydrogenase (GabD1) from *M. tuberculosis*. *Arch Biochem Biophys* **509**, 90–99.

Castanie-Cornet, M.-P. and Foster, J.W. (2001) *E. coli* acid resistance: cAMP receptor protein and a 20Å bp cis-acting sequence control pH and stationary phase expression of the *gadA* and *gadBC* glutamate decarboxylase genes. *Microbiology* **147**, 709–715.

Castanie-Cornet, M.P., Penfound, T.A., Smith, D., Elliott, J.F. and Foster, J.W. (1999) Control of acid resistance in *E. coli*. *J Bacteriol* **181**, 3525–3535.

Chevrot, R., Rosen, R., Haudecoeur, E., Cirou, A., Shelp, B.J., Ron, E. and Faure, D. (2006) GABA controls the level of quorum-sensing signal in *A. tumefaciens*. *PNAS* **103**, 7460–7464.

Clark, S.M., Di Leo, R., Dhanoa, P.K., Van Cauwenberghe, O. R., Mullen, R.T. and Shelp, B.J. (2009) Biochemical characterization, mitochondrial localization, expression, and potential functions for an *Arabidopsis* γ-aminobutyrate transaminase that utilizes both pyruvate and glyoxylate. *J Exp Bot* **60**, 1743–1757.

Cole, S.T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S.V., Eiglmeier, K. *et al.* (1998) Deciphering the biology of *M. tuberculosis* from the complete genome sequence. *Nature* **393**, 537–544.

Coleman, S.T., Fang, T.K., Rovinsky, S.A., Turano, F.J. and Moye-Rowley, W.S. (2001) Expression of a glutamate decarboxylase homologue is required for normal oxidative stress tolerance in *S. cerevisiae*. *J Biol Chem* **276**, 244–250.

Cotter, P.D., Gahan, C.G.M. and Hill, C. (2001) A glutamate decarboxylase system protects *L. monocytogenes* in gastric fluid. *Mol Microbiol* **40**, 465–475.

Cotter, P.D., Ryan, S., Gahan, C.G.M. and Hill, C. (2005) Presence of GadD1 glutamate decarboxylase in selected *L. monocytogenes* strains is associated with an ability to grow at low pH. *Appl Environ Microbiol* **71**, 2832–2839.

Csonka, L.N. (1989) Physiological and genetic responses of bacteria to osmotic stress. *Microbiol Rev* **53**, 121–147.

Donnelly, M.I. and Cooper, R.A. (1981a) Succinic semialdehyde dehydrogenases of *E. coli*. *Eur J Biochem* **113**, 555–561.

Donnelly, M.I. and Cooper, R.A. (1981b) Two succinic semialdehyde dehydrogenases are induced when *E. coli* K-12 Is grown on γ-aminobutyrate. *J Bacteriol* **145**, 1425–1427.

Dover, S. and Halpern, Y.S. (1972) Control of the pathway of γ-aminobutyrate breakdown in *E. coli K-12*. *J Bacteriol* **110**, 165–170.

Ferson, A.E., Wray, J.L.V. and Fisher, S.H. (1996) Expression of the *B. subtilis gabP* gene is regulated independently in response to nitrogen and amino acid availability. *Mol Microbiol* **22**, 693–701.

Foster, J.W. (2004) *E. coli* acid resistance: tales of an amateur acidophile. *Nat Rev Microbiol* **2**, 898–907.

Francis, G.A., Scollard, J., Meally, A., Bolton, D.J., Gahan, C.G. M., Cotter, P.D., Hill, C. and O'Beirne, D. (2007) The glutamate decarboxylase acid resistance mechanism affects survival of *L. monocytogenes* LO28 in modified atmosphere-packaged foods. *J Appl Microbiol* **103**, 2316–2324.

Fuhrer, T., Chen, L., Sauer, U. and Vitkup, D. (2007) Computational prediction and experimental verification of the gene encoding the $NAD^+$/$NADP^+$-dependent succinate

semialdehyde dehydrogenase in *E. coli*. *J Bacteriol* **189**, 8073–8078.

Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., Amend, A., Baquero, F., Berche, P., Bloecker, H. *et al.* (2001) Comparative genomics of *Listeria* species. *Science* **294**, 849–852.

Gobbetti, M., Cagno, R.D. and De Angelis, M. (2010) Functional microorganisms for functional food quality. *Crit Rev Food Sci Nutr* **50**, 716–727.

Gong, S., Ma, Z. and Foster, J.W. (2004) The Era-like GTPase TrmE conditionally activates *gadE* and glutamate-dependent acid resistance in *E. coli*. *Mol Microbiol* **54**, 948–961.

Goude, R., Renaud, S., Bonnassie, S., Bernard, T. and Blanco, C. (2004) Glutamine, glutamate, and α-glucosylglycerate are the major osmotic solutes accumulated by *Erwinia chrysanthemi* strain 3937. *Appl Environ Microbiol* **70**, 6535–6541.

Gralla, J.D. and Vargas, D.R. (2006) Potassium glutamate as a transcriptional inhibitor during bacterial osmoregulation. *EMBO J* **25**, 1515–1521.

Hayakawa, K., Kimura, M., Kasaha, K., Matsumoto, K., Sansawa, H. and Yamori, Y. (2004) Effect of a γ-aminobutyric acid-enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar-Kyoto rats. *Br J Nutr* **92**, 411–417.

Hommais, F., Krin, E., Coppee, J., Lacroix, C., Yeramian, E., Danchin, A. and Bertin, P. (2004) GadE (YhiE): a novel activator involved in the response to acid environment in *E. coli*. *Microbiology* **150**, 61–72.

Inoue, K., Shirai, T., Ochiai, H., Kasao, M., Hayakawa, K., Kimura, M. and Sansawa, H. (2003) Blood-pressure-lowering effect of a novel fermented milk containing γ-aminobutyric acid (GABA) in mild hypertensives. *Eur J Clin Nutr* **57**, 490–495.

Jakoby, W.B. and Scott, E.M. (1959) Aldehyde oxidation. III. Succinic semialdehyde dehydrogenase. *J Biol Chem* **234**, 937–940.

Jung, I.L. and Kim, I.G. (2003) Polyamines and glutamate decarboxylase-based acid resistance in *E. coli*. *J Biol Chem* **278**, 22846–22852.

Jydegaard-Axelsen, A.M., Hoiby, P.E., Holmstrom, K., Russell, N. and Knochel, S. (2004) $CO_2$ – and anaerobiosis-induced changes in physiology and gene expression of different *L. monocytogenes* strains. *Appl Environ Microbiol* **70**, 4111–4117.

Karatzas, K.A.G., Valdramidis, V.P. and Wells-Bennik, M.H.J. (2005) Contingency locus in *ctsR* of *L. monocytogenes* Scott A: a strategy for occurrence of abundant piezotolerant isolates within clonal populations. *Appl Environ Microbiol* **71**, 8390–8396.

Karatzas, K.A.G., Zervos, A., Tassou, C.C., Mallidis, C.G. and Humphrey, T.J. (2007) Piezotolerant small-colony variants with increased thermotolerance, antibiotic susceptibility, and low invasiveness in a clonal *Staphylococcus aureus* population. *Appl Environ Microbiol* **73**, 1873–1881.

Karatzas, K.A.G., Hocking, P.M., Jørgensen, F., Mattick, K., Leach, S. and Humphrey, T.J. (2008) Effects of repeated cycles of acid challenge and growth on the phenotype and virulence of *S. enterica*. *J Appl Microbiol* **105**, 1640–1648.

Karatzas, K.A.G., Brennan, O., Heavin, S., Morrissey, J. and O'Byrne, C.P. (2010) Intracellular accumulation of high levels of γ-aminobutyrate by *L. monocytogenes* 10403S in response to low pH: uncoupling of γ-aminobutyrate synthesis from efflux in a chemically defined medium. *Appl Environ Microbiol* **76**, 3529–3537.

Karatzas, K.A.G., Suur, L. and O'Byrne, C.P. (2012) Characterisation of the intracellular-glutamate decarboxylase system ($GAD_i$): analysis of its function, transcription and role in the acid resistance of various strains of *L. monocytogenes*. *Appl Environ Microbiol* **78**, 3571–3579.

Kezmarsky, N.D., Xu, H., Graham, D.E. and White, R.H. (2005) Identification and characterization of an L-tyrosine decarboxylase in *Methanocaldococcus jannaschii*. *Biochim Biophys Acta* **1722**, 175–182.

Krastel, K., Senadheera, D.B., Mair, R., Downey, J.S., Goodman, S.D. and Cvitkovitch, D.G. (2010) Characterization of a glutamate transporter operon, *glnQHMP*, in *Streptococcus mutans* and its role in acid tolerance. *J Bacteriol* **192**, 984–993.

Kurihara, S., Kato, K., Asada, K., Kumagai, H. and Suzuki, H. (2010) A putrescine-inducible pathway comprising PuuE-YneI in which γ-aminobutyrate is degraded into succinate in *E. coli* K-12. *J Bacteriol* **192**, 4582–4591.

Lei, G.-S., Syu, W.-J., Liang, P.-H., Chak, K.-F., Hu, W. and Hu, S.-T. (2011) Repression of *btuB* gene transcription in *E. coli* by the GadX protein. *BMC Microbiol* **11**, 33.

Maras, B., Sweeney, G., Barra, D., Bossa, F. and John, R.A. (1992) The amino acid sequence of glutamate decarboxylase from *E. coli*. *Eur J Biochem* **204**, 93–98.

Measures, J.C. (1975) Role of amino acids in osmoregulation of non-halophilic bacteria. *Nature* **257**, 398–400.

Metzer, E., Levitz, R. and Halpern, Y.S. (1979) Isolation and properties of *E. coli* K-12 mutants impaired in the utilization of γ-aminobutyrate. *J Bacteriol* **137**, 1111–1118.

Metzner, M., Germer, J. and Hengge, R. (2004) Multiple stress signal integration in the regulation of the complex $\sigma^S$-dependent *csiD-ygaF-gabDTP* operon in *E. coli*. *Mol Microbiol* **51**, 799–811.

Nomura, M., Kobayashi, M. and Okamoto, T. (2002) Rapid PCR-based method which can determine both phenotype and genotype of *Lactococcus lactis* subspecies. *Appl Environ Microbiol* **68**, 2209–2213.

O'Byrne, C.P. and Karatzas, K.A.G. (2008) The role of SigB in the stress adaptations of *L. monocytogenes*: overlaps between stress adaptation and virulence. In *Adv Appl Microbiol* eds Allen, S., Laskin, I. and Geoffrey, M.G. **65**, pp. 115–140. New York: Academic Press.

Padmanabhan, R. and Tchen, T.T. (1969) Nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate-linked succinic semialdehyde dehydrogenases in a *Pseudomonas* species. *J Bacteriol* **100**, 398–402.

Petroff, O.A.C. (2002) Book Review: GABA and glutamate in the human brain. *Neuroscientist* **8**, 562–573.

Prell, J.R., Boesten, B., Poole, P. and Priefer, U.B. (2002) The *Rhizobium leguminosarum* bv. *viciae* VF39 $\gamma$-aminobutyrate (GABA) aminotransferase gene (*gabT*) is induced by GABA and highly expressed in bacteroids. *Microbiology* **148**, 615–623.

Richard, H. and Foster, J.W. (2007) Sodium regulates *E. coli* acid resistance, and influences GadX- and GadW-dependent activation of *gadE*. *Microbiology* **153**, 3154–3161.

Roop, R.M., Gee, J.M., Robertson, G.T., Richardson, J.M., Ng, W.L. and Winkler, M.E. (2003) *Brucella* stationary-phase gene expression and virulence. *Annu Rev Microbiol* **57**, 57–76.

Rutberg, B. and Hoch, J.A. (1970) Citric acid cycle: gene-enzyme relationships in *Bacillus subtilis*. *J Bacteriol* **104**, 826–833.

Satorhelyi, P. (2005) Microarray-analyse der pH-stressantwort von *L. monocytogenes* und *Corynebacterium glutamicum*. PhD Thesis, Technische Universität München, München.

Sayed, A., Odom, C. and Foster, J. (2007) The *E. coli* AraC-family regulators GadX and GadW activate *gadE*, the central activator of glutamate-dependent acid resistance. *Microbiology* **153**, 2584–2592.

Schneider, B.L., Ruback, S., Kiupakis, A.K., Kasbarian, H., Pybus, C. and Reitzer, L. (2002) The *E. coli gabDTPC* operon: specific $\gamma$-aminobutyrate catabolism and nonspecific induction. *J Bacteriol* **184**, 6976–6986.

Shelp, B.J., Bown, A.W. and McLean, M.D. (1999) Metabolism and functions of $\gamma$-aminobutyric acid. *Trends Plant Sci* **4**, 446–452.

Siragusa, S., De Angelis, M., Di Cagno, R., Rizzello, C.G., Coda, R. and Gobbetti, M. (2007) Synthesis of $\gamma$-aminobutyric acid by lactic acid bacteria isolated from a variety of italian cheeses. *Appl Environ Microbiol* **73**, 7283–7290.

Smith, D.K., Kassam, T., Singh, B. and Elliott, J.F. (1992) *E. coli* has two homologous glutamate decarboxylase genes that map to distinct loci. *J Bacteriol* **174**, 5820–5826.

Solomon, P. and Oliver, R. (2002) Evidence that $\gamma$-aminobutyric acid is a major nitrogen source during *Cladosporium fulvum* infection of tomato. *Planta* **214**, 414–420.

Stancik, L.M., Stancik, D.M., Schmidt, B., Barnhart, D.M., Yoncheva, Y.N. and Slonczewski, J.L. (2002) pH-Dependent expression of periplasmic proteins and amino acid catabolism in *E. coli*. *J Bacteriol* **184**, 4246–4258.

Storz, G. and Hengge, R. (2000) *Bacterial Stress Responses*. Washington, DC: ASM Press.

Su, M., Schlicht, S. and Ganzle, M. (2011) Contribution of glutamate decarboxylase in *Lactobacillus reuteri* to acid resistance and persistence in sourdough fermentation. *Microb Cell Fact* **10**, S8.

Tian, J., Bryk, R., Itoh, M., Suematsu, M. and Nathan, C. (2005a) Variant tricarboxylic acid cycle in *M. tuberculosis*: identification of $\alpha$-ketoglutarate decarboxylase. *PNAS* **102**, 10670–10675.

Tian, J., Bryk, R., Shi, S., Erdjument-Bromage, H., Tempst, P. and Nathan, C. (2005b) *M. tuberculosis* appears to lack $\alpha$-ketoglutarate dehydrogenase and encodes pyruvate dehydrogenase in widely separated genes. *Mol Microbiol* **57**, 859–868.

Tramonti, A., Visca, P., De Canio, M., Falconi, M. and De Biase, D. (2002) Functional characterization and regulation of *gadX*, a gene encoding an AraC/XylS-like transcriptional activator of the *E. coli* glutamic acid decarboxylase system. *J Bacteriol* **184**, 2603–2613.

Tramonti, A., De Canio, M., Delany, I., Scarlato, V. and De Biase, D. (2006) Mechanisms of transcription activation exerted by GadX and GadW at the *gadA* and *gadBC* gene promoters of the glutamate-based acid resistance system in *E. coli*. *J Bacteriol* **188**, 8118–8127.

Tucker, D.L., Tucker, N. and Conway, T. (2002) Gene expression profiling of the pH response in *E. coli*. *J Bacteriol* **184**, 6551–6558.

Tucker, D., Tucker, N., Ma, Z., Foster, J., Miranda, R., Cohen, P. and Conway, T. (2003) Genes of the GadX-GadW regulon in *E. coli*. *J Bacteriol* **185**, 3190–3201.

Ventura, M., Turroni, F., Zomer, A., Foroni, E., Giubellini, V., Bottacini, F., Canchaya, C., Claesson, M.J. *et al.* (2009) The *Bifidobacterium dentium* Bd1 genome sequence reflects its genetic adaptation to the human oral cavity. *PLoS Genet* **5**, e1000785.

Waagepetersen, H.S., Sonnewald, U. and Schousboe, A. (1999) The GABA paradox. *J Neurochem* **73**, 1335–1342.

Waterman, S.R. and Small, P.L.C. (2003a) The glutamate-dependent acid resistance system of *E. coli* and *S. flexneri* is inhibited in vitro by L-trans-pyrrolidine-2,4-dicarboxylic acid. *FEMS Microbiol Lett* **224**, 119–125.

Waterman, S.R. and Small, P.L.C. (2003b) Transcriptional expression of *E. coli* glutamate-dependent acid resistance genes *gadA* and *gadBC* in an *hns rpoS* mutant. *J Bacteriol* **185**, 4644–4647.

Weber, H., Polen, T., Heuveling, J., Wendisch, V. and Hengge, R. (2005) Genome-wide analysis of the general stress response network in *E. coli:* sigmaS-dependent genes, promoters, and sigma factor selectivity. *J Bacteriol* **187**, 1591–1603.

Welsh, D.T. (2000) Ecological significance of compatible solute accumulation bu microorganisms from single cells to global climate. *FEMS Microbiol Rev* **24**, 263–290.

Wemekamp-Kamphuis, H.H., Wouters, J.A., de Leeuw, P.P.L. A., Hain, T., Chakraborty, T. and Abee, T. (2004) Identification of sigma factor $\sigma^B$-controlled genes and their impact on acid stress, High Hydrostatic Pressure,

and freeze survival in *L. monocytogenes* EGD-e. *Appl Environ Microbiol* **70**, 3457–3466.

WHO Campylobacter (2012) Fact sheet No 255 – October 2011. [Online] Available at: http://www.who.int/mediacentre/factsheets/fs255/en/index.html. [Accessed 08 July 2012].

Wood, J.M. (2011) Bacterial osmoregulation: a paradigm for the study of cellular homeostasis. *Annu Rev Microbiol* **65**, 215–238.

Zaboura, M. and Halpern, Y.S. (1978) Regulation of $\gamma$-aminobutyric acid degradation in *E. coli* by nitrogen metabolism enzymes. *J Bacteriol* **133**, 447–451.

Zhu, L., Peng, Q., Song, F., Jiang, Y., Sun, C., Zhang, J. and Huang, D. (2010) Structure and regulation of the *gab* gene cluster, involved in the $\gamma$-aminobutyric acid shunt, are controlled by a $\sigma^{54}$ factor in *Bacillus thuringiensis*. *J Bacteriol* **192**, 346–355.