**INTRODUCTION**

*Bacillus cereus* is a Gram positive, facultative anaerobic bacterium belonging to the genus *Bacillus* which may produce endospores. The *B. cereus* group comprises seven recognized species: *B. cereus* and *B. anthracis*, known as human pathogens, *B. thuringiensis* used as biopesticide, *B. mycoides*, *B. pseudomycoides* characterized by chisoidal formations, *B. weihenstephanensis* including psychrotolerant strains and *B. cereus* cytotoxicus which is the last identified species (Guinebretière et al., 2013). Furthermore, Guinebretière et al. (2008) proposed a division of the *Bacillus cereus* sensu lato into seven major groups (I–VII) using both genetic and phenotypic criteria. Each group corresponds to different virulence characteristics (Stenfors Arnesen et al., 2008). Within the seven major groups of *Bacillus* sensu lato the involvement of groups II, III, IV, V and VII in food outbreaks have been reported (Cadel Six et al., 2012). *B. cereus* is associated to a large number of food products such as rice, pasta and milk or mayonnaise-based ready-to-eat (RTE) food salad (Mortimer and McCann, 1974; Parry and Gilbert, 1980; Mortimer and McKee, 1979; Kozanecka et al., 2009).

Microorganisms are able to adapt to different environments and evolve rapidly, allowing them to cope with their new environments. Such adaptive response and associated protections toward other lethal stresses, is a crucial survival strategy for a wide spectrum of microorganisms, including food spoilage bacteria, pathogens, and organisms used in functional food applications. The growing demand for minimal processed food yields to an increasing use of combination of hurdles or mild preservation factors in the food industry. A commonly used hurdle is low pH which allows the decrease in bacterial growth rate but also the inactivation of pathogens or spoilage microorganisms. *Bacillus cereus* is a well-known food-borne pathogen leading to economical and safety issues in food industry.

Because survival mechanisms implemented will allow bacteria to cope with environmental changes, it is important to provide understanding of *B. cereus* stress response. Thus this review deals with the adaptive traits of *B. cereus* cells facing to acid stress conditions. The acid stress response of *B. cereus* could be divided into four groups (i) general stress response (ii) pH homeostasis, (iii) metabolic modifications and alkali production and (iv) secondary oxidative stress response. This current knowledge may be useful to understand how *B. cereus* cells may cope to acid environment such as encountered in food products and thus to find some molecular biomarkers of the bacterial behavior. These biomarkers could be furthermore used to develop new microbial behavior prediction tools which can provide insights into underlying molecular physiological states which govern the behavior of microorganisms and thus opening the avenue toward the detection of stress adaptive behavior at an early stage and the control of stress-induced resistance throughout the food chain.

**Keywords:** *Bacillus cereus*, acid stress response, general stress response, pH homeostasis, metabolic rearrangement, oxidative stress response

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**Bacillus cereus** cell response upon exposure to acid environment: toward the identification of potential biomarkers

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**General Stress Response**

The ability of bacteria to respond rapidly to changing environmental conditions is a prerequisite for survival in their habitats. This bacterial stress response is triggered by a change in the microorganism growth conditions. Such a change triggers a cascade of events that will lead to increase stress resistance of the bacterial cell, most often against not the stress to which it was exposed but also other stresses, thereby ensuring its survival under a variety of conditions. A common strategy that bacteria use to counter stressful conditions is to activate a specific alternative sigma factor, which leads to the transcription of a set of genes (a so-called regulon), the products of which protect the cell against adverse conditions (Kazmierczak et al., 2005). In several Gram-positive bacteria, the alternative sigma factor σ^B is the key sigma factor controlling the general stress response (van Schaik et al., 2004b; Kazmierczak et al., 2005). This factor is a secondary subunit of RNA polymerase that is known to play an important role in regulating gene expression when major changes in the environment occur. Upon binding σ^B to core of RNA polymerase, genes located downstream a promoter that can be recognized by the σ^B-RNA polymerase complex are transcribed. The role of σ^B^ and its regulation has been extensively studied in the Gram-positive model-organism *B. subtilis* (for a comprehensive review, see Hecker et al., 2007). In *B. cereus*, σ^B^ is activated in several stress conditions such as ethanol, NaCl exposure or H_2O_2 and acid shock. However, the largest up-regulation of σ^B^ is observed in response to heat shock (van Schaik et al., 2004a).

*σ^B^ Regulation*

In all Gram-positive bacteria, the activation of σ^B^ confers protection to cell against adverse conditions. In *B. subtilis*, σ^B^ activity is controlled by RsbW partner-switching, a mechanism which is highly conserved in species that contain σ^B^, including *B. cereus* strains (de Been et al., 2010, 2011). In non-stressed cells, σ^B^ is present in an inactive form by complexation with the anti-sigma factor RsbV. This factor is a secondary subunit of RNA polymerase that is known to play an important role in regulating gene expression when major changes in the environment occur. Upon binding σ^B^ to core of RNA polymerase, genes located downstream a promoter that can be recognized by the σ^B^-RNA polymerase complex are transcribed. The role of σ^B^ and its regulation has been extensively studied in the Gram-positive model-organism *B. subtilis* (for a comprehensive review, see Hecker et al., 2007). In *B. cereus*, σ^B^ is activated in several stress conditions such as ethanol, NaCl exposure or H_2O_2 and acid shock. However, the largest up-regulation of σ^B^ is observed in response to heat shock (van Schaik et al., 2004a).

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**B. cereus** exhibits many differences with the Gram-positive model *B. subtilis*, such as the regulation of the general stress response (Anderson et al., 2005; de Been et al., 2011): this review will detail the vegetative cells stress response of *B. cereus* while data on the response of other Gram-positive bacteria upon exposure to low pH have been reviewed by Cotter and Hill (2003).

Here, mechanisms of acid resistance in *B. cereus* are reviewed as (i) the involvement of the general stress response in acid stress response, (ii) the pH homeostasis maintaining, (iii) metabolic rearrangements and alkali production, and (iv) the secondary oxidative stress response observed upon exposure to low pH.

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This part...
of this regulatory is conserved in Bacilli (van Schaik et al., 2005), Staphylococcus aureus (Palma and Cheung, 2001; Senn et al., 2005; Pant-Farré et al., 2006) and L. monocytogenes (Wiedmann et al., 1998; Ferreira et al., 2004). However, there are considerable differences in the upstream part of the σB activation pathway (Ferreira et al., 2004; van Schaik et al., 2004a), reflecting differences in the mechanisms of stress sensing and signaling in the various bacteria.

In the human pathogen R. cereus, the mechanism of σB activation has only been studied more recently (van Schaik et al., 2004a, 2005, 2007; de Been et al., 2010, 2011). It has been shown that σB activation is governed by a single PP2C-type phosphatase, RsbY, which carries an N-terminal response receiver (REC) domain (van Schaik et al., 2004a) indicating that RsbK and RsbY should constitute one functional module for the control of σB, allowing bacterial resistance. van Schaik et al. (2004a) demonstrated that upon stress conditions, the level of σB raised rapidly. Thereby they showed that an addition of 4% ethanol, 2.5% NaCl as well as heat (42°C) or acid shock (pH 5.2) have an impact on σB level. They also highlighted a limited effect of ATP depletion on σB level, showing that, unlike in B. subtilis, the σB response could occur solely in response to changes of environmental conditions.

**σB REGULATED GENES AND ACID STRESS**

As mentioned, the activation of the σB response will allow the transcription of the set of genes coding for proteins with specific functions, which will protect the cell against stress. The identification of the complete σB regulon in B. subtilis (Helmann et al., 2001; Petersohn et al., 2001; Price et al., 2001), L. monocytogenes (Kazmierczak et al., 2003), and Staphylococcus aureus (Bischoff et al., 2004a; Pant-Farré et al., 2006) by DNA microarray technology revealed that a low number of genes have an obvious role in the stress response of these organisms and may contribute to stress resistance. For instance, L. monocytogenes σB regulates the gadB gene (Kazmierczak et al., 2003) that is involved in acid stress resistance (discuss in the see Amino-Acids Decarboxylase Systems) and into several metabolic pathways like the amino-acids pathway (Ferreira et al., 2004). Orthologous RsbKY signaling modules were found in four other Bacilli outside the B. cereus group. However, the RsbKY modules in these other Bacilli strains were not connected to σB in terms of genomic context (de Been et al., 2010). Analysis of the transcriptional organization of the σB operon revealed that this operon is transcribed as a 2.1-kb transcript encompassing rsbV, rsbW, sigB and orf3 (Figure 2). orf3, encoding a bacterioferritin (Wang et al., 2009), is also under the control of an additional σB-dependent promoter and is a member of the σB regulon in R. cereus. The rsbY gene was found directly downstream of the σB operon in B. cereus group and is transcribed both from σB and σB-RNA polymerase (van Schaik et al., 2004a; Wang et al., 2009). Once stress conditions is sensed and signaled through the regulatory cascade, the activation of σB as well as the transcription of the set of σB-regulated genes occurs allowing bacterial resistance. van Schaik et al. (2004a) demonstrated that upon stress conditions, the level of σB raised rapidly. Thereby they showed that an addition of 4% ethanol, 2.5% NaCl as well as heat (42°C) or acid shock (pH 5.2) have an impact on σB level. They also highlighted a limited effect of ATP depletion on σB level, showing that, unlike in B. subtilis, the σB response could occur solely in response to changes of environmental conditions.

**σB OPERON STRUCTURE**

FIGURE 1| Schematical representation of the activation of Bacillus cereus σB. Upon different stressing conditions, RsbK auto-phosphorylates a conserved histidine residue. The phosphoryl group is then transferred to RsbY, resulting in the inactivation of RsbK and the activation of σB. The anti-sigma factor RsbY also acts as a kinase of σB, thereby providing a negative feedback on σB activation.

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genes in *L. monocytogenes* may be higher, as the microarray used in that study had only limited genome coverage (Kazmierczak et al., 2003).

The comparison of *B. cereus*, *B. subtilis*, *L. monocytogenes* and *Staphylococcus aureus* σB regulons showed that genes are generally conserved among the four Gram-positive bacteria to same extent as all the other genes of the genome. However, only three genes (rsbV, rsbW, and sigB) are conserved in their σB dependency in all four bacteria, suggesting that the σB regulon of the different Gram positive bacteria has evolved to perform niche specific functions (van Schaik et al., 2007). Composed of 14 unknown proteins, the role of the σB regulon of *B. cereus* to counteract the acid stress has not been studied in detail. Microarray analyses were performed on *B. cereus* ATCC 14579 cells exposed 10 min to a lethal (pH 4.5) or sublethal (pH 5.4) acid stress (Mols et al., 2010b): the variation of B. cereus

σB-stress response remains unclear, whereas the second gene encodes a heat-shock inducible both in *B. cereus* and *B. subtilis* but its role in stress response remains unclear, whereas the second gene encodes the YfkM protease that may acts to degrade incorrectly folded proteins (Helmann et al., 2001; van Schaik et al., 2004b). The genes BC1000 and BC1009 are over-regulated (≥1.5 fold in both conditions). In the same way, BC0863 and BC0998 genes are over-expressed: the first one encoding the YfhI protein is known to be heat-shock inducible both in *B. cereus* and *B. subtilis* but its role in stress response remains unclear, whereas the second gene encodes the YfkM protease that may acts to degrade incorrectly folded proteins (Helmann et al., 2001; van Schaik et al., 2004b). The genes BC1154 encoding a ferrochelatase and BC1155 encoding the main vegetative catalase KatA were up-regulated both in lethal and non-lethal conditions but more upon mild acid exposure (from 7.8 to 44 fold) than upon lethal acid stress (from 1.5 to 3.7 fold).

Eight other σB-dependent genes confirm that the response differ with the intensity of acid stress: for example, BC0863 and BC3129 genes, encoding respectively the catalase KatE and a magnesium cobalt transporter CorA, were up-regulated upon non-lethal acid stress whereas they were down regulated in lethal acid stress.

Furthermore, clp, groEL, and dnaK genes encoding repair and chaperone proteins and heat stress regulators cpxR and hrcC (van de Gucht et al., 2002) were also shown to be up-regulated upon exposure to acid conditions in *B. cereus* (Mols et al., 2010b, Mols and Abebe, 2011b).

**pH HOMEOSTASIS**

The external pH as well as the presence of weak acid determines the intracellular pH (pH_{int}). Weak acids under their unprotonated form can diffuse into the cell and dissociate, releasing a proton and leading to acidification of the cytoplasm. Strong acids are not able to permeate through the cell membrane but by lowering the external pH and increasing the pH gradient, they increase the proton permeability and thereby lead to the reduction of pH_{int} (Krebh et al., 1983; Abebe and Wouters, 1999; Cotter et al., 2000; Beales, 2004). Bacteria can survive thanks to their ability to regulate their pH_{int}, a process primarily driven by controlled movement of cations across the membrane (Beales, 2004). However, this ability to maintain pH_{int} (pH homeostasis) can be overtaken at low extracellular pH value, leading to the cell death (Booth and Kroll, 1989; Beales, 2004). For instance, *B. cereus* ATCC 14579 cells grown at pH 7.0 or 5.5 showed pH_{int} values of 7.10 and 6.22, respectively, whereas a 40 min exposure of cells at pH 4.0 decreased the pH_{int} of 2.02 units and was combined to a population reduction of 2.35 log (Senouci-Rezkallah et al., 2011).

Therefore to survive in acidic conditions, many microorganisms activate enzymes contributing to maintain their pH homeostasis (Mols and Abebe, 2011b), such as proton pumps and consuming proton reactions like glutamate decarboxylation.

**F_{1}F_{0}-ATPase AND PROTON TRANSPORTER**

In aerobic organisms, the active transport of H^+ is coupled with electron transport in respiratory chains whereas anaerobic bacteria carry out H^+ transport via H^+-ATPase molecules using energy from ATP hydrolysis (Shahbala et al., 2002; Gandhi and Chikhadas, 2007). In *L. monocytogenes*, a facultative anaerobic bacterium, the use of both processes has been demonstrated (Shahbala et al., 2002). The multi-subunit enzyme F_{1}F_{0}-ATPase, that is highly conserved, serves as a channel for proton translocation coupled with ATP synthase or hydrolysis (Fillinger and Doun, 1999; Yoshida et al., 2001). ATP synthesis is generated by the expense of proton motive force (PMF) whereas ATP hydrolysis generates a PMF that can facilitate the extrusion of proton from the cytoplasm (Cotter and Hill, 2003).

Since *B. cereus* is a facultative anaerobic bacterium, it could be supposed that this bacterium may use both ATP hydrolysis and synthesis to maintain its pH homeostasis as shown in *L. monocytogenes*. In fact, F_{1}F_{0}-ATPase encoding genes were down-regulated...
Staphylococcus aureus et al., 2010b). This down-regulation has also been demonstrated proton via this ATPase upon exposure to acid conditions (Mols explained by the cells trying to prevent excessive inward flux of

B. cereus Desriac et al., 2010a,b). Down regulation of F0F1-ATPase genes could be tant role by pumping H+ out of the cells, since Arikado et al. (1999) suggest that the enzyme regulation occurs mainly at the

Other proton transporters may also play key role in pH homeostasis: indeed, in early 1970s, it has been shown that an activity in biological membranes couples the fluxes of Na+ and H+ (Mitchell, 1961; Mitchell and Moyle, 1967; West and Mitchell, 1974). They suggested that Na+/H+ antiporters are involved in the homeostasis of both Na+ and H+ in cells. Since then, Na+/H+ as well as K+/H+ antipporter activity has been found in cytoplasmic membranes of many cells. These monovalent cation/proton antipporters are especially known to be involved in bacterial pH homeostasis under alkaline challenge (Krulwich et al., 1999). Interestingly, Mols et al. (2010b) showed that napA encoding a Na+/H+ antipporter was highly up-regulated in B. cereus cells exposed to lethal pH conditions whereas it was down regulated upon non-lethal acid exposure (Mols et al., 2010b).

Thus, F0F1-ATPase and antipporters gene regulations under lethal and non-lethal conditions in B. cereus cells indicated a fine balance between ATP synthesis on one hand and proton pumps regulating pHint at the expense of ATP on the other hand (Mols and Aber, 2011b).

AMINO-ACIDS DECARBOXYLASE SYSTEMS
Amino acid decarboxylases function to control the pH of the bacterial environment by consuming hydrogen ions as part of the decarboxylation reaction (Gale and Epps, 1942; Cotter and Hill, 2003; Beales, 2004; Pearson et al., 1997). Examples of this are the lysine, arginine, and glutamate decarboxylases (GADs), which operate by combining an internalized amino acid with a proton and exchanging the resultant product for another amino acid substrate. Furthermore, the products of amino acid decarboxylases consist of basic amines (pKa value around 10) which may be responsible of a slight increase of extracellular pH (Olson, 1993). Lysine decarboxylase is an enzyme that converts lysine to cadaverine and was found to be an important system involved in acid resistance and in pH homostasis in several Gram-negative bacteria including E. coli, Vibrio vulnificus, Vibrio para-hemolyticus, and Salmonella typhimurium (Pearson et al., 1997; Eun Rhode et al., 2002; Moreau, 2007; Tanaka et al., 2008). Arginine decarboxylase enzyme has one substrate (L-arginine) and two products, CO2 and arginine, which has been showed to be a competitive inhibitor in E. coli (Belfon et al., 1968). GAD converts glutamate in y-amino-butyrate (GABA) and is well described in L. monocytogenes for instance (Cotter et al., 2001; Gandhi and Chikindas, 2007; Karatzas et al., 2010, 2012; Feehily et al., 2013).

Today, only few pieces of information are available on those systems in B. cereus. Mols et al. (2010b) showed that the glutamate decarboxylase gene (gad) is not present in the genome of B. cereus ATCC 14579 whereas it is present in B. cereus ATCC 10987 and many other strains. However, they did not see any difference in growth under acidic conditions between these strains. As previously described for L. monocytogenes (Cotter et al., 2001), it may be possible that the GAD does not contribute to the growth of B. cereus ATCC 10987 in acidic environments because the gene encoding the glutamate/GABA antipporter is lacking.
Senouci-Rezkallah et al. (2011) studied the impact of amino-acid presence on the acid tolerance response of B. cereus ATCC 14579 strain. They observed that survival to an acid shock at pH 4.0 of B. cereus cells grown at pH 7.0 was enhanced in the presence of glutamate and strongly enhanced in the presence of arginine or lysine. However, the presence of these amino-acids had no impact on the acid tolerance of pre-adapted cells when submitted to acid shock. They also observed that the presence of glutamate, arginine or lysine increased the pH of B. cereus cells grown at pH 7.0 during exposure at pH 4.0 whereas it had no significant influence on pH of B. cereus cells grown at pH 5.5 during acid shock, suggesting an induction of these systems under these growth conditions (Figure 3). As B. cereus ATCC 14579 does not possess the gene encoding for the GAD, the glutamate may be decarboxylated by the arginine decarboxylase, as described in Lathyrus sativus (Ramakrishna and Adiga, 1975).

**METABOLISM MODIFICATION AND ALKALI PRODUCTION**

Depending on their environment, bacteria will establish specific pathways allowing their survival or growth. Several metabolic pathways have been associated with bacterial growth at low pHs. Upon acid stress, alkal production is often observed and well established in oral bacteria such as Streptococcus gordonii, Streptococcus parasanguinis, Streptococcus rattus, Streptococcus sanguis (Burne and Marquis, 2010). Urea and arginine are two major substrates for alkal generation by oral biofilms colonizing the teeth (Lemos and Burne, 2008). Urea is in all salivary gland secretions and is rapidly hydrolyzed to ammonia and CO2 by bacterial ureases which arginine is primarily catabolized to ornithine ammonia and CO2. The increased tolerance of cells results from the production of NH3, which combines with protons to form ammonium. Furthermore, the generated ATP can enable extrusion of cytoplasmic protons by the Na+ / H+ - ATPase as shown in L. monocytogenes or in LAB (van de Guchte et al., 2002).

**AMMONIA PRODUCING MECHANISM**

Arginine deiminase or the ADI pathway (for review, see Lu, 2006) has been identified in a variety of Gram-positive bacteria, including Bacillus spp., L. monocytogenes, and several lactic acid bacteria (LAB; Cumin et al., 1986; Ryan et al., 2009). The ADI pathway converts arginine to citrulline and ammonia. Subsequently, citrulline is metabolized into ornithine generating carbon dioxide, ammonia and ATP. Arginine and ornithine are exchanged via an antiporter importing arginine and exporting ornithine. The resulting NH3 rapidly reacts with H+ and helps to alkalize the environment consuming proton and forming ammonium. Furthermore, the generated ATP can enable extrusion of cytoplasmic protons by the Na+ / H+ - ATPase.

Another ammonia producing mechanism associated with low pH resistance is urease. The urease enzyme catalyzes the hydrolysis of urea, generating two molecules of ammonia and one molecule of carbon dioxide. Urea is well studied in the human pathogen Helicobacter pylori in which urease plays an important role in the ability of stomach colonization and in virulence (Eaton et al., 1991). Urea is present in various environments in which B. cereus can be found, including soil, food, and the human body, where urea is present in all fluids and is finally excreted in the urine as a detoxification product (Mобиль and Hauinger, 1989; Burne and Chen, 2000). Rasko et al. (2004) identified an urease cluster composed of nine genes (from BCE3637 to BCE3666) in the genome of B. cereus strain ATCC 10987 that is not present in all sequenced strains belonging to B. cereus (Mols and Abee, 2008). This cluster harbors three genes ureA, urd and ureC, encoding structural enzymes, four genes (ureEFGD) encoding accessory proteins, and two additional genes ureD and mukT respectively encoding a putative urea (acetamine) transporter and a nickel transporter. Mols...
Fermentative pathways

The metabolic pathways involved in fermentative growth, such as lactate, alcohol, and butanediol pathways are involved in B. subtilis cell acid stress response as shown by their up-regulation upon low pH of growth (Cruz Ramos et al., 2000; Wilks et al., 2009). AlsS condenses two molecules of pyruvate to form acetolactate, condensing two molecules of pyruvate to form acetolactate, condensing two molecules of pyruvate to form acetolactate, condensing two molecules of pyruvate to form acetolactate, condensing two molecules of pyruvate to form acetolactate. Lactate dehydrogenase, a cytoplasmic NADH-linked enzyme that metabolizes pyruvate into acetate and ammonia. Arginine and ornithine are exchanged via an antiporter importing arginine and exporting ornithine. Ammonia reacts with proton forming ammonium.

**FIGURE 4** Graphical representation of the arginine deiminase (ADI) pathway implied in the acid stress response of Bacillus cereus. The ADI pathway converts arginine to citrulline and ornithine. Citrulline is then metabolized into ornithine generating carbon dioxide and ammonia. Arginine and ornithine are exchanged via an antiporter importing arginine and exporting ornithine. Ammonia reacts with proton forming ammonium.

Acetoin can then be excreted by the cells. Alcohol dehydrogenase is an NAD(P)-dependent dehydrogenase that may remove acidity and transfer electrons to the electron transport chain (ETC, Reid and Fewson, 1994). Lactate dehydrogenase, encoded by ldh, is a cytoplasmic NADH-linked enzyme that converts pyruvate to lactate to remove acidic compounds, restoring the NAD+/NADH balance. During fermentation, Ldh is the key enzyme involved in respiration of the NADH formed by glycolysis (Cruz Ramos et al., 2000). In Streptococcus oralis and Streptococcus mutans, it was shown that many enzymes involved in glycolysis are up-regulated during growth at low pH (Wilkins et al., 2001, 2002). It has been suggested that the increase in the amount of these proteins may result in an increase in ATP production and consequently increased proton extrusion via the F1F0-ATPase. In B. subtilis, acid conditions also up-regulate a large number of NAD(P)-dependent dehydrogenases such as alanine dehydrogenase (alsD), succinato-semialdehyde dehydrogenase (gasD), and several putative formate dehydrogenases (foxD, yqgC, yrhE, and yrhG). These enzymes are able to remove acidity through NAD(P)H which transfers electrons to the electron transport system and pumps protons out of the cell (Wilks et al., 2009).

Mols et al. (2010a) showed that a set of 25 genes of B. cereus ATCC 14579 are differentially expressed in lethal or non-lethal organic or inorganic acid conditions and a set of 146 genes for all non-lethal acid conditions. Up-regulation concerned mainly genes involved in energy metabolism, oxidative and general stress response. Pyruvate metabolism, the tricarboxylic acid cycle (TCA) and fermentation pathways were induced to maintain intracellular ATP levels and/or the redox balance. In lethal acid conditions, increase of lactate dehydrogenase (ldh) and cytochrome bd oxidase (cydAB) gene expression are shown. In B. subtilis, these genes are co-ordinately expressed together with the lactate permease gene lctP and the formate-nitrite transporter gene yjwC and under control of the negative regulator YqfH (René Larsson et al., 2005). Lactate dehydrogenase in concert with the cytochrome bd oxidase has been proposed to function as an alternative electron transport chain (Chai et al., 2009). The alsSD is also up-regulated upon exposure to lethal or non-lethal acid stresses, but this up-regulation is less pronounced upon lethal conditions than under non-lethal acid stress. Together with the alsSD genes, cydAB, ldh, and lctP form a distinct regulon, which is part of the Fnr regulon (René Larsson et al., 2006). By analogy with B. subtilis, the induction of these genes may be associated to a changing in NADH/NAD + ratio. It could be noted that under lethal concentration of acetic acid B. cereus showed some resemblance with the response of Staphylococcus aureus cells deficient of murF which exhibit a reduced peptidoglycan synthesis. Indeed, these cells down-regulated iron uptake associated genes and, induced ifd, lactate permease, and formate/nitrite transporter protein genes (Sobral et al., 2007).

Mols et al. (2010b) also investigated the impact of lethal hydrochloric acid stress on B. cereus ATCC 14579 cells. Genes encoding for Adhs and lactate dehydrogenases appeared to be also induced upon exposure to lethal acid stresses (Figure 5). Therefore, the conversion of pyruvate to ethanol or lactate, generating CO2 and dissipating H+, may be an ultimate futile response...
of *B. cereus* to deal with low intracellular pH or restoration of NAD/*NADH* balance. Furthermore, some metabolism rearrangements were also found to be correlated with lactic acid or acetic acid stress. For instance, several genes involved in glycolysis are moderately up-regulated upon exposure to lactic acid stress (2 mM undissociated acid; Mols et al., 2010a). Nevertheless, their functions remain to be elucidated.

**SECONDARY OXIDATIVE STRESS**

The limited tolerance for oxygen is evident in the cases of obligate anaerobes and microaerophilic microorganisms, which cannot grow in air saturated media, but it applies as well to aerobes bacteria which also deal with the toxic side-effect of O2. Indeed, aerobic organisms use molecular oxygen (O2) for respiration or oxidation of nutrient to obtain energy. Reactive by products of oxygen, such as superoxide (O2−), hydrogen peroxide (H2O2), and the highly reactive hydroxyl radicals (OH·), are generated continuously in cells growing aerobically. Therefore, aerobic microorganisms could survive only because they contain antioxidant defenses. The biological targets for the reactive oxygen species (ROS) are DNA, RNA, proteins, and lipids. In some bacteria, such as *Borrelia burgdorferi*, the membrane could be the primary targets of ROS (Boylan et al., 2008). However, it has been shown that the most damaging effect of ROS in bacteria result from the interactions of H2O2 with Fe2+, generating reactive OH by the Fenton reaction. Because the Fe2+ is localized along the phosphodiester backbone of nucleic acid, DNA is a major target of OH (reviewed by Imlay, 2003). The effect of ROS on proteins is the oxidation of thiols, resulting in disulfide bond formation (Leichert et al., 2003). Living organisms have developed various defenses to protect themselves against ROS damage, some are enzymatic (catalases, superoxide dismutases, SOD), thiolredoxins, and peroxidases) and others are non-enzymatic (glutathione, vitamin A, C). In bacteria, oxidative stress is sensed and lead to the activation of specific transcriptional regulators which will induce defense mechanisms when ROS concentration exceeds a critical level (Demple, 1991; Farr and Kogoma, 1991; Storz and Imlay, 1999). Furthermore, it is known that some other stressors could generate a bacterial response similar to the oxidative stress response. Indeed, this secondary oxidative stress response have been described upon exposure to salt, heat or cold temperatures, bile, starved and more recently upon lethal antibiosis (McMenik et al., 2008; Havelaar et al., 2010; Rantsiou et al., 2011; Brul et al., 2012). Up to now, two main different approaches to integrate bacterial behavior into predictive modeling are known with the identification and quantification of (i) signaling and metabolic pathway with flux balance analysis (Kaufman et al., 2005; Metris et al., 2012), or (ii) biomarkers (den Besten et al., 2010, 2015; Pestricek et al., 2012, 2013). The official US National Institute of Health definition of a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (Atkinson et al., 2001). When applied to industrial process conditions, we propose to adapt this commonly medical oriented definition as a characteristic that is objectively measured and evaluated as an indicator of bacterial responses to food processes and stress conditions. den Besten et al. (2010) described a strategy to identify biomarkers for cell robustness of *B. cereus*. Shortly, both unstressed and mild stress treated cells were exposed to lethal stress conditions (severe heat, acid and oxidative stress) to quantify the robustness of *B. cereus* using the ratio of log10N/log10N0, with N being the survivors quantified on agar plate at a defined time and N0 the inoculum (unstressed cells) used for inactivation treatment. This framework enabled the identification of candidate biomarkers, i.e., enzymatic activity, protein levels and genes expression obtained by reverse transcription followed by quantitative PCR (RT-qPCR) quantifications. Linear correlations between induced biomarker and induced robustness upon exposure to mild stress, revealed three kinds of biomarkers defined as “no-response biomarker,”
“short-term biomarker” and “long-term biomarkers.” If this first approach proposed linear correlation, in 2012 we proposed an integrative approach encompassing both gene expression quantification throughout bacterial inactivation and mathematical modeling of the bacterial behavior to identify different molecular biomarkers to further predict the acid resistance of *B. weihenstephanensis*. The sigB gene was proposed as biomarker to track moderate acid resistance whereas katA was identified as biomarker to track high acid resistance. Indeed, fitting surviving bacterial counts of *B. weihenstephanensis* under lethal acid conditions (pH 4.6), allowed the identification of a biphasic patterns meaning that the bacterial population could be divided into two subpopulations with different acid resistances. By correlating the proportion of the two subpopulations with the gene expression, it could be shown that the highest expression of sigB was observed when the sensitive subpopulation represented the majority of the bacterial population. At the opposite the katA up regulation was correlated to the more resistant subpopulation. In 2013, we also proposed both linear and non-linear correlations between gene expression and acid survival ability of short adapted cells. Two kinds of biomarkers were defined with (i) direct biomarker genes for which the expression patterns upon mild stress treatment were linearly correlated to induced acid resistance; and (ii) long-acting biomarker genes which were upregulated for mild stress adaptation times of 30 min at maximum and linked to increased resistance over studied time (60 min).

However, if the selection of potential biomarkers offers new perspectives for the prediction of bacterial behavior and physiology, one of the key challenges will be to increment these data into mathematical model to predict growth or inactivation, during industrial processes to offer decision making tools for food safety and quality management (Breul et al., 2012; Desriac et al., 2012, 2013). The development of such models will contribute to support the food business sector competitiveness by optimizing inactivation process monitoring, eco efficient processing and accurate shelf life products. Thus, the integration of bacterial physiological state into predictive microbiology behavior will offer new tailor made decision making tools which could be used for getting the proper balance between food safety and food quality.

**CONCLUSION**

Nowadays, food industries use mild preservation and processing techniques, in response to consumers demand for fresher, healthier and better foods and because mild preservation techniques save energy and are more environmental friendly. These mild preservation techniques, such as hurdles, may lead to the survival of spoilage and/or pathogenic microorganisms. Therefore the adaptive stress response and the physiology of bacteria is an important subject. *B. cereus* which can spoil food and cause food-borne illnesses encounter acid conditions in foods and upon ingestion and has to overcome the acid barrier of the human stomach (Clavel et al., 2004; Wijnands et al., 2009). For its control in food, it is
especially relevant to understand how B. cereus cells grow and survive in adverse conditions.

The acid stress response of B. cereus could be divided into four groups (i) general stress response (ii) pH homeostasis, (iii) metabolic rearrangements and (iv) secondary oxidative stress response (Figure 6). The current knowledge, presented in this review, may lead to the identification of biomarkers of bacterial behavior. Indeed the finding of molecular biomarkers to characterize the bacterial physiological state under specific conditions remains a key issue for food industry (Kort et al., 2009; den Besten et al., 2010, den Besten et al., 2012, 2013). Actually, the combination of molecular tools and predictive microbiology concepts appears as an interesting challenge for food formulation and preservation optimization (McMenkin et al., 2009; Havlaur et al., 2010; Rantsiou et al., 2011; Brul et al., 2012; Desric et al., 2012).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commer- cial or financial relationships that could be construed as a potential con- flict of interest.

Received: 17 July 2013; paper pending publication: 18 August 2013; accepted: 09 September 2013; published online: 02 October 2013

 Citation: Desriac N, Broussolle V, Postollec M, Mathot A-G, Sohier D, Coroller L, van Schaik W, Tempelaars M, H., De Vos W, M., and Boor KJ (2013) Bacillus cereus: potential biomarkers. Front. Microbiol. 4:204. doi: 10.3389/fmicb.2013.00204

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