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Minireview

Bacillus cereus responses to acid stress

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Summary

Coping with acid environments is one of the prerequisites for the soil saprophytic and human pathogenic lifestyle of *Bacillus cereus*. This minireview highlights novel insights in the responses displayed by vegetative cells and germinating spores of *B. cereus* upon exposure to low pH as well as organic acids, including acetic acid, lactic acid and sorbic acid. Insights regarding the possible acid-inflicted damage, physiological responses and protective mechanisms have been compiled based on single cell fluorescence microscopy, flow cytometry and transcriptome analyses.

Introduction

Bacillus cereus is a common human pathogen that can cause two distinct types of food-borne diseases and other types of infection (Kotiranta *et al*., 2000). Upon ingestion, diarrhoeic strains can produce enterotoxins, such as haemolysin BL, cytotoxin K and non-haemolytic enterotoxin (Schoeni and Wong, 2005), causing abdominal pain and watery diarrhoea (Stenfors Arnesen *et al*., 2008). The other type of food-borne illness involves intoxication caused by the emetic toxin cereulide produced by some *B. cereus* strains (Ehling-Schulz *et al*., 2004). Cereulide is pre-formed in food and because it remains stable upon heat and acid exposures, the toxin is still active after cooking and stomach transit (Kramer and Gilbert, 1989). Upon ingestion of cereulide typical symptoms may occur within 1–6 h that resemble *Staphylococcus aureus* intoxication (Le Loir *et al*., 2003), including nausea, vomiting and general malaise. The symptoms are generally mild; however, in rare cases liver failure has been noted resulting in fatalities (Mahler *et al*., 1997; Dierick *et al*., 2005). Besides being an important food-borne pathogen, *B. cereus* is also a notorious food spoilage organism. Food spoilage is caused by growth of unwanted bacteria in food and causes enormous expenses for food industry (Gram *et al*., 2002). *Bacillus cereus* mainly causes spoilage of milk and dairy products, because it is able to form endospores. These spores are survival vehicles formed upon nutrient shortage and are metabolically inactive (de Vries, 2006). Spores are extremely resistant to stress conditions, such as radiation, high temperature, freezing, drying and acid conditions (Setlow, 2006).

Spores and vegetative cells of *B. cereus* can be found in a wide range of environments (Fig. 1), such as soil (von Stetten *et al*., 1999; Vilain *et al*., 2006), plant rhizosphere (Berg *et al*., 2005) and various foods (Choma *et al*., 2000; Rosenquist *et al*., 2005). *Bacillus cereus* can also be isolated from faeces of healthy adults (Ghosh, 1978), suggesting that *B. cereus* can be part of the microbiota found in the human gastrointestinal tract. The human stomach and small intestine are acidic environments that have to be overcome by spores and/or vegetative cells to become infectious. Outside the human host, *B. cereus* may also be frequently exposed to acidic conditions including a vast array of foods at low pH, where in specific cases organic acids have been added as preservatives (Keijser *et al*., 2007). Additionally, the natural reservoir of the soil saprophyte *B. cereus* may also be acidic upon the exudation of protons and organic acids in the plant rhizosphere (Neumann and Martinoia, 2002). The antimicrobial activity of organic acids is pH-dependent with the maximum effect occurring at low pH values. At these low pH values organic acids are in undissociated states. Because undissociated acid molecules are uncharged and lipophilic, they will penetrate plasma membranes and thus enter cells. Theoretically, the higher-pH environment of the cell's cytoplasm promotes the rapid dissociation of acid molecules into charged protons and anions. These charged molecules cannot subsequently diffuse back across the plasma membrane. Thus, a permeant organic acid stresses the cell by importing protons, depressing

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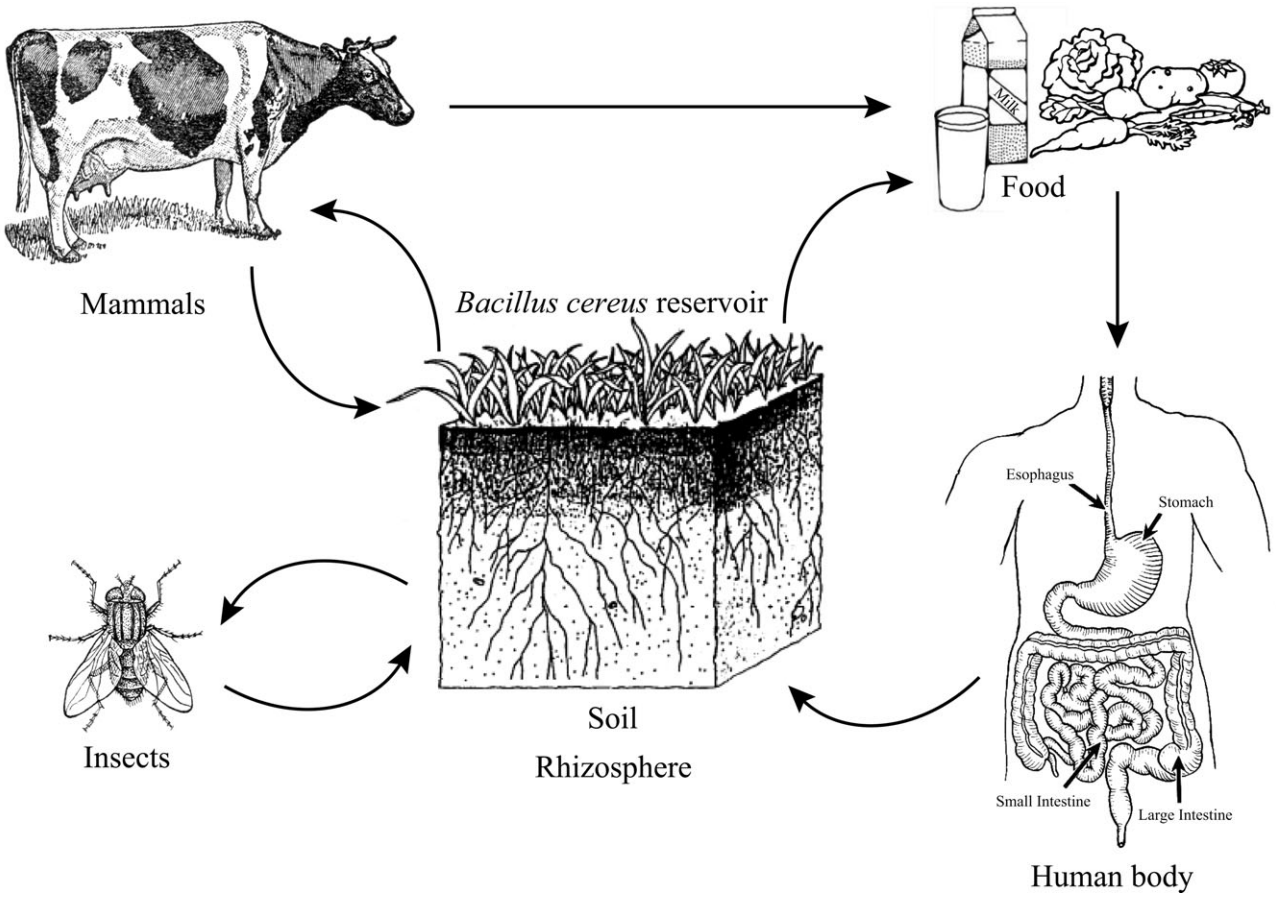

Fig. 1. Transmission routes of the food-borne human pathogen *Bacillus cereus*, with a variety of niches indicated from which vegetative cells and/or spores can be isolated (Mols, 2009).

cytoplasmic pH, and by concentrating the organic anion within the cytoplasm in proportion to the transmembrane pH difference (Brul and Coote, 1999). These effects may be counteracted by the cell at the expensive ATP when it tries to extrude protons or metabolize undissociated organic acid molecules (Mols *et al*., 2010b). Apparently, coping with acid conditions is a determining factor in *B. cereus*' successful colonization of different niches.

Acid stress responses of Gram-negative organisms, such as *Escherichia coli* and *Salmonella* Typhimurium (Richardson *et al*., 2001), and in a select number of Gram-positive bacteria, such as lactic acid bacteria and *Listeria monocytogenes* (van de Guchte *et al*., 2002; Cotter and Hill, 2003; Ryan *et al*., 2009) have been reviewed. These reviews highlight the importance of proton pumps, i.e. F_1F_0-ATPase, transcriptional regulators, such as RpoS (Gram-negatives) and σ^B (Gram-positives), proteins involved in protection of macromolecules, such as DnaK and GroESL, and enzymes that produce alkaline compounds, such as the ammonium-forming enzymes urease and arginine deiminase. Until recently, no detailed information was available on the acid stress responses of *B. cereus*. Fluorescence techniques, physiological studies and transcriptome analyses elucidated acid stress responses of vegetative cells and germinating spores of *B. cereus*, including novel observations such as the formation of reactive oxygen species (ROS) and the induction of a secondary oxidative stress response (Thomassin *et al*., 2006; Mols *et al*., 2009; 2010a,b; den Besten *et al*., 2010; Biesta-Peters *et al*., 2010a,b; van Melis *et al*., 2011a). The aim of this minireview is to provide an overview in the physiological responses, possible acid-inflicted damage and protective mechanisms displayed by *B. cereus* upon exposure to acid conditions.

Response of vegetative cells to acid stress

The physiological response of vegetative cells of *B. cereus* upon exposure to acid conditions, including exposure to organic acids, the putative protective mechanisms and the acid-induced secondary oxidative stress are discussed in the following section.

Physiological response to acid conditions

Upon exposure to acid conditions the growth of *B. cereus* is readily affected (Biesta-Peters *et al*., 2010a,b; Mols *et al*., 2010a,b). The growth rate declines and the lag-phase increases when vegetative cells are subjected to a lowered pH with or without additional organic acids (Biesta-Peters *et al*., 2010a,b). To what extent the growth rate and lag-phase are affected is highly dependent on initial growth rate, strain and acidulant used. During the acid-induced lag-phase *B. cereus* cells are repairing damage, increasing the internal pH and increasing ATP concentration before growth resumes (E.G. Biesta-Peters, M. Mols, M.W. Reij and T. Abee, unpubl. results). The presence of organic acids next to the lowered pH of the medium increases the growth-diminishing effects of the acid-exposed culture. Exposure to 15 mM lactic acid and 2 mM acetic at pH 5.5 acid stops the growth while without the additional organic acids only the growth rate was decreased (Fig. 2A). When the pH of the environment is acidified further, vegetative cells cannot resume growth and are eventually inactivated (Fig. 2B).

Bacillus cereus displays a so-called acid tolerance response. When vegetative cells were previously exposed to a mildly lowered pH, e.g. pH 6.3, they become resistant to normally lethal pH values, for instance pH 4.6 (Jobin *et al*., 2002; Thomassin *et al*., 2006). Additionally, exposure to mildly acidic environments may result in enhanced protection when cells are subsequently exposed to lethal heat or hydrogen peroxide stress, a process often referred to as cross-protection (den Besten *et al*., 2010). Acid tolerance responses and cross-protection phenomena can have great implications for controlling *B. cereus* growth and occurrence in food products and food-processing equipment. Therefore, it is necessary to understand the acid stress response of *B. cereus* and the putative mechanisms deployed to protect against acidic conditions.

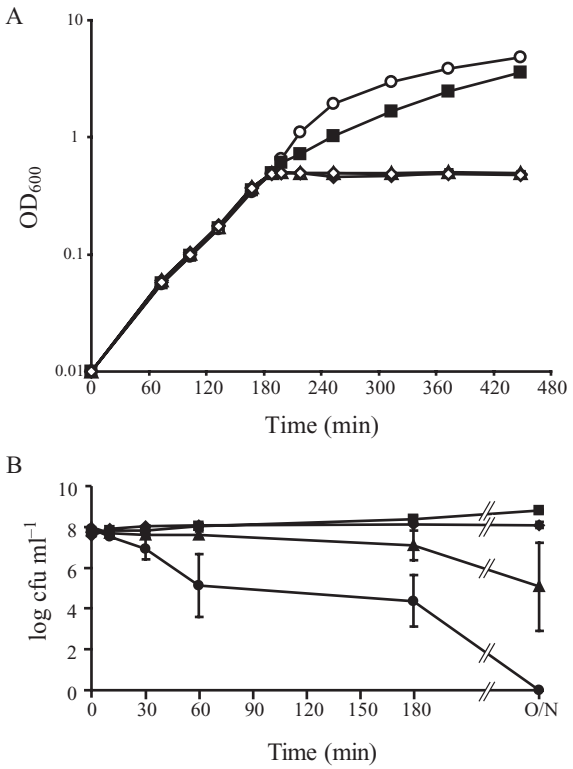

Fig. 2. Physiological responses of *B. cereus* upon exposure to various low-pH conditions. Upon reaching OD 0.5, the pH of the cultures was adjusted to pH 5.5 using HCl (filled squares), 2 mM undissociated lactic acid (open triangles), 2 mM undissociated acetic acid combined with HCl (filled diamonds) or 15 mM undissociated acetic acid (open diamonds) as acidulants. The non-stressed control culture is depicted with open circles (A) (Mols *et al*., 2010b). Upon reaching OD 0.5, the pH of the cultures was adjusted to pH 5.4 (squares), pH 5.0 (diamonds), pH 4.8 (triangles) and pH 4.5 (circles). Subsequently colony-forming units (cfu) were determined at different time points (B) (Mols *et al*., 2010a). O/N represents data obtained from samples taken after incubation over night.

Protective mechanisms

General stress response. Mechanisms of acid resistance in other Gram-positive organisms have been reviewed by Cotter and Hill (2003). Most studies were performed on the acid stress response of non-respiring lactic acid bacteria in the presence of oxygen. In contrast, *B. cereus* actively respires in the presence of oxygen and therefore the results obtained from these lactic acid bacteria should only be extrapolated cautiously to *B. cereus.* Besides differences between the organisms reviewed by Cotter and Hill (2003) and *B. cereus*, there are common mechanisms putatively playing a role in acid resistance as indicated by induction of the corresponding genes (Fig. 3 and Table 1). Genes involved not only in acid stress response but also in responses to other stresses, including protein repair chaperones *groESL* and *dnaK* and *clp* genes, were shown to be upregulated upon exposure to acid conditions in *B. cereus*. Furthermore, several transcriptional regulators are putatively involved in the acid stress response. The expression of *sigB*, the gene encoding for alternative sigma factor σ^B, was induced, which is in agreement with previous studies (van Schaik *et al*., 2004). Heat stress regulators *ctsR* and *hrcA* (van de Guchte *et al*., 2002) were also upregulated upon exposure to low pH in *B. cereus*, indicating possible common damaging factors and protective mechanisms in different stress conditions. These general stress response mechanisms have been described to be involved in cross-protection of *B. cereus* exposed to various stress conditions, including low pH, and can be used as biomarkers for bacterial robustness (den Besten *et al*., 2010).

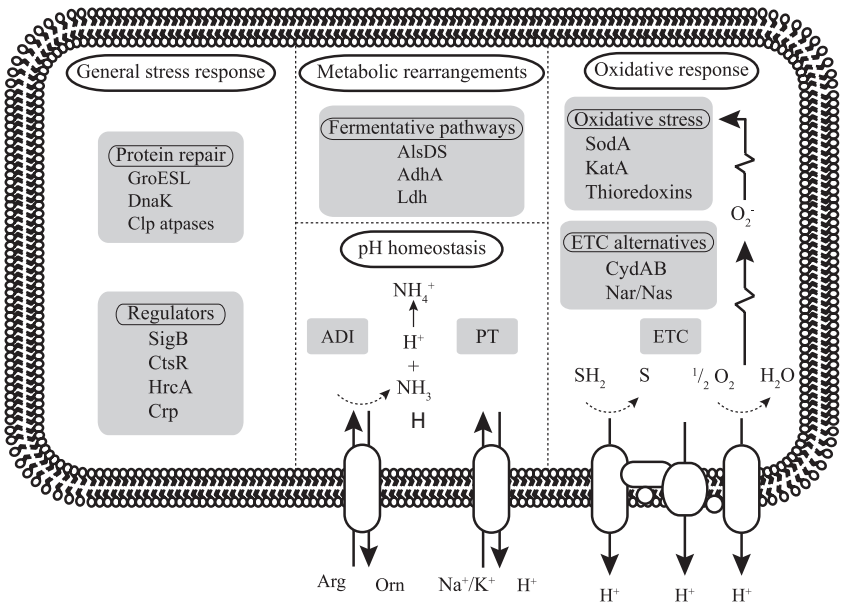

Fig. 3. Graphical representation of general acid stress-associated mechanisms in *B. cereus* divided in four different groups: (i) general stress response, (ii) metabolic rearrangements, (iii) pH homeostasis and (iv) oxidative response. The general stress response group involves genes that are putatively not only induced by low pH, but may be involved in a more general response to stresses. The transcription of protein repair mechanisms, including the chaperones GroESL and DnaK and the Clp proteases, as well as several transcriptional regulators, such as σ^B (SigB), CtsR, HrcA and Crp, was changed upon low pH exposure. The most notable metabolic rearrangements shown upon exposure to mainly organic acid stress were fermentative pathways, such as acetoin production (AlsDS), alcohol (AdhA) and lactate dehydrogenases (Ldh) and rerouting of pyruvate metabolism. pH homeostasis involves proton-dependant transporters (PT) that may transport protons inwards and outwards. The arginine deiminase (ADI) pathway mediates intracellular proton consumption and this pathway is induced in *B. cereus* upon low pH exposure. Oxidative response may not be directly involved in the resistance to acid stress; however, genes involved in oxidative stress were shown to be heavily upregulated upon exposure to low pH. The electron transfer chain (ETC) is conceivably disturbed by a low pH, generating superoxide. Superoxide can lead to the formation of other reactive oxygen species and may induce oxidative stress mechanisms, including thioredoxins, catalase (KatA) and superoxide dismutase (SodA). Furthermore, the perturbation of the ETC is corroborated by the expression of alternatives for the ETC, such as cytochrome *d* ubiquinol oxidase (CydAB) and nitrate/nitrite reductase (Nar/Nas). Other abbreviations used: S, substrate; Arg, arginine; Orn, ornithine.

Table 1. Acid stress responses of *B. cereus*.

Putative acid resistance mechanism	Expression upon acid shocks	Presence in genome of *B. cereus* ATCC 14579	Presence in genome of *B. cereus* ATCC 10987
General stress response			
σ^B	Induced	Yes	Yes
Clp protease	Induced	Yes	Yes
Chaperones	Induced	Yes	Yes
Metabolic rearrangements			
Acetoin biosynthesis	Induced	Yes	Yes
Alcohol dehydrogenase	Induced	Yes	Yes
pH homeostasis			
F_1F_0-ATPase	Repressed	Yes	Yes
Proton pump	Induced[a]	Yes	Yes
Urease	Induced[b]	No	Yes
Glutamate decarboxylase	Unchanged	No	Yes
Secondary oxidative stress			
Catalase	Induced	Yes	Yes
Superoxide dismutase	Induced	Yes	Yes
Nitrate/nitrite reductase	Induced	Yes	No

a. Repressed upon mild acid shocks and induced upon lethal acid shocks.
b. Induced at mild pH; however, urease activity did not lead to increased resistance (Mols and Abee, 2008).

Metabolic rearrangements. Metabolic rearrangements, known to be involved in acid resistance in lactic acid bacteria, were also induced by *B. cereus* upon low pH exposures (Fig. 3 and Table 1). Genes encoding for enzymes catalysing the reaction from pyruvate to acetoin and butanediol, i.e. *alsDS*, were induced upon exposure to acid shocks. Although such reaction is at the expense of pyruvate, it removes intracellular protons and forms carbon dioxide (CO_2). Also in *Bacillus subtilis alsSD* genes are strongly induced under mild acid stress conditions (Wilks *et al*., 2009) and in *Lactobacillus plantarum* activation of the corresponding enzymes contributed to pH homeostasis (Tsau *et al*., 1992). Genes encoding for alcohol dehydrogenases and lactate dehydrogenases were induced upon exposure to lethal acid shocks. Therefore, the conversion of pyruvate to ethanol or lactate, generating CO_2 and consuming protons, may be an ultimate futile response of *B. cereus* to deal with low intracellular pH (pHi) or restoration of NAD^+/NADH balance. Some metabolic rearrangements were found specifically correlated with lactic acid or acetic acid stress, such as metabolic pathways for amino acid metabolism (Mols *et al*., 2010b), but their functions remain to be established. Metabolomics and mutant analysis will aid in unravelling the role of these metabolic changes in organic acid resistance.

pH homeostasis. Upon exposure to acid conditions, many bacteria activate enzymes contributing to pH homeostasis (Fig. 3 and Table 1). Cells may pump protons out of the cell, prevent protons from leaking in and counteract acidification of the cytoplasm by producing alkaline compounds. Aerobic bacteria, such as *B. cereus*, use their electron transport machinery to transport protons over the cell membrane generating an excess of protons on the outside of the cell thus generating a proton motive force (PMF). The PMF is subsequently used to generate ATP by inward flux of protons via F_1F_0-ATPase. In lactic acid bacteria, and presumably also in anaerobically growing *B. cereus* cells, F_1F_0-ATPase can also transport protons outside the cell at the expensive of ATP in acid conditions. *Bacillus cereus* represses the expression of genes encoding for subunits of F_1F_0-ATPase upon exposure to mild acidic environments (Mols *et al*., 2010a). Conceivably, *B. cereus* does not use F_1F_0-ATPase to pump protons out of the cell in aerobic acid conditions and by repressing F_1F_0-ATPase genes and lowering the amount of active ATPase, the influx of protons is limited. Notably, upon exposure to lethal levels of acidity these genes are not repressed. Also other proton transporters, such as *napA* and *nhaC*, were downregulated upon exposure to mild acid stress. Interestingly, these genes were (highly) induced upon exposure to lethal pHs, indicating a fine balance between proton influx and ATP synthesis on one hand and on the other hand proton pumps regulating pHi at the expense of ATP. In addition, amino acid decarboxylases may contribute to homeostasis of pHi in bacteria (Cotter and Hill, 2003; Foster, 2004). In Gram-positives, especially *L. monocytogenes*, glutamate decarboxylase (GAD) has been associated with acid resistance (Cotter *et al*., 2001). Glutamate is converted to gamma-aminobutyric acid (GABA) consuming an intracellular proton by GAD. Subsequently, the product GABA is exchanged with extracellular glutamate by a glutamate/GABA antiporter. Such a transporter has been found to be necessary for optimal GAD-dependant acid resistance in *L. monocytogenes*. In the genome of the *B. cereus* type strain ATCC 14579 no GAD system could be identified. Another sequenced *B. cereus* strain, ATCC 10987, does harbour a glutamate decarboxylase gene (Mols *et al*., 2007) that was however not found to be differentially expressed upon exposure to low pH values (Mols *et al*., 2010a), indicating that the role of GAD in the acid resistance of *B. cereus* ATCC 10987 is limited. An explanation for this phenomenon is the fact that a glutamate/GABA antiporter gene is lacking in the genome of ATCC 10987. Bacteria can also counteract a low internal pH by the production of alkaline compounds, such as ammonia. One of the mechanisms known to produce ammonia and involved in acid resistance in other bacteria is the arginine deiminase pathway (ADI) (Ryan *et al*., 2009). The ADI pathway converts arginine into ammonia and CO_2 via citrulline and carbamoylphosphate. Although the role of ADI in acid resistance in other bacteria is evident, in *B. cereus* the ADI genes are only moderately upregulated upon exposure to acid in aerobic conditions, whereas the ADI pathway was found highly upregulated under mildly acidic anaerobic conditions, suggesting that this system may play a role in acid stress survival in anaerobic conditions (van der Voort and Abee, 2009). Arginase, which also converts arginine to citrulline producing ammonia, was highly induced upon exposure to low pHs, indicating that arginine catabolism may support acid tolerance in *B. cereus.* Another well-known mechanism of alkali production is the hydrolysis of urea into ammonia and CO_2 by the enzyme urease. Urease is known to be involved in the acid resistance of several bacteria and well-studied in *Helicobacter pylori* and *B. subtilis* (Mobley *et al*., 1995; Wray *et al*., 1997). Urease and concomitant ureolytic activity is shown by strain ATCC 10987, in contrast to type strain, which does not harbour the urease genes. The genes encoding for the urease enzyme were somewhat induced upon exposure to sublethal pH 5.4 in ATCC 10987 (Mols *et al*., 2010a). However, it was shown that the ureolytic activity in a variety of *B. cereus* strains, including ATCC 10987, did not provide for acid

resistance and that its role was solely in nitrogen metabolism (Mols and Abee, 2008).

Secondary oxidative stress response

The exposure of *B. cereus* to inorganic acid as well as organic acids, lactic acid and acetic acid in aerobic conditions revealed a major oxidative response (Mols *et al*., 2010a,b). This secondary oxidative stress response (Mols and Abee, in press) was indicated by the induction of oxidative stress associated genes, including genes encoding for thioredoxins, catalases, superoxide dismutase and the major oxidative stress regulator PerR, upon exposure to acid shocks. Thioredoxins are known to control the reduced state of thiol groups that can be oxidized upon exposure to oxidative stress (Holmgren, 1985). Superoxide dismutase and catalase convert superoxide and hydrogen peroxide into water (Imlay, 2003) and superoxide dismutase has been suggested to be involved in the acid tolerance response of *B. cereus* (Browne and Dowds, 2002; Jobin *et al*., 2002). PerR is a hydrogen peroxide sensing transcriptional regulator associated with the expression of genes encoding catalases and peroxidases (Mongkolsuk and Helmann, 2002). The induction of these oxidative stress-associated genes suggests that oxidative compounds are generated upon exposure to low pHs in *B. cereus*. Indeed, ROS such as hydroxyl (OH·), peroxynitrite ($ONOO^-$) (Mols *et al*., 2010a) and superoxide (O_2^-) (M. Mols, M. Ceragioli and T. Abee, unpubl. results) are shown to be generated when *B. cereus* cells are exposed to bactericidal pHs. These ROS may be generated at specific sites in the aerobic electron transfer chain (ETC). Acid shocks may affect ETC activity since expression of genes encoding alternative electron donor and acceptor mechanisms was found to be induced. In the *B. cereus* type strain the most prominent induction upon exposure to acid shocks was that of nitrate and nitrite reductase genes (Mols *et al*., 2010a). Nitrate can act as an alternative electron acceptor and is converted to nitrite in a reaction consuming a proton (Richardson *et al*., 2001). Subsequently the resultant nitrite can be reduced to ammonium consuming five protons. Whether nitrate and nitrite reductases are induced because they form an alternative ETC, restore NAD^+/NADH balance, and/or because the reactions they catalyse consume intracellular protons remains to be elucidated. Also other alternative components of the ETC were associated with mainly lethal levels of organic and inorganic acid shocks (Mols *et al*., 2010a,b). Cytochrome *bd* oxidase (*cydAB*) genes, which may act as an alternative complex IV of the ETC, were highly induced upon exposure to 15 mM acetic acid and at pH 4.5. Cytochrome *bd* oxidase has been proposed to function in an alternative electron transport chain together with NAD(P)H-dependant dehydrogenases, such as lactate (*ldh*) and alcohol dehydrogenase (*adhA*) (Chai *et al*., 2009). Lactate dehydrogenase (*ldh*) and cytochrome *bd* oxidase genes are coordinately expressed together with the lactate permease gene *lctP* and formate-nitrite transporter gene *ywcJ* and under control of the negative regulator YdiH (Rex) in *B. subtilis* (Larsson *et al*., 2005). Together with the *alsSD* genes, *cydAB*, *ldh* and *lctP* form a distinct regulon, which is part of the larger anaerobic-responsive Fnr regulon (Reents *et al*., 2006), indicating a clear association between these upregulated genes and anaerobic conditions. Furthermore, *B. cereus* is more resistant to acid stress when grown and exposed under oxygen limitation (Mols *et al*., 2009). Whether activation of these enzymes and pathways contribute to higher acid resistance of *B. cereus* when grown and exposed without oxygen remains to be elucidated.

Response of spores germinating in acid conditions

Bacillus cereus is able to form endospores that allow the organism to survive adverse conditions. These spores are formed upon nutrient shortage and are metabolically inactive (de Vries, 2006). They can be isolated from many environments and are very resistant to harsh conditions, including low pH and high organic acid concentrations. When spores encounter more favourable conditions, they can germinate into vegetative cells and subsequently grow (Setlow, 2003; Hornstra *et al*., 2006; Paredes-Sabja *et al*., 2011). The process of germination is of interest, because it is the transition between inactive spores to metabolically active and possibly virulent vegetative cells. Recently, the effect of low pH with the addition of sorbic acid on the germination of *B. cereus* has been studied (van Melis *et al*., 2011a). The germination of *B. cereus* spores in mildly acidic pH (pH 5.5) is not affected (Fig. 4). The rate of outgrowth into dividing vegetative cells, on the other hand, is decreased. In the presence of sorbic acid such decreased outgrowth rate is also observed. Furthermore, the presence of sorbic acid delays the germination and decreases the germination efficiency. With microscopic observations and flow cytometry, the transition from dormant phase bright spores to phase dark spores was shown to be delayed and the process of germination and outgrowth is stuck at the phase dark spore with no outgrowth visible. The reduced germination efficiency may relate to the hydrophobicity of sorbic acid, causing it to accumulate in the spore's inner membrane. This accumulation may interfere with the signalling cascade that is required for germinant receptor-mediated germination (van Melis *et al*., 2011b). Transcriptome analysis of spores germinated in the presence of sorbic acid indeed revealed genes involved in membrane biogenesis and cell envelope modifications. Notably, the transcriptome analyses

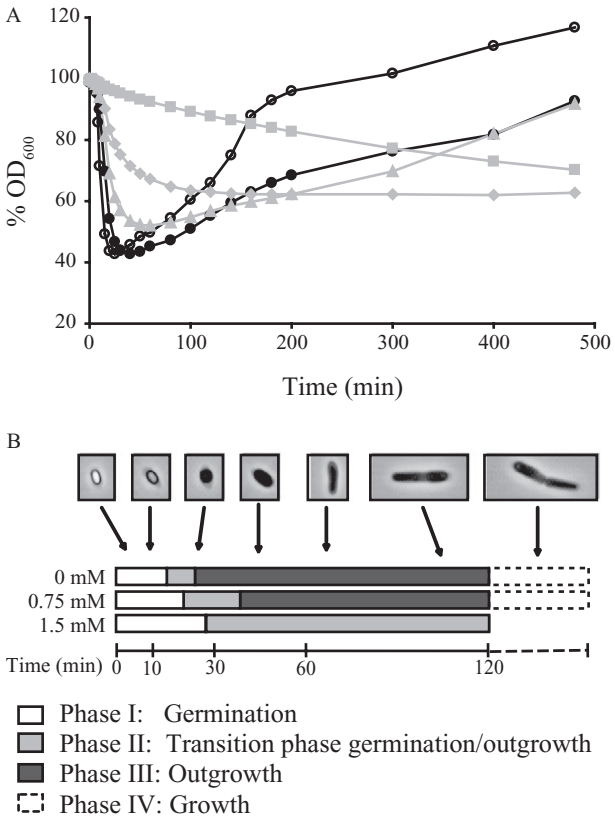

Fig. 4. The impact of sorbic acid on germination and outgrowth of *B. cereus* spores (adapted from van Melis *et al*., 2011a). Germination and outgrowth were followed in time by the transitions of phase bright (dormant) spores to phase dark (germinated spores) by the change in optical density (A). Spores were germinated either at pH 7.1 (black, open circles), at pH 5.5 without added sorbic acid (black, closed circles) or with 0.75 mM (grey, closed triangles), 1.5 mM (grey, closed diamonds) or 3.0 mM (grey, closed squares) undissociated sorbic acid. The *y*-axis shows the change in optical density (OD) relative to the OD at initiation of germination. Germination and outgrowth was followed in time using microscopy (B) showing the transition of phase bright spores, to phase dark spores and eventually to dividing vegetative cells. Germination at pH 5.5 shows a prolonged outgrowth phase, germination at pH 5.5 with 0.75 mM undissociated sorbic acid is slower indicated by a prolonged germination phase, and spores germinated at pH 5.5 with 1.5 mM undissociated sorbic acid are not capable of growing out to vegetative cells.

revealed that spores are triggered to germinate and initiate vegetative growth irrespective of whether conditions for outgrowth were acidic or not. Gene expression data showed that genes that are induced in the control and sorbic acid stressed spores largely overlap in the initial stage of germination. This corroborates observations made in *B. subtilis*, where detailed transcriptome analysis revealed spore germination to occur via a tightly controlled spore outgrowth programme (Keijser *et al*., 2007). The spore outgrowth programme of *B. cereus* indeed shows high similarity with that of *B. subtilis* and is not influenced by the presence of organic acids or a low pH.

Concluding remarks

The responses of *B. cereus* vegetative cells to acid environments resemble the responses seen in other Gram-positive organisms. However, there are several crucial differences including the findings that *B. cereus* does not utilize all possible protective mechanisms, such as transporting protons outwards via F_1F_0-ATPase and producing ammonium via urease, in aerobic conditions. Furthermore, the rearrangements in energy production and conversion are striking. The induction of alternative ETC components may indicate a protective mechanism; however, the correlation with the generation of a secondary oxidative stress response is more evident. This secondary oxidative stress response originates from acid-induced malfunctioning of the ETC resulting in ROS formation. These ROS are proposed to be part of a common mechanism of cellular death in *E. coli* exposed to bactericidal antibiotics (Kohanski *et al*., 2007). Although actively respiring cells and germinating spores induce oxidative stress-associated genes upon exposure to acid environments and ROS are formed, the role of ROS in the cellular death remains to be established. However, not only ROS formation and the induction of oxidative stress responses determine the cell fate, as indicated by the inactivation of anaerobically grown and exposed *B. cereus* cells. The insights obtained in recent studies and reviewed here contribute to our understanding of *Bacillus*' physiology in various acid environments and may provide leads to optimize the efficiency of existing and new food preservation strategies.

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