RESEARCH ARTICLE

The pathogenic biomarker alcohol dehydrogenase protein is involved in Bacillus cereus virulence and survival against host innate defence

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Abstract

Bacillus cereus is a spore forming bacteria recognized among the leading agents responsible for foodborne outbreaks in Europe. B. cereus is also gaining notoriety as an opportunistic human pathogen inducing local and systemic infections. The real incidence of such infection is likely underestimated and information on genetic and phenotypic characteristics of the incriminated strains is generally scarce. We have recently analyzed a large strain collection of varying pathogenic potential. Screening for biomarkers to differentiate among clinical and non-clinical strains, a gene encoding an alcohol dehydrogenase-like protein was identified among the leading candidates. This family of proteins has been demonstrated to be involved in the virulence of several bacterial species. The relevant gene was knocked out to elucidate its function with regards to resistance to host innate immune response, both in vitro and in vivo. Our results demonstrate that the adhB gene plays a significant role in resistance to nitric oxide and oxidative stress in vitro, as well as its pathogenic ability with regards to in vivo toxicity. These properties may explain the pathogenic potential of strains carrying this newly identified virulence factor.

Introduction

Bacillus cereus is an ubiquitous spore forming human pathogen. It is present in soil, foods, almost all surfaces in hospital settings, and human skin. It is the second leading cause of collective foodborne outbreaks in France after Staphylococcus aureus and the third in Europe [1–3]. B. cereus was associated with 155 outbreaks, 1,636 illnesses and 44 hospitalizations in Europe in 2019 according to reports by 27-member states. B. cereus can induce two types of gastrointestinal diseases, leading to emetic or diarrhoeal syndromes. B. cereus can also cause severe systemic infections, especially in immunocompromised patients leading to patient death in approximately 10% of cases [4–9]. However, some B. cereus strains can cause severe and even fatal infections in healthy people [10]. The pathogenic potential of B. cereus is thus extremely variable, with some strains being harmless and others lethal [11].
B. cereus produces toxins such as Hbl, Nhe, and CytK that induce cell toxicity [12–14]. In addition, other factors such as HlyII, InhA1, CwpFM or Mfd have been implicated in B. cereus resistance against the host immune system [15–21]. These toxins provide an indication of the strain toxicity potential [13, 22–24]. However, these factors do not allow the discrimination of strains according to their pathogenicity. Indeed, several studies have shown that the Nhe production by hazardous strains is variable and that non-pathogenic strains can also produce it in large quantities [1, 24]. Moreover, these toxins do not appear to be suitable markers for strains causing non-gastrointestinal infections [22].

B. cereus strains that induced severe gastrointestinal or non-gastrointestinal disorders do not carry neither hbl, ces, hlyII, cytK1 nor cytK2 genes and did not produce the Nhe protein, implying that other still unknown factors were responsible for their pathogenicity [1, 11].

Accordingly, we have recently analyzed a large strain collection comparing strains that induced an infection (intestinal or otherwise) with non-pathogenic strains [11, 25]. The large strain screening allowed to identify a combination of four as yet undescribed biomarkers, wherein their presence/absence allows an accurate identification of clinical B. cereus strains [26]. Three of these genes are located on the bacterial chromosome, and the fourth one is located on a large plasmid in a region that could be defined as a novel pathogenicity island for B. cereus [27]. These findings constitute a huge step in the understanding of the B. cereus pathogenic potential and complexity and may provide tools to better assess the risks associated with B. cereus contamination. Among these genes, adhB, was identified as a leading candidate [26]. This adhB gene encodes an alcohol dehydrogenase-like protein (ADH). This family of enzymes is involved in oxidation-reduction biological process. ADH are involved in metabolic and physiological processes in a variety of organisms, including fermentative metabolism [28], the oxidation of alcohols as carbon and energy sources [29], protection against anaerobic stress [30], and maintenance of the intracellular redox balance [31].

In this study, the adhB gene was knocked out to better elucidate its function during B. cereus virulence. Our results demonstrate that adhB plays a significant role in resistance to nitric oxide (NO) and oxidative stress in vitro, as well as its pathogenic ability with regards to in vivo infection and toxicity. These properties may explain the pathogenic potential of strains carrying this newly identified virulence factor.

**Materials and methods**

**Bacterial strains**

This study includes 35 B. cereus strains isolated from human patients following systemic or local infections and 21 non-pathogenic strains (Table 1). The 35 strains of the clinical collection were isolated from patient samples (biopsy, blood culture, etc) from nine French voluntary hospitals between 2008 and 2014. The samples and information were collected for a previous study and were treated anonymously and thus not subjected to personal consent [22]. The non-pathogenic strains have been isolated from food, where no infection was reported in humans. They were further tested in cell and animal models and did not induce any pathologies [23, 25]. We have previously shown a correlation between cytotoxicity and virulence [11]. Nevertheless, although these strains had previously been shown to be weakly cytotoxic to human cells and to have reduced virulence in an insect infection model, this does not rule out their potential ability to produce symptoms in specific vulnerable populations (i.e. the elderly, immunocompromised, or premature/new-born babies).
Table 1. Characteristics of non-pathogenic (A) and clinical (B) strains.

A

| Non-pathogenic strains | Source                                      | adhB |
|------------------------|---------------------------------------------|------|
| INRA-PF_S09            | Milk protein                               | 0    |
| I13_S10                | Cooked rice                                | 1    |
| INRA-5_S11             | Pasteurized zucchini puree                  | 0    |
| INRA-C64_S12           | Pasteurized vegetables                      | 0    |
| ADRIA-I3_S13           | Cooked foods                               | 0    |
| INRA-BN_S36            | Vegetable                                  | 1    |
| INRA-PA_S37            | Milk protein                               | 0    |
| INRA-A3_S38            | Starch                                     | 1    |
| I23_S39                | Cooked apple                               | 0    |
| SB_S40                 | Soil from a vegetable field                 | 0    |
| I11_S41                | Cooked food                                | 1    |
| INRA-C1_S42            | Pasteurized vegetables                      | 0    |
| INRA-C46_S43           | Pasteurized vegetables                      | 0    |
| INRA-SL_S44            | Soil                                       | 0    |
| INRA-SO_S45            | Soil                                       | 0    |
| INRA-BC_S47            | Vegetable                                  | 1    |
| I2_S48                 | Dried fruit                                | 0    |
| INRA-RL_S49            | Vegetable                                  | 0    |
| ADRIA 121_S50          | Cooked foods                               | 0    |
| INRA-SV_S51            | Soil                                       | 0    |
| WSBC 10204_S52         | Pasteurized milk                           | 0    |

B

| Clinical strains       | Age of patients   | Type of sampling | Symptoms                                                                 | Outcomes                      | adhB |
|------------------------|-------------------|------------------|--------------------------------------------------------------------------|-------------------------------|------|
| 09CEB13BAC_S6          | Premature newborn | Blood culture    | Brain abscess                                                            | Recovery                      | 1    |
| 09CEB14BAC_S7          | Premature newborn | Blood culture    | Bacteremia                                                               | Recovery                      | 1    |
| 09CEB33BAC_S8          | Newborn           | Axilla-later feces| Skin infection                                                           | Recovery                      | 1    |
| 12CEB31BAC_S14         | Premature newborn | Blood culture    | Organ failure and pulmonary and cerebral abscesses                      | Death                         | 1    |
| 13CEB06BAC_S15         | 86                | Blood culture from catheter | Heart failure, ventilator-associated pneumonia, ischemic stroke        | Recovery                      | 1    |
| 09CEB11BAC_S16         | Premature newborn | Blood culture    | Meningitis, infection in the liver, both lungs                          | Death                         | 1    |
| 09CEB16BAC_S17         | Newborn           | Umbilical        | Local colonization                                                       | Recovery                      | 1    |
| 12CEB30BAC_S18         | Premature newborn | Blood culture    | Sepsis                                                                   | Recovery                      | 1    |
| 12CEB40BAC_S20         | 63                | Blood culture    | Bacteremia and central venous catheter-linked infection                 | Recovery                      | 1    |
| 12CEB46BAC_S21         | 61                | Blood culture    | Sepsis (patient with an acute myeloid leukemia)                         | Recovery                      | 1    |
| 12CEB47BAC_S22         | 43                | Blood culture    | Bacteremia                                                               | Recovery                      | 1    |
| 12CEB51BAC_S23         | 60                | Blood culture    | Sternal abscess, absent fever                                            | Sequela of osteitis           | 1    |
| 13CEB01BAC_S24         | 31                | Prosthesis from tibia | No clinical sign of infection                                   | Recovery                      | 1    |
| 09CEB12BAC_S53         | Premature newborn | Cerebrospinal fluid | Meningitis, infection in the liver, both lungs                          | Death                         | 1    |
| 09CEB34BAC_S59         | Premature-        | Stomach-tube feeding | Premature birth                                                        | Recovery                      | 1    |
|                         | newborn           |                  |                                                                          |                               |      |
| 09CEB36BAC_S61         | Premature-        | Central venous catheter | Bacteremia                                                          | Recovery                      | 1    |

(Continued)
For all the strains, a single colony was picked, resuspended in 100 μL Tris-EDTA NaCl buffer (TEN) and incubated at 98˚C for 10 min. After centrifugation to pellet cell debris, 1 μl of supernatant was used as DNA matrix. The PCR mixture for gene detection contained 1 μl DNA matrix, 0.5 μM primer (forward: TTATTATCTATTCTTTCGTGTGATGC, and reverse CTATTTGTAGCAGAACATTC), 10 μL DreamTaq Green PCR Master Mix (2X) (Thermo Scientific) in a final volume of 20 μL. Thermal cycling was carried out in a Mastercycler nexus (Eppendorf) with the following program: a start cycle of 3 min at 98˚C, followed by 30 cycles of 20 s at 98˚C, 30 s at 55˚C, and 1 min at 72˚C, and a final extension time of 10 min at 72˚C. PCR fragment sizes were revealed on 1.5% agarose gels containing Midori Green, and visualised by a UV imaging device as previously described [26].

**adhB gene detection by PCR**

For all the strains, a single colony was picked, resuspended in 100 μL Tris-EDTA NaCl buffer (TEN) and incubated at 98˚C for 10 min. After centrifugation to pellet cell debris, 1 μl of supernatant was used as DNA matrix. The PCR mixture for gene detection contained 1 μl DNA matrix, 0.5 μM primer (forward: TTATTATCTATTCTTTCGTGTGATGC, and reverse CTATTTGTAGCAGAACATTC), 10 μL DreamTaq Green PCR Master Mix (2X) (Thermo Scientific) in a final volume of 20 μL. Thermal cycling was carried out in a Mastercycler nexus (Eppendorf) with the following program: a start cycle of 3 min at 98˚C, followed by 30 cycles of 20 s at 98˚C, 30 s at 55˚C, and 1 min at 72˚C, and a final extension time of 10 min at 72˚C. PCR fragment sizes were revealed on 1.5% agarose gels containing Midori Green, and visualised by a UV imaging device as previously described [26].

**adhB mutant generation**

The Bt407 Cry with the reference genome Bacillus thuringiensis Bt407: NC_018877.1 was used as a model for B. cereus and was renamed Bc 407.

Knock-out of the adhB gene (WP_000438843) was accomplished by double-cross over gene substitution by use of the pMAD vector [32]. Briefly, using the available sequencing information of the Bc407 strain, 600 bp regions upstream and downstream of the identified gene of interest were synthesized surrounding a tetracycline-resistance cassette by the GeneCust company (Boynes, France). The upstream nucleotide coordinates used are 2,575,680 to 2,576,279, and the downstream nucleotide coordinates are 2,577,204 to 2,577,802. The synthesized region was then ligated into the pMAD vector. This vector was further transformed by heat shock into chemically competent NEB-10 beta cells. The plasmid was then extracted and transformed

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**Table 1.** (Continued)

| Strain ID | Gene | Disease | Infection Site | Outcome |
|-----------|------|---------|----------------|---------|
| 12CEB34BAC_S64 | 80 | Thoracentesis | Pulmonary infection | not known |
| 12CEB37BAC_S90 | 30 | Blood culture | Endocarditis | Death |
| 12CEB38BAC_S91 | 65 | Blood culture | Sepsis | Death |
| 12CEB39BAC_S92 | 54 | Blood culture | Sepsis | Recovery |
| 12CEB42BAC_S94 | 63 | Blood culture | Bacteremia and central venous catheter-linked infection | Recovery |
| 12CEB43BAC_S95 | 63 | Blood culture | Bacteremia and central venous catheter-linked infection | Recovery |
| 12CEB44BAC_S96 | 34 | Blood culture | Bacteremia | Recovery |
| 12CEB45BAC_S97 | newborn | Blood culture | Kidneys and urinary infections | Recovery |
| 12CEB48BAC_S98 | 66 | Blood culture | Bacteremia (patient with a colorectal cancer) | Recovery |
| 12CEB49BAC_S99 | 24 | Blood culture+ skin infection | Sepsis and aplastic anemia caused by drugs | Recovery |
| 12CEB50BAC_S100 | 77 | Blood culture | Bacteremia (patient with breast cancer) | Recovery |
| 12CEB52BAC_S101 | 40 | Blood culture | Bacteremia (immunocompromised patient) | Recovery |
| 13CEB03BAC_S102 | 76 | Blood culture | Community acquired pneumonia | Recovery |
| 13CEB07BAC_S105 | 24 | Blood culture | Abdominal pain, shivering, vomiting, fever, diarrhea | Recovery |
| 13CEB09BAC_S106 | 85 | Liver abscess | Sepsis, hepatitis c and liver abscess, abdominal pain, diarrhea | Recovery |
| 13CEB30BAC_S107 | not known | Blood culture | Nausea, abdominal pain and vomiting | not known |
| 14CEB16BAC_S114 | Premature newborn | Blood culture from peripheral veins | Septic shock, multiple organ failure, pulmonary and cerebral abscesses | Death |
| 14CEB17BAC_S115 | Premature newborn | Bronchial aspiration (lung) | Septic shock and pneumonia | Death |
| 14SBC1987_S116 | not known | Biopsy (kidney) | Vomiting and diarrhea | Death |

The absence (0) or presence (1) of the adhB gene was detected by PCR.

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into *E. coli* strain ET to facilitate de-methylation of the plasmid, increasing subsequent transformation into *B. cereus Bc407* as previously described [16]. Resulting colonies were then subjected to temperature stress at 40˚C to force the incorporation of the resistance cassette leading to the stable knock-out of the *adhB* gene, which was verified by PCR with oligonucleotide sequences flanking the cloned region. The mutation was stable and sequencing revealed that the mutation occurred at the corrected place and did not affect the flanking regions. The resulting strain was designated as Δ*aadhB*.

Wild type and mutant strains were streaked onto BHI agar from 20% glycerol stocks to obtain isolated colonies. Colonies were inoculated into BHI broth and grown at 37˚C, 200 rpm until mid to late-exponential phase for phenotypic analysis. Cultures in mid-exponential phase were used for microscopy to determine cellular morphology. For growth assays, stocks were inoculated into BHI broth and followed by sampling for CFU/ml at regular intervals.

**Nitric oxide (NO) stress survival**

*B. cereus Bc407* and the Δ*aadhB* mutant were grown to late-exponential phase. Cultures were harvested and diluted 1:1000 in RPMI (Gibco Glutamax, Fisher Scientific, Illkirch Cedex, France) and further grown at 37˚C without agitation with differing doses of the NO donor, NOC-5 (3-[2-hydroxy-1-(1-methylethyl)-2-nitrosohydrazino]-1-propanamine (Calbiochem, Sigma-Aldrich, Saint-Louis, MO, USA). NOC-5 was dissolved in NaOH 0.01 M and used at the following concentrations: 0, 15.6, 25, 31.25, 50, 62.5, 100, 125, 250, 500 μM. After 1 h, bacteria were agitated to avoid sedimentation and the survival rate was quantified after 4 h by plating serial dilutions on LB agar plates. Data are pooled from two to four independent experiments and presented as % survival = (NO-treated/Buffer-treated) × 100.

**Oxidative stress survival**

Oxidative stress-resistance was determined as previously described [33]. Briefly, wild-type and Δ*aadhB* mutant strains were grown and 2 h post-inoculation, 500 μl of each culture was added to 100 μl of either sterile water or hydrogen peroxide at final concentrations of 2 mM or 10 mM. Treated (2 mM or 10 mM H₂O₂) and control (H₂O) cultures were incubated for 10 min at 37˚C and then serially diluted in phosphate-buffered saline (PBS) and plated on BHI to stop the reaction and count CFU/ml. Data are pooled from two independent experiments and presented as % survival = (H₂O₂-treated/H₂O-treated) × 100.

**Insect infection trial**

*B. cereus Bc407* and the Δ*aadhB* mutant were grown to exponential phase. Cultures were harvested and serially diluted 1:4 in peptone water prior to injection. 10 to 20 last instar *Galleria mellonella* larvae were used following a 24 h fast as previously described [34, 35]. 10 μl of bacterial preparations at various doses were injected between the second and third body segment from the rear of the insect. Injected insects were incubated at 37˚C for 24 h, following which survival was assessed. Peptone water was injected as negative control. Data are pooled from three independent experiments and presented as % survival = (injected with strain/injected with water) x 100.

**Protein bioinformatic analysis**

The protein sequences of the ADH protein (WP_000438843) was analysed with Pfam to find functional domains. E-values are based on searching the Pfam-A family against UniProtKB 2018_04 using HMM search.
**Statistical analysis**

Statistical analysis was performed with GraphPad Prism version 7. Insect survival curves were assessed by non-linear regression, constraining the bottom to 0.

Bacterial survival rate following stresses were also analysed by non-linear regression, and the statistical differences were calculated with a Wilcoxon test between the conditions with or without stress.

**Results**

**adhB as a marker of clinical B. cereus strains**

The presence/absence of the adhB gene was assessed by PCR on a collection of strains of varying pathogenic potential: 21 non-pathogenic strains and 35 clinical strains (Table 1). adhB was present in 34/35 (97%) clinical isolates, whereas it was present in 5 of 21 (24%) non-pathogenic isolates. We thus hypothesised that adhB may be a new and important virulence factor of *B. cereus*.

The amino acid sequences of the Bc407 gene WP_000438843 coding for a protein of the AdhB family was analysed using the Uniprot database (Fig 1). This enzyme of 308 amino acids belongs to the zinc-containing alcohol dehydrogenase family. The software identified two domains, with the catalytic domain of the alcohol dehydrogenase containing an inserted zinc-binding domain. This domain has a GroES-like structure [36, 37]. The co-factor-binding domain of the enzyme is located proximal to the C-terminus. Structural studies indicate that it forms a classical motif called Rossmann fold that reversibly binds NAD(H) as a co-factor [38, 39].

**Growth characteristics and morphology**

*B. cereus* Bc407 and the ΔadhB mutant were grown in BHI medium at 37˚C, 200 rpm and bacterial growth was followed by measuring the OD_{600} and CFU/mL determined by serial dilution and plating (Fig 2A and 2B). The two strains presented similar rates of growth with no significant differences in growth curves. The strains were observed under the microscope and bacterial morphology shows that the two strains are similar in cellular shape and size, with the adhB mutant often making longer chains of cells (6–8 cells) (Fig 2C and 2D).
Nitric oxide (NO) and oxidative stress resistance

To assess the role of AdhB in the resistance to the host immune system response, *B. cereus* Bc407 and ΔadhB strains were incubated with the NO donor to test their resistance against NO stress (Fig 3). Several doses of NO were assessed and the dose inhibiting 50% of bacterial growth (IC50) was calculated. The IC50 of *B. cereus* wild type (WT) strain is approximately 4 times higher than that of the mutant (193 vs 45 μM of NO) and the survival rate of the mutant is lower at each concentration of NO tested. Thus, the mutant adhB is more sensitive to nitric oxide than the wild type strain.

Then, oxidative stress resistance of *B. cereus* Bc407 WT and ΔadhB strains was determined after exposure to 2 mM or 10 mM H2O2 for 10 min at 37˚C (Fig 4). Wildtype Bc407 demonstrated increased resistance at both concentrations, with survival percentage being 14-fold higher at 2 mM, and 20-fold higher at 10 mM.

Insect model of *B. cereus* toxicity

The role of AdhB in the pathogenicity of *B. cereus* was assessed in an insect model of infection. *B. cereus* Bc407 and ΔadhB mutant strains were injected at various doses into *Galleria mellonella* larvae (Fig 5). At 24 h post-injection, survival of the insects was assessed. Insects infected with the ΔadhB mutant strain demonstrated higher rates of survival in relation to the wildtype strain, demonstrating a reduced virulence of the mutant strain. Further, statistical analysis of the survival curves reveals a significant difference in the LD50 values between the strains: 4.2 10^3 CFU/injection for the wildtype and 1.5 10^4 CFU/injection for the ΔadhB mutant. HillSlope determined the curves to be distinct at 99.94% probability.
Alcohol dehydrogenase (ADH) is an enzyme involved in oxidation-reduction biological process. It catalyses the reversible oxidation of alcohols and induces the formation of their corresponding acetaldehyde or ketone with the reduction of NAD (Fig 6). This class of enzyme typically has a broad spectrum of action [40, 41]. Here we characterized AdhB as a protein involved in B. cereus resistance to nitric and oxidative stresses, two major components of the host immune system, and in its pathogenicity.

Currently three types of alcohol dehydrogenases are known, that differ structurally and catalytically: Zinc-containing ‘long-chain’ alcohol dehydrogenases, ‘short-chain’ alcohol dehydrogenases, and iron-containing alcohol dehydrogenases [42, 43]. The AdhB (WP_000438843) protein in B. cereus is a zinc-containing ADH. These enzymes are typically dimeric or tetrameric proteins, which require two atoms of zinc per subunit to be functional, however, catalytic activity is maintained in the presence of a single zinc atom. The zinc atoms interact with either cysteine or histidine residues; the catalytic zinc being coordinated by two cysteines and one histidine. Zinc-containing ADH’s are found in bacteria, mammals, plants, and fungi. Normally, there is more than one isozyme per species (e.g. humans possess at least six isozymes and yeast have three). Consistently, we identified three Zinc-containing ADH’s in the Bc407 strain (WP_000438843, WP_000649129.1, WP_000645827.1). These three isozymes share common structures with two identified domains (not shown). The first is the catalytic domain that might contain an inserted zinc-binding domain. This domain has a GroES-like structure; a name derived from the superfamily of proteins with a GroES fold. Proteins with a GroES fold structure have a highly conserved hydrophobic core and a glycy1-aspartate dipeptide,
Fig 4. H₂O₂ sensitivity. The wild-type and ΔadhB mutant strains were grown and subsequently exposed to either 2 mM or 10 mM of hydrogen peroxide for 10 min at 37°C. Bacterial survival was assessed by plating and normalized against buffer-treated controls. Data points correspond to the mean ± SEM of the values obtained from 2 biological replicates.

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Fig 5. Insect infection. Bacterial virulence was determined as Galleria mellonella survival percentage following injection with varying CFU/mL of wild type (triangles, black line) or ΔadhB (circles, dashed line) mutant strains. Survival was measured as live insects following 24 h post-injection. Calculation of the LD50 was done using Graphpad software.

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which is thought to maintain the fold. The second is the domain that binds its cofactor NAD owing to its motif denoted as a Rossman fold [38, 39].

In order to specify the role of AdhB in *B. cereus*, the virulence of the wild type and ΔadhB mutant was tested in an insect infection model. *G. mellonella* larvae were used as a model of infection as *B. cereus* is both a human and an insect pathogen [25, 44]. This study reveals that adhB plays an essential role during *B. cereus* virulence and could thus be considered as a new pathogenic factor.

During human or insect infections, *B. cereus* is able to resist the host immune system and persist. It can indeed survive phagocytosis by macrophages and can induce their apoptosis [20, 45]. The primary mechanism of macrophage-induced cytotoxicity is through the massive production of nitric oxide and oxidative stress at the peak of inflammation leading to bacterial death [46, 47]. Thus, bacterial response to NO is of major importance for bacterial survival and several pathogenic bacteria have developed means for detoxification and repair of the damages caused by NO [48]. We have previously shown that *B. cereus* is particularly resistant to NO [15, 18, 45, 49]. Here, we show that the ΔadhB mutant was more sensitive than the wild-type strain to both oxidative and nitric stresses. Accordingly, this sensitivity may be implicated in the reduced mutant virulence in the insect model.

The initial step of bacterial response to NO and oxidative response is the detection of reactive oxygen and nitrogen species (ROS and RNS), which will permit to activate the detoxification and repair pathways. It has been previously shown that virulence factor production by *B. cereus* is dynamic and shaped by cellular oxidation [50]. ADH proteins have been previously shown to be involved in the reduction of alcohol and the production of NADH. NADPH is required to maintain and regenerate the cellular detoxifying and anti-oxidative defense systems [51]. The antioxidant defense system of *B. cereus* is constituted by an elaborate, often overlapping network of enzymes [52], but to the best of our knowledge, there was no evidence of ADH implication in the resistance of oxidative or NO stress. As oxidative and NO response overlap during the immune response, it is not surprising that mechanisms of bacterial resistance against ROS and RNS share similarities. The reduction capacity of ADH may be involved in NO detoxification. Bacterial capacity to detoxify NO through reduction is widely distributed in denitrifying bacteria but is also present in pathogens. For denitrifying bacteria, the reduction of nitrate to N₂ is part of the nitrogen cycle and prevents NO high toxicity; for pathogenic bacteria, NO detoxification might be a mean to survive under oxygen limited environments and to survive to nitrogen stress [46, 47, 53].

Taken together, we have identified a new virulence factor implicated in *B. cereus* resistance to host immunity whose activities may explain the pathogenic potential of clinical strains carrying this newly identified pathogenic biomarker.

**Author Contributions**

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