Investigative Genetics

REVIEW Open Access

Identifying experimental surrogates for *Bacillus anthracis* spores: a review

David L Greenberg, Joseph D Busch, Paul Keim, David M Wagner*

Abstract

Bacillus anthracis, the causative agent of anthrax, is a proven biological weapon. In order to study this threat, a number of experimental surrogates have been used over the past 70 years. However, not all surrogates are appropriate for *B. anthracis*, especially when investigating transport, fate and survival. Although *B. atrophaeus* has been widely used as a *B. anthracis* surrogate, the two species do not always behave identically in transport and survival models. Therefore, we devised a scheme to identify a more appropriate surrogate for *B. anthracis*. Our selection criteria included risk of use (pathogenicity), phylogenetic relationship, morphology and comparative survivability when challenged with biocides. Although our knowledge of certain parameters remains incomplete, especially with regards to comparisons of spore longevity under natural conditions, we found that *B. thuringiensis* provided the best overall fit as a non-pathogenic surrogate for *B. anthracis*. Thus, we suggest focusing on this surrogate in future experiments of spore fate and transport modelling.

Background

Bacillus anthracis, the causative agent of anthrax, has received much attention in the past decade due to its use in 2001 as a biological weapon distributed through the USA mail system. However, *B. anthracis* spores have been used as a weapon for close to 100 years and, historically, this pathogen was an important disease model [1]. This bacterium also provides a nearly perfect model of prokaryotic clonal evolution, with rare genomic recombination and extremely low levels of homoplasy [2]. The body of research acquired for *B. anthracis* provides key insights into its biology, epidemiology and the risks associated with its release into a civilian environment [3]. However, an important gap still remains in our empirical understanding of *B. anthracis* spore survival and mobility. As a result, it is necessary to examine and develop more accurate fate and transport models of anthrax spores in order to better understand public health risks and develop methods for emergency response to a mass release.

Mathematical fate and transport models provide a means of predicting the distribution of pathogenic particles after their release into air or water. Clearly, such information is an important asset in risk assessment following a terrorist attack or a biological accident. Scenarios for intentional release into a civilian area include infecting the water supply or releasing aerosolized spores [4,5]. In a 1970 report, the World Health Organization predicted that 50 kg of spores released upwind of 500,000 civilians would result in 95,000 fatalities; likewise, a single subway attack could lead to over 10,000 deaths if carried out during rush hour [6]. Model scenarios and the 2001 events demonstrate that non-targeted individuals are also vulnerable. However, models may lack predictive power if their critical parameters are not based on real world values. Therefore, it is necessary to collect experimental data that will lead to greater model accuracy of spore behaviour. For example, our laboratory group is performing experiments to measure attenuation values for spore survivability in natural and artificial environments (such as water, soil and fomites). These and other experiments will help to validate the predictions of current mathematical models, thereby increasing model accuracy and improving our response to natural, accidental or intentional releases of anthrax.

Fully virulent *B. anthracis* must be handled under biosafety level (BSL)-3 conditions and requires secure containment. Therefore, we cannot experimentally release this organism into the environment nor use it in experiments outside of a BSL3 facility. In order to conduct experiments that inform release models, we must use a

* Correspondence: Dave.Wagner@nau.edu
Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ 86011-4073, USA

non-pathogenic bacterium that can accurately represent *B. anthracis*. Surrogates of this type have been used for many years in military release experiments, water supply studies and food protection assessment. However, little attention has been focused on the criteria used to select surrogates. Our synthesis makes use of existing empirical evidence to present an informed decision for the best choice of a *B. anthracis* surrogate.

History of surrogate use for *B. anthracis*

Before selecting an appropriate surrogate for *B. anthracis*, it is useful to review the history of surrogate use for this organism. This information, though anecdotal in some cases, provides valuable information useful for surrogate selection such as (1) comparative survival and behavioural data, (2) an initial list of potential surrogate candidates and (3) baseline data to compare against current experiments. Over the years a number of surrogates have been used, including an attenuated *B. anthracis* strain (Sterne) and several phylogenetic relatives: *B. atrophaeus* (formerly *B. globigii* and *B. subtilis niger* [7,8]), *B. cereus*, *B. megaterium*, *B. mycoides*, *B. subtilis*, *B. thuringiensis* and *Geobacillus* (Figure 1). Table 1 indicates the number of times each has been utilized in published studies. *B. atrophaeus* has been employed most frequently; *B. cereus*, *B. subtilis* and *B. thuringiensis* have been used moderately; and the others have been used just a few times (*B. megaterium*, *B. mycoides* and *Geobacillus*).

Both the USA and Japanese governments used pathogenic simulants in biological warfare test studies. For example, Yoshi Iishi of Japan confessed after World War II to using *B. anthracis* surrogates in his biological warfare programme, which was initiated in 1935 [9]. The USA began using *B. atrophaeus* as their major non-pathogenic surrogate for *B. anthracis* in July of 1943 at Camp Detrick [9]. This surrogate has been used for many experiments in order to ascertain potential outcomes of using anthrax as a biological weapon [10-12]. In 1949 the USA Army experimentally sprayed *B. atrophaeus* and *Serratia marcescens* over the coastal population centers of Hampton, Virginia and San Francisco, California [9]. *B. atrophaeus* was also disseminated in Greyhound bus and New York subway terminals via covert spray generators hidden in briefcases during the mid-1960 s [11]. More recent work at national laboratories has emphasized the detection and identification of spores in the environment.

The earliest in-depth comparison of related *Bacillus* species was done by Schneiter and Kolb [13,14], who tested heat processing methods to destroy 'industrial' spores of *B. anthracis*, *B. subtilis* and *B. cereus* found on shaving brush bristles. Brazis *et al.* [15] made a direct comparison of the effect of free available chlorine on *B. anthracis* and *B. atrophaeus* spores and found that *B. atrophaeus* was more resistant to chlorine. In these early works, no mention is made of the potential for these species to be used as *B. anthracis* surrogates. However, their results provide valuable comparative data (for example, *B. atrophaeus* is more resistant to chlorine and therefore is a conservative surrogate for estimating *B. anthracis* survival in tap water).

More recent experiments have examined the effects of various environmental challenges and disinfectants on *B. anthracis* surrogates, including studies of food protection or decontamination in the wake of a release event. Faille *et al.* [16] used *B. thuringiensis* as a non-pathogenic representative for *B. cereus* and indicated that *B. thuringiensis* has been used in this capacity for many years. Others have used *B. atrophaeus*, *B. thuringiensis*, *B. cereus* and *B. subtilis* to examine decontamination strategies using various bactericidal compounds such as

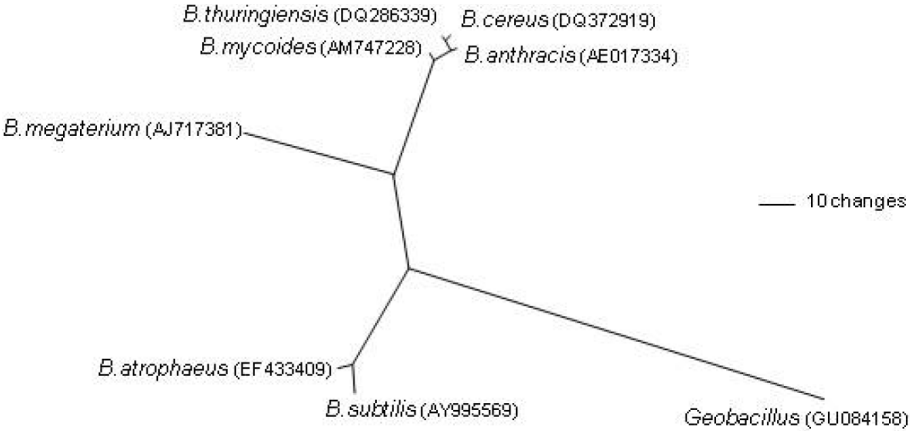

Figure 1 Unrooted phylogenetic tree of *Bacillus anthracis* and potential near-neighbour surrogates. Reconstruction is based on neighbour-joining analysis of 16 s rRNA gene sequences using Jukes-Cantor correction. GenBank accession numbers are provided in parentheses.

Table 1 Number of historical uses for each potential surrogate with references

Species*	No. of uses†	References
Bacillus atrophaeus	40	[15,17,18,27,29,34,40-42,48,50,52,54,68,71,72,75,76,78,83,86-88,94,95,101,102,104,107,109,112-115,174,208,219-222]
B. cereus	29	[22,26,40-43,48,54,58,59,65,66,68-70,72,73,77,82,88,95,103,104,174,213,223-226]
B. subtilis	26	[19,37,40,42-44,48,60,70,82,84,85,88,94,96,100,104-106,174,209,213,216,219,224,226]
B. thuringiensis	26	[16,22,26,27,40-43,48,58,60,66,68,72,81,82,88,94,95,99,100,111,174,192,227,228]
B. anthracis Sterne	20	[25,26,40,43,48,49,58-60,68,72,75,81,103,174,213,223,224,226,229]
B. megaterium	8	[40-42,48,94,102,104,174]
B. mycoides	4	[43,60,72,226]
Geobacillus	3	[37,174,209]

*Strains not identified.
†References through January 2010.

chlorine, hydrogen peroxide, dyes, neutral oxone chloride, formaldehyde, gluteraldehyde and antibiotics [15,17-43]. Additional decontamination methods used against these surrogates include ultraviolet irradiation [39,44-50], plasma [51], electron beam radiation [52,53] and heat [39,54-63].

B. anthracis stand-ins have also played an important role in evaluating the broad arsenal of techniques used to detect and identify bio-threat agents in the environment. At least 17 methods have been employed to detect spores of *B. anthracis* and its relatives, including: electron microscopy [64], atomic force microscopy [65-68], photothermal spectroscopy [69], microcalorimetric spectroscopy [70], biochip sensors [71,72], Raman spectroscopy [73], polymerase chain reaction methods [74-80], optical chromatography [81], differential mobility spectroscopy [82], laser induced breakdown spectroscopy [83-86], flow cytometry sorting [87], mass spectroscopy [88-96], proteomics [97,98], luminescence analysis [99], long-wave biosensors [100], lytropic liquid sensors [101] and fluorescent labelling [102-105]. Although most of these studies used *B. anthracis* directly, some included close relatives for comparisons of detectability across species.

Lastly, surrogates have played an important role in several types of aerosol studies. They have been used to evaluate electrical forces [106,107], examine the effect of filter material on bioaerosol collection [108] and to determine if bees could be deployed to detect anthrax spores in the air [109]. Other studies have used stand-ins such as *B. thuringiensis* to test spore movement in aerial spray [4,110,111], transport and deposition efficiency of spores in ventilation ducts [112], engineered aerosol production [113] and re-aerosolization of spores [114]. *B. atrophaeus* has been used to reproduce an anthrax letter event, demonstrating how an individual swine located 1.5 m from an opened letter inhaled >21,000 spores [115]. This is a lethal dose for humans exposed to *B. anthracis* and validates the significant bio-threat of passive spore dispersion.

From the diverse experimental uses of anthrax surrogates during the last 70 years, it is obvious that non-pathogenic representatives are indispensable for conducting safe inquiries into the behaviour and mobility of pathogen spores. However, not all species are equally appropriate stand-ins for *B. anthracis*. In the remainder of this review we outline our selection criteria, present pertinent literature for surrogate selection in *B. anthracis* and identify gaps in our knowledge of a surrogate's ability to mimic the behaviour of this pathogen. Whenever possible, we present quantified values to provide robust justification of any surrogate to be used in future fate and transport experiments.

Selection criteria

We used several criteria for selection, including (1) the risk of use (pathogenicity), (2) genetic similarity to *B. anthracis*, (3) morphology and (4) response to various chemical and environmental challenges. Our initial list began with microbes in the family Bacillaceae that have been used as surrogates in the past. Practical attributes of potential surrogates are summarized in Table 2. It is important to select appropriate representatives with regard to the specific experiments one wishes to conduct. As an example, if we were interested in studying the disinfectant capacity of a substance we would use a surrogate that has greater survivability than our target organism. The results would then provide conservative estimates of appropriate disinfectant levels. In our case, we are interested in physical experiments of mobility in water and air media. Hence, we determined that the physical properties of the spores are of greatest interest, including size, shape, density, surface morphology, surface structure and surface hydrophobicity. Behavioural responses to stress and natural conditions are also relevant to spore survival.

Surrogate pathogenicity

The risks associated with surrogate use are of critical concern. Table 3 lists the biosafety designations for the

Table 2 Practical attributes in surrogate selection

Attribute	Remarks
Safety	Should not cause illness or infection in animals or plants
Ease of culture	Able to produce with standard microbiological methods in a reasonable timeframe and have reproducibility
History of use	Possibility of attaining comparative information from the literature and judging surrogate behaviour
Ease and speed of detection	Allows large numbers of samples to be processed for rapid feedback of results
Cost	Surrogate production and detection should not be excessive
Stability or persistence	No long-term persistence, or easily decontaminated
Practical for industrial testing	Should not damage equipment or processes

potential surrogates. Surrogates are typically used to replace a pathogen that, if used, would present a potential threat to public health. *B. anthracis* is classified as a BSL-3 organism and work must be conducted under highly contained conditions not suitable for fate and transport experiments. Ideally, an attenuated strain of *B. anthracis* would be a good surrogate because it should behave similarly to the pathogenic strains and pose little risk. However, our knowledge of plasmid exchange rates and the environmental effects of these strains remains very limited - they may still pose a risk despite being classified as BSL-2 organisms. In addition, detection of *B. anthracis* in the environment, even of an attenuated strain, could cause a public relations issue. Worse, released surrogates might mask a real attack or create high background positives and unnecessary emergency responses. Therefore, we feel that non-pathogenic *B. anthracis* strains are not good surrogates for fate and transport experiments.

Another surrogate of interest is *B. cereus*. This species is an opportunistic food-borne pathogen that can infect humans [116,117] and the CDC recommends the handling of the organism at BSL-2 standards. Although it is naturally found in the environment, additional releases of this potential pathogen are deemed unsafe. As such, this organism cannot be used as a replacement for *B. anthracis* in spore release studies. The same is true for *B. megaterium* and *Geobacillus stearothermophilus*, which are treated as BSL-2 organisms.

Table 3 Biosafety levels for the potential *Bacillus anthracis* surrogates (from the Biodefense and Emerging Infections Research Resources Repository)

Species	Biosafety laboratory rating
Bacillus anthracis Ames	BSL 3
B. anthracis Sterne	BSL-2
B. cereus	BSL-2
B. megaterium	BSL-2
B. atrophaeus	BSL-1
B. subtilis	BSL-1
B. thuringiensis	BSL-1
Geobacillus stearothermophilus	BSL-2

BSL, biosafety level.

The other potential surrogates, including *B. atrophaeus*, *B. mycoides*, *B. subtilis* and *B. thuringiensis*, are not typically regarded as potential human pathogens or select agents. They are BSL-1 organisms and are safe candidates. *B. thuringiensis* is used as an insecticide throughout the world, and has been shown to pose no health risk to humans in some studies [118,119]. Infections do occasionally occur, however. These include a case from using commercial *B. thuringiensis* var. *kurstaki* [120], a wound infection identified as *B. thuringiensis* strain 97-27 [74,121], and an isolate recovered from a gastrointestinal illness [122]. That said, the overall the use of most *B. thuringiensis* strains appears to be safe and this species provides a good potential surrogate for *B. anthracis* [118,119]. *B. atrophaeus* is commonly found in soil throughout the world, is considered non-pathogenic and has been used extensively as a surrogate for *B. anthracis* [40,123]. *B megaterium* and *B. subtilis* are also found in the soil and are non-pathogenic to humans. Based on safety concerns, most candidates except *B. cereus* could serve as a surrogate for *B. anthracis*.

Genetics of the potential surrogates

Genetic relationships are important when selecting a surrogate because, theoretically, a phylogenetic relative should be morphologically and behaviorally more similar and have comparable physical characteristics to the target organism. There have been many genetic studies that elucidate the phylogenetic relationships of organisms related to *B. anthracis* [74,98,124-143]. The results of these studies indicate that *B. anthracis* is most closely related to *B. cereus*, *B. thuringiensis* and *B. mycoides*, which are grouped together as the *B. cereus* group (Figure 1). In contrast, *B. subtilis*, *B. atrophaeus*, *B. megaterium*, and *Geobacillus* are more distant relatives of *B. anthracis*. As their chromosomal genomes are very similar, some authors have suggested that *B. cereus*, *B. thuringiensis* and *B. anthracis* are actually a single species separated only by different plasmid composition [130]. However, highly informative genetic markers such as single nucleotide polymorphisms can resolve *B. anthracis* from these near neighbor species [144,145]. The identification of

closely related surrogates does not present a problem when these powerful genetic tools are used. The importance of genetic similarity on spore composition is demonstrated by the *BclA* gene, which is unique to the *B. cereus* group. This protein is found in the exosporium and helps determine the adhesive properties of the spore [146,147]. As *B. atrophaeus* and *B. megaterium* are lacking this gene, we would expect important changes in behavior compared to *B. anthracis*.

Morphology of the potential surrogates

Morphological characters are important to consider when choosing a surrogate because physical behaviours are the foundation of transport models. As stated earlier, genetic relatedness is a good indicator of morphological similarity, so we expect organisms within the *B. cereus* group to be morphologically similar to *B. anthracis*. Microscopy examination reveals few morphological features that can be used to definitively distinguish the various species from one another [64,65,68]. However, spores present measurable differences among surrogates, including the structure of the exosporium, the presence/absence of filamentous appendages and size variation.

The spores of the *B. cereus* group all possess a specific type of exosporium surrounding the outer spore coat. It is a balloon-like sac that envelopes the spore, is made of crystal lattices and, typically, has a short nap of hair-like projections extending off the surface [64-68,146,148-154]. The exosporium can be highly variable, both among *B. anthracis* relatives [155-157] and within *B. anthracis*, as shown by differences between the Vollum and Sterne strains [158]. Some species also have long appendages that extend off the exosporium, known as filaments. *B. cereus*, *B. megaterium* and *B. thuringiensis* all possess filaments, whereas *B. anthracis* has none [64,149-152,158-161]. More distant relatives such as *B. atrophaeus* and *B. subtilis* have neither a nap nor filaments [67,68,152,162]. Likewise, *B. atrophaeus* and *B. megaterium* have an atypical exosporium-like layer that is distinct but does not extend off the surface of the outer coat [64,67,148,152,162-165]. *B. thuringiensis* has a similar nap to *B. anthracis* but the presence or absence of filaments in *B. thuringiensis* is variable [152,166-168]. It is important to note that the exosporium is strongly hydrophobic [169] and that this chemical property may influence flow dynamics in aqueous solutions. Therefore, species with less hydrophobic spores (*B. subtilis*) are probably not appropriate simulants compared to the *B. cereus* group. As differences in exterior morphology will influence the mobility of pathogen spores in air and water, the investigation of these dynamics is a much-needed focus of future research.

Size, shape and density of the spore are also considered important factors that can influence surrogate behavior in release experiments. The spores of the *B. cereus* group have similar ratios of length to width and similar diameters, whereas the spores of *B. atrophaeus* are smaller and those of *B. megaterium* are larger [65,68,170,171]. Although the difference in size is not great, it does exist and may require different coefficients for various model parameters (such as, Reynolds number, diffusion coefficient and sedimentation velocity) [172,173]. Spore volume is strongly correlated to density (R = 0.95) when spores are wet and in a moistened state the smaller spores of *B. atrophaeus* and *B. subtilis* are much more dense than *B. anthracis* [174]. Such differences are likely to affect the behaviour of these particles in air or water. Wet *B. thuringiensis* spores have densities and volumes within the range of *B. anthracis*, making this simulant a better match for the measurement of liquid dispersion. Interestingly, dry spore density is similar among the surrogates listed in Table 1, despite volume differences [174]. Thus, the right choice of surrogate appears to depend on the dispersion medium under consideration.

Comparative survivability among surrogates

Previous experiments comparing the survivability of various spore-formers provide valuable information to the surrogate selection process. Comparative experiments of spore survival under natural conditions or exposure to heat, ultraviolet and chemical disinfectants can illuminate which species may behave similarly to *B. anthracis* in experiments. In this section we review the literature for comparative spore survival.

Quantitative data relating inactivation kinetics of the natural survival of spores would be of great value when comparing potential surrogates. Unfortunately, most of the available data are qualitative. Past studies with *B. anthracis* have revealed that spores may survive for years under natural conditions [175-190]. The data are mostly qualitative, not directly comparable, and primarily exist only for *B. anthracis*. Experimental evidence that quantifies survival rates in both the short and long term are missing. Several studies examined the attenuation rate of *B. thuringiensis* spores on leaves, soil and snow [191-197]; *B. cereus* was included in a survival study measuring the effects of soil pH, moisture, nutrients and presence of other microbes [198]. In addition to two aerosol field studies [110,199], we found no other studies that investigated natural attenuation rates of the potential surrogates for *B. anthracis* or that compared several species at once. Another drawback to using these data is that spore behaviour is variable due to factors such as purification method, sporulation conditions and strain type, and in many of these studies different purification protocols and strains are used, which makes direct comparisons of the values mostly pointless.

Nevertheless these values do have some comparative information that can be used for surrogate selection. For example, natural attenuation values have been quantified for *B. cereus* and *B. thuringiensis* demonstrating that, after 135 days, the number of viable *B. thuringiensis* spores falls to about a quarter of the original inoculum [194]. The same may be true for *B. anthracis* but data are lacking. Although some spores remain active for a long time, the rate at which they lose viability is unknown, which suggests that additional experimental evidence is required to confirm the decay rates for *B. anthracis* spores and the potential surrogates.

Many experiments have been conducted that examine the effects of heat on spores [39,54,57,63,200-208]. However, very few studies have focused on quantifying differences in the survival of spores with regards to surrogate selection. More recent studies have compared the affect of heat on spores with the intention to understand differences among species. The main focus of most of these experiments is related to industrial sanitation, particularly disinfection in the food industry [58-60,62,209-211]. Montville and coworkers [60] have published the only study that specifically compares attenuation values among several surrogates. Whitney *et al.* [39] review some of the studies on the thermal survival of *B. anthracis*, whereas Mitscherlich and March [212] provide a very comprehensive review on the overall survival of *B. anthracis* and many of the potential surrogate candidates. However, it is apparent that the variability of D values (decimal reduction times) within species is large enough that we cannot make any robust decisions based upon this comparative information [60]. Rather, from these data we realize that each strain may behave differently with regards to survivability. As a result, each potential surrogate species should be compared directly with *B. anthracis* in future experimental studies.

Experiments to compare the effect of disinfectants can also be useful for examining parallels in spore resilience. Whitney *et al.* [39] reviewed many of the studies that have performed disinfectant trials on *B. anthracis*. Brazis *et al.* [15] compared the effects of chlorine on *B. atrophaeus* and *B. anthracis* spores and found *B. atrophaeus* survival to be a conservative indicator for *B. anthracis* survival. *B. cereus* spores reasonably simulate *B. anthracis* spore inactivation by peroxyacetic acid-based biocides, but are less reliable for hydrogen peroxide, sodium hypochlorite, and acidified sodium chlorite [213]. Rice *et al.* [26] examined the affect of chlorine on several *B. anthracis* strains and potential surrogates and found that *B. thuringiensis* behaviour was most similar to a virulent *B. anthracis* strain. However, they also found a difference between the attenuated and virulent *B. anthracis* strains, indicating that even very close organisms may behave differently when conditions vary. More recently, Sagripanti *et al.* [40] investigated the effects of various chlorides and other decontaminants on virulent *B. anthracis* and several potential surrogates on glass, metal, and polymeric surfaces.

Over the years many studies have focused on different bactericidal techniques for *B. anthracis* and their comparative effect on survival, including ultraviolet [44,48-50,214] and various chemicals [15,34,39,215]. Two of the ultraviolet studies were geared toward surrogate selection. Nicholson and Galeano [44] validated *B. subtilis* as a good ultraviolet surrogate for *B. anthracis* using the attenuated Sterne strain. However, another study found *B. subtilis* spores were highly resilient to ultraviolet ionizing radiation when immersed in water and concluded this species would be a poor surrogate for *B. anthracis* [216]. Menetrez and coworkers [48] found that *B. anthracis* Sterne was more resistant to ultraviolet than other surrogates, including *B. thuringiensis*, *B. cereus* and *B. megaterium*. Therefore, the data remain equivocal for choosing a stand-in with similar ultraviolet survival characteristics.

The results from the literature search on survivability are useful, but must be used with caution when comparing surrogates. Several authors have noted the high variability observed between spore batches and experiments [26,44]. This variability makes the translation of results from different researchers difficult. Stringent testing of differences between strains can only take place when careful experimental designs are employed, including sporulation under identical conditions and strictly conserved methods for purification and survival estimates. The overall conclusions drawn from the results of previous survivability experiments suggest that any of our potential surrogates may behave similarly to *B. anthracis*. As a result, individual laboratory testing is also required in order to empirically validate a surrogate choice based on theoretical considerations.

Choice of surrogate

Our goal was to examine the various possible surrogates for *B. anthracis*, review the criteria for selecting an appropriate surrogate, compare the potential surrogates by these criteria and, ultimately, choose the most appropriate surrogate for our purposes. After examination of the first criteria, safety of use, we are left with *B. atrophaeus*, *B. thuringiensis*, *B. megaterium* and *B. subtilis* as potential surrogates. However, after further examination of genetic relatedness and the consequential morphological differences, *B. thuringiensis* emerges as the most appropriate candidate for a *B. anthracis* surrogate. This may be a surprising choice for some researchers, based on the traditional preference for *B. atrophaeus*. However, further examination of published comparisons

Table 4 Gaps in our knowledge related to surrogate selection and model parameters

Gaps	Recommended action
No quantitative comparisons of spore survival on fomites	Conduct experiments using steel, laminar, plastic and other surfaces
No quantitative comparisons of spore survival in soil	Conduct experiments across soil types
No quantitative comparisons of spore survival in buffer/water	Conduct survival experiments in water or buffer
No long-term studies	Perform spore survival studies that are over a year long
Only one comparative study examining the effect of heat in various buffers	Reconfirm results
Only one comparative study with UV	Reconfirm results
Only a few studies with virulent *Bacillus anthracis*	Use virulent *B. anthracis* and compare directly to potential surrogates

also supports *B. thuringiensis* as a good surrogate for *B. anthracis*.

We recommend *B. thuringiensis* as the most appropriate surrogate based upon existing empirical data. As a result of the phenotypic similarity within the *B. cereus* group it will be important to utilize a *B. thuringiensis* strain that has a publically available genome sequence, such as *B. thuringiensis* serovar *israelensis* (ATCC 35646; GenBank No. AAJM01000000). This will allow for strain-specific markers to be identified [217,218] which can be used as the basis for assays that can readily detect this strain and distinguish it from con-specifics as well as near neighbour species. We stress that additional experimental evidence is needed to confirm that *B. thuringiensis* and *B. anthracis* have similar behaviours. Data on spore survival and mobility are extremely lacking and we have identified several important knowledge gaps (Table 4). We have found only a few studies comparing spores from *Bacillus* species with the goal of surrogate validation and comparison [26,40,44,48,60]. We are aware of no studies that provide comparative survival of the surrogate candidates in soil or on different types of fomites, both under natural conditions and with heat, pH variance or UV radiation. In addition, there are no quantitative studies on the long-term survival of the spores in any medium. We also find very few studies that use virulent *B. anthracis* strains. The current literature suggests that there can be differences between the attenuated strains and the virulent strains. Therefore, in order to truly quantify and thereby confirm that our selected surrogate is the correct choice, we recommend conducting additional comparative experiments.

Abbreviation

BSL: biosafety level.

Acknowledgements

This study was supported by the Center for Advancing Microbial Risk Assessment, which is funded by the US Environmental Protection Agency Science to Achieve Results programme and the US Department of Homeland Security University Programs (grant R3236201).

Authors' contributions

DG and DW conceived the study. DG, JB, PK and DW drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 15 January 2010 Accepted: 1 September 2010
Published: 1 September 2010

References

1. Tournier JN, Ulrich RG, Quesnel-Hellmann A, Mohamadzadeh M, Stiles BG: **Anthrax, toxins and vaccines: a 125-year journey targeting Bacillus anthracis.** *Expert Rev Anti Infect Ther* 2009, **7**:219-236.
2. Pearson T, Busch JD, Ravel J, Read TD, Rhoton SD, U'Ren JM, Simonson TS, Kachur SM, Leadem RR, Cardon ML, Van Ert MN, Huynh LY, Fraser CM, Keim P: **Phylogenetic discovery bias in Bacillus anthracis using single-nucleotide polymorphisms from whole-genome sequencing.** *PNAS* 2004, **101**:13536-13541.
3. Turnbull PCB: **Introduction: Anthrax history, disease and ecology.** *Anthrax. Current Topics In Microbiology And Immunology* 2002, **271**:1-19.
4. Levin DB, Valadares de Amorim G: **Potential for aerosol dissemination of biological weapons: lessons from biological control of insects.** *Biosecurity and bioterrorism: Biodefense strategy, practice, and science* 2003, **1**:37-42.
5. Meinhardt PL: **WATER AND BIOTERRORISM: Preparing for the Potential Threat to U.S. Water Supplies and Public Health.** *Annual Review of Public Health* 2005, **26**:213-237.
6. WHO Group of Consultants: *Health Aspects of Chemical and Biological Weapons* Geneva: WHO 1970.
7. Fritze D, Pukall R: **Reclassification of bioindicator strains Bacillus subtilis DSM 675 and Bacillus subtilis DSM 2277 as Bacillus atrophaeus.** *Int J Syst Evol Microbiol* 2001, **51**:35-37.
8. Nakamura LK: **Taxonomic Relationship of Black-Pigmented Bacillus subtilis Strains and a Proposal for Bacillus atrophaeus sp. nov.** *Int J Syst Bacteriol* 1989, **39**:295-300.
9. Regis E: *The Biology of Doom* New York: Henry Holt and Company 1999.
10. Carey LF, Amant DCS, Guelta MA, Proving E: **Production of Bacillus Spores as a Simulant for Biological Warfare Agents.** EDGEWOOD CHEMICAL BIOLOGICAL CENTER ABERDEEN PROVING GROUND 2004, 40.
11. Regis E: *The Biology of Doom. The History of America's Secret Germ Warfare Project* New York: Henry Holt & Co 1999.
12. Stuart AL, Wilkening DA: **Degradation of biological weapons agents in the environment: implications for terrorism response.** *Environ Sci Technol* 2005, **39**:2736-2743.
13. Kolb RW, Schneiter R: **The germicidal and sporicidal efficacy of methyl bromide for Bacillus anthracis.** *J Bacteriol* 1950, **59**:401-412.
14. Schneiter R, Kolb RW: **Heat resistance studies with spores of *Bacillus anthracis* and related aerobic bacilli in hair and bristles.** In *Supplement No. 207 to the Public Health Reports* Edited by: NPHS 1948, 1-24.
15. Brazis AR, Leslie JE, Kabler PW, Woodward RL: **The inactivation of spores of *Bacillus globigii* and *Bacillus anthracis* by free available chlorine.** *Appl Microbiol* 1958, **6**:338-342.
16. Faille C, Dennin L, Bellon-Fontaine MN, Benezech T: **Cleanability of stainless steel surfaces soiled by Bacillus thuringiensis spores under various flow conditions.** *Biofouling* 1999, **14**:143-151.

17. Buttner MP, Cruz P, Stetzenbach LD, Klima-Comba AK, Stevens VL, Cronin TD: **Determination of the efficacy of two building decontamination strategies by surface sampling with culture and quantitative PCR analysis.** *Appl Environ Microbiol* 2004, **70**:4740-4747.
18. Weber DJ, Sickbert-Bennett E, Gergen MF, Rutala WA: **Efficacy of selected hand hygiene agents used to remove Bacillus atrophaeus (a surrogate of Bacillus anthracis) from contaminated hands.** *Jama* 2003, **289**:1274-1277.
19. Radziminski C, Ballantyne L, Hodson J, Creason R, Andrews RC, Chauret C: **Disinfection of Bacillus subtilis spores with chlorine dioxide: a bench-scale and pilot-scale study.** *Water Res* 2002, **36**:1629-1639.
20. Gorman SP, Scott EM, Hutchinson EP: **Hypochlorite effects on spores and spore forms of Bacillus subtilis and on a spore lytic enzyme.** *J Appl Bacteriol* 1984, **56**:295-303.
21. Wyatt LR, Waites WM: **The effect of chlorine on spores of Clostridium bifermentans, Bacillus subtilis and Bacillus cereus.** *J Gen Microbiol* 1975, **89**:337-344.
22. Beuchat LR, Pettigrew CA, Tremblay ME, Roselle BJ, Scouten AJ: **Lethality of chlorine, chlorine dioxide, and a commercial fruit and vegetable sanitizer to vegetative cells and spores of Bacillus cereus and spores of Bacillus thuringiensis.** *Ind Microbiol Biotechnol* 2005, **32**:301-308.
23. Young SB, Setlow P: **Mechanisms of killing of Bacillus subtilis spores by hypochlorite and chlorine dioxide.** *J Appl Microbiol* 2003, **95**:54-67.
24. Cortezzo DE, Koziol-Dube K, Setlow B, Setlow P: **Treatment with oxidizing agents damages the inner membrane of spores of Bacillus subtilis and sensitizes spores to subsequent stress.** *J Appl Microbiol* 2004, **97**:838-852.
25. Rose LJ, Rice EW, Jensen B, Murga R, Peterson A, Donlan RM, Arduino MJ: **Chlorine inactivation of bacterial bioterrorism agents.** *Appl Environ Microbiol* 2005, **71**:566-568.
26. Rice EW, Adcock NJ, Sivaganesan M, Rose LJ: **Inactivation of spores of Bacillus anthracis Sterne, Bacillus cereus, and Bacillus thuringiensis subsp. israelensis by chlorination.** *Appl Environ Microbiol* 2005, **71**:5587-5589.
27. Delcomyn CA, Bushway KE, Henley MV: **Inactivation of biological agents using neutral oxone-chloride solutions.** *Environ Sci Technol* 2006, **40**:2759-2764.
28. Kreske AC, Ryu JH, Beuchat LR: **Evaluation of chlorine, chlorine dioxide, and a peroxyacetic acid-based sanitizer for effectiveness in killing Bacillus cereus and Bacillus thuringiensis spores in suspensions, on the surface of stainless steel, and on apples.** *Journal Of Food Protection* 2006, **69**:1892-1903.
29. Szabo JG, Rice EW, Bishop PL: **Persistence and decontamination of Bacillus atrophaeus subsp. globigii spores on corroded iron in a model drinking water system.** *Appl Environ Microbiol* 2007.
30. Cross JB, Currier RP, Torraco DJ, Vanderberg LA, Wagner GL, Gladen PD: **Killing of bacillus spores by aqueous dissolved oxygen, ascorbic acid, and copper ions.** *Appl Environ Microbiol* 2003, **69**:2245-2252.
31. Melly E, Cowan AE, Setlow P: **Studies on the mechanism of killing of Bacillus subtilis spores by hydrogen peroxide.** *J Appl Microbiol* 2002, **93**:316-325.
32. Loshon CA, Melly E, Setlow B, Setlow P: **Analysis of the killing of spores of Bacillus subtilis by a new disinfectant, Sterilox.** *J Appl Microbiol* 2001, **91**:1051-1058.
33. Marquis RE, Shin SY: **Mineralization and responses of bacterial spores to heat and oxidative agents.** *FEMS Microbiol Rev* 1994, **14**:375-379.
34. Sagripanti JL, Bonifacino A: **Comparative sporicidal effects of liquid chemical agents.** *Appl Environ Microbiol* 1996, **62**:545-551.
35. Sagripanti JL, Bonifacino A: **Comparative sporicidal effect of liquid chemical germicides on three medical devices contaminated with spores of Bacillus subtilis.** *Am J Infect Control* 1996, **24**:364-371.
36. Young SB, Setlow P: **Mechanisms of killing of Bacillus subtilis spores by Decon and Oxone, two general decontaminants for biological agents.** *J Appl Microbiol* 2004, **96**:289-301.
37. Rogers JV, Sabourin CL, Choi YW, Richter WR, Rudnicki DC, Riggs KB, Taylor ML, Chang J: **Decontamination assessment of Bacillus anthracis, Bacillus subtilis, and Geobacillus stearothermophilus spores on indoor surfaces using a hydrogen peroxide gas generator.** *J Appl Microbiol* 2005, **99**:739-748.
38. Armstrong G, Watson I, Stewart-Tull D: **Inactivation of *B. cereus* spores on agar, stainless steel or in water with a combination of Nd: YAG laser and UV irradiation.** *INNOVATIVE FOOD SCIENCE & EMERGING TECHNOLOGIES* 2006, **7**:94-99.
39. Whitney EAS, Beatty ME, Taylor TH, Weyant R, Sobel J, Arduino MJ, Ashford DA: **Inactivation of Bacillus anthracis spores.** *Emerging Infectious Diseases* 2003, **9**:623-627.
40. Sagripanti JL, Carrera M, Insalaco J, Ziemski M, Rogers J, Zandomeni R: **Virulent spores of Bacillus anthracis and other Bacillus species deposited on solid surfaces have similar sensitivity to chemical decontaminants.** *Journal Of Applied Microbiology* 2007, **102**:11-21.
41. Demidova TN, Hamblin MR: **Photodynamic inactivation of Bacillus spores, mediated by phenothiazinium dyes.** *Appl Environ Microbiol* 2005, **71**:6918-6925.
42. Demidova TN, HamblinI MR: **Anthrtax surrogate spores are destroyed by PDT mediated by phenothiazinium dyes.** In *Prodceedings of SPIE; Bellingham, WA* Edited by: Kessel D 2005.
43. Montville TJ, De Siano T, Nock A, Padhi S, Wade D: **Inhibition of Bacillus anthracis and potential surrogate bacilli growth from spore inocula by nisin and other antimicrobial peptides.** *Journal Of Food Protection* 2006, **69**:2529-2533.
44. Nicholson WL, Galeano B: **UV resistance of Bacillus anthracis spores revisited: validation of Bacillus subtilis spores as UV surrogates for spores of B. anthracis Sterne.** *Appl Environ Microbiol* 2003, **69**:1327-1330.
45. Setlow P: **Resistance of spores of Bacillus species to ultraviolet light.** *Environ Mol Mutagen* 2001, **38**:97-104.
46. Myasnik M, Manasherob R, Ben-Dov E, Zaritsky A, Margalith Y, Barak Z: **Comparative sensitivity to UV-B radiation of two Bacillus thuringiensis subspecies and other Bacillus sp.** *Curr Microbiol* 2001, **43**:140-143.
47. Griego VM, Spence KD: **Inactivation of Bacillus thuringiensis spores by ultraviolet and visible light.** *Appl Environ Microbiol* 1978, **35**:906-910.
48. Menetrez MY, Foarde KK, Webber TD, Dean TR, Betancourt DA: **Efficacy of UV irradiation on eight species of Bacillus.** *Journal Of Environmental Engineering And Science* 2006, **5**:329-334.
49. Blatchley ER, Meeusen A, Aronson AI, Brewster L: **Inactivation of Bacillus spores by ultraviolet or gamma radiation.** *Journal Of Environmental Engineering-Asce* 2005, **131**:1245-1252.
50. Rice JK, Ewell M: **Examination of peak power dependence in the UV inactivation of bacterial spores.** *Appl Environ Microbiol* 2001, **67**:5830-5832.
51. Lee K, Paek KH, Ju WT, Lee Y: **Sterilization of bacteria, yeast, and bacterial endospores by atmospheric-pressure cold plasma using helium and oxygen.** *J Microbiol* 2006, **44**:269-275.
52. Helfinstine SL, Vargas-Aburto C, Uribe RM, Woolverton CJ: **Inactivation of Bacillus endospores in envelopes by electron beam irradiation.** *Appl Environ Microbiol* 2005, **71**:7029-7032.
53. Niebuhr SE, Dickson JS: **Destruction of Bacillus anthracis strain Sterne 34F2 spores in postal envelopes by exposure to electron beam irradiation.** *Lett Appl Microbiol* 2003, **37**:17-20.
54. Schneiter R, Kolb RW: **Heat resistance studies with spores of *Bacillus anthracis* and related aerobic bacilli in hair and bristles.** In *Supplement No. 207 to the Public Health Reports* Edited by: Public Health Service N 1948, 1-24.
55. Paik WW, Sherry EJ, Stern JA: **Thermal Death Of Bacillus Subtilis Var Niger Spores On Selected Lander Capsule Surfaces.** *Applied Microbiology* 1969, **18**:901.
56. Beaman TC, Gerhardt P: **Heat resistance of bacterial spores correlated with protoplast dehydration, mineralization, and thermal adaptation.** *Appl Environ Microbiol* 1986, **52**:1242-1246.
57. Palop A, Manas P, Condon S: **Sporulation temperature and heat resistance of Bacillus spores: A review.** *Journal Of Food Safety* 1999, **19**:57-72.
58. Rice EW, Rose LJ, Johnson CH, Boczek LA, Arduino MJ, Reasoner DJ: **Boiling and Bacillus spores.** *Emerg Infect Dis* 2004, **10**:1887-1888.
59. Novak JS, Call J, Tomasula P, Luchansky JB: **An assessment of pasteurization treatment of water, media, and milk with respect to Bacillus spores.** *Journal Of Food Protection* 2005, **68**:751-757.
60. Montville TJ, Dengrove R, De Siano T, Bonnet M, Schaffner DW: **Thermal resistance of spores from virulent strains of Bacillus anthracis and potential surrogates.** *J Food Prot* 2005, **68**:2362-2366.
61. Turnbull PC, Frawley DA, Bull RL: **Heat activation/shock temperatures for Bacillus anthracis spores and the issue of spore plate counts versus true numbers of spores.** *J Microbiol Methods* 2006.

62. Scurrah KJ, Robertson RE, Craven HM, Pearce LE, Szabo EA: **Inactivation of Bacillus spores in reconstituted skim milk by combined high pressure and heat treatment.** *J Appl Microbiol* 2006, **101**:172-180.
63. Leuschner RG, Lillford PJ: **Effects of temperature and heat activation on germination of individual spores of Bacillus subtilis.** *Lett Appl Microbiol* 1999, **29**:228-232.
64. Bulla LA, St Julian G, Rhodes RA, Hesseltine CW: **Scanning electron and phase-contrast microscopy of bacterial spores.** *Appl Microbiol* 1969, **18**:490-495.
65. Plomp M, Leighton TJ, Wheeler KE, Malkin AJ: **The high-resolution architecture and structural dynamics of Bacillus spores.** *Biophys J* 2005, **88**:603-608.
66. Plomp M, Leighton TJ, Wheeler KE, Malkin AJ: **Architecture and high-resolution structure of Bacillus thuringiensis and Bacillus cereus spore coat surfaces.** *Langmuir* 2005, **21**:7892-7898.
67. Plomp M, Leighton TJ, Wheeler KE, Pitesky ME, Malkin AJ: **Bacillus atrophaeus outer spore coat assembly and ultrastructure.** *Langmuir* 2005, **21**:10710-10716.
68. Zolock RA, Li G, Bleckmann C, Burggraf L, Fuller DC: **Atomic force microscopy of Bacillus spore surface morphology.** *Micron* 2006, **37**:363-369.
69. Wig A, Arakawa E, Passian A, Thundat T: **Photothermal spectroscopy of *Bacillus anthracis* and *Bacillus cereus* with microcantilevers.** *Sensors and Actuators* 2004, **B114**:206-211.
70. Arakawa ET, Lavrik NV, Datskos PG: **Detection of anthrax simulants with microcalorimetric spectroscopy: *Bacillus subtilis* and *Bacillus cereus* spores.** *Appl Opt* 2003, **42**:1757-1762.
71. Stratis-Cullum DN, Griffin GD, Mobley J, Vass AA, Vo-Dinh T: **A miniature biochip system for detection of aerosolized Bacillus globigii spores.** *Anal Chem* 2003, **75**:275-280.
72. Ulrich MP, Christensen DR, Coyne SR, Craw PD, Henchal EA, Sakai SH, Swenson D, Tholath J, Tsai J, Weir AF, Norwood DA: **Evaluation of the Cepheid GeneXpert system for detecting Bacillus anthracis.** *J Appl Microbiol* 2006, **100**:1011-1016.
73. Farquharson S, Grigely L, Khitrov V, Smith W, Sperry JF, Fenerty G: **Detecting Bacillus cereus spores on a mail sorting system using Raman spectroscopy.** *Journal Of Raman Spectroscopy* 2004, **35**:82-86.
74. Radnedge L, Agron PG, Hill KK, Jackson PJ, Ticknor LO, Keim P, Andersen GL: **Genome differences that distinguish Bacillus anthracis from Bacillus cereus and Bacillus thuringiensis.** *Appl Environ Microbiol* 2003, **69**:2755-2764.
75. Kane SR, Letant SE, Murphy GA, Alfaro TM, Krauter PW, Mahnke R, Legler TC, Raber E: **Rapid, high-throughput, culture-based PCR methods to analyze samples for viable spores of *Bacillus anthracis* and its surrogates.** *J Microbiol Methods* 2009, **76**:278-284.
76. Saikaly PE, Barlaz MA, de Los Reyes FL: **Development of quantitative real-time PCR assays for detection and quantification of surrogate biological warfare agents in building debris and leachate.** *Appl Environ Microbiol* 2007, **73**:6557-6565.
77. Yang S, Rothman RE, Hardick J, Kuroki M, Hardick A, Doshi V, Ramachandran P, Gaydos CA: **Rapid polymerase chain reaction-based screening assay for bacterial biothreat agents.** *Acad Emerg Med* 2008, **15**:388-392.
78. McBride MT, Masquelier D, Hindson BJ, Makarewicz AJ, Brown S, Burris K, Metz T, Langlois RG, Tsang KW, Bryan R, Anderson DA, Venkateswaran KS, Milanovich FP, Colston BW Jr: **Autonomous detection of aerosolized Bacillus anthracis and Yersinia pestis.** *Anal Chem* 2003, **75**:5293-5299.
79. Hindson BJ, McBride MT, Makarewicz AJ, Henderer BD, Setlur US, Smith SM, Gutierrez DM, Metz TR, Nasarabadi SL, Venkateswaran KS, Farrow SW, Colston BW Jr, Dzenitis JM: **Autonomous detection of aerosolized biological agents by multiplexed immunoassay with polymerase chain reaction confirmation.** *Anal Chem* 2005, **77**:284-289.
80. Stachowiak JC, Shugard EE, Mosier BP, Renzi RF, Caton PF, Ferko SM, Van de Vreugde JL, Yee DD, Haroldsen BL, VanderNoot VA: **Autonomous microfluidic sample preparation system for protein profile-based detection of aerosolized bacterial cells and spores.** *Anal Chem* 2007, **79**:5763-5770.
81. Hart SJ, Terray A, Leski TA, Arnold J, Stroud R: **Discovery of a significant optical chromatographic difference between spores of Bacillus anthracis and its close relative, Bacillus thuringiensis.** *Anal Chem* 2006, **78**:3221-3225.
82. Krebs MD, Mansfield B, Yip P, Cohen SJ, Sonenshein AL, Hitt BA, Davis CE: **Novel technology for rapid species-specific detection of Bacillus spores.** *Biomol Eng* 2006, **23**:119-127.
83. Gibb-Snyder E, Gullett B, Ryan S, Oudejans L, Touati A: **Development of size-selective sampling of Bacillus anthracis surrogate spores from simulated building air intake mixtures for analysis via laser-induced breakdown spectroscopy.** *Appl Spectrosc* 2006, **60**:860-870.
84. Gottfried JL, De Lucia FC Jr, Munson CA, Miziolek AW: **Standoff detection of chemical and biological threats using laser-induced breakdown spectroscopy.** *Appl Spectrosc* 2008, **62**:353-363.
85. Munson CA, Gottfried JL, Snyder EG, De Lucia FC Jr, Gullett B, Miziolek AW: **Detection of indoor biological hazards using the man-portable laser induced breakdown spectrometer.** *Appl Opt* 2008, **47**:G48-57.
86. Snyder EG, Munson CA, Gottfried JL, De Lucia FC Jr, Gullett B, Miziolek A: **Laser-induced breakdown spectroscopy for the classification of unknown powders.** *Appl Opt* 2008, **47**:G80-87.
87. Laflamme C, Verreault D, Ho J, Duchaine C: **Flow cytometry sorting protocol of Bacillus spore using ultraviolet laser and autofluorescence as main sorting criterion.** *Journal Of Fluorescence* 2006, **16**:733-737.
88. Hathout Y, Demirev PA, Ho YP, Bundy JL, Ryzhov V, Sapp L, Stutler J, Jackman J, Fenselau C: **Identification of Bacillus spores by matrix-assisted laser desorption ionization-mass spectrometry.** *Appl Environ Microbiol* 1999, **65**:4313-4319.
89. Hathout Y, Setlow B, Cabrera-Martinez RM, Fenselau C, Setlow P: **Small, acid-soluble proteins as biomarkers in mass spectrometry analysis of Bacillus spores.** *Appl Environ Microbiol* 2003, **69**:1100-1107.
90. Elhanany E, Barak R, Fisher M, Kobiler D, Altboum Z: **Detection of specific Bacillus anthracis spore biomarkers by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.** *Rapid Commun Mass Spectrom* 2001, **15**:2110-2116.
91. Warscheid B, Fenselau C: **Characterization of Bacillus spore species and their mixtures using postsource decay with a curved-field reflectron.** *Anal Chem* 2003, **75**:5618-5627.
92. Pribil PA, Patton E, Black G, Doroshenko V, Fenselau C: **Rapid characterization of Bacillus spores targeting species-unique peptides produced with an atmospheric pressure matrix-assisted laser desorption/ionization source.** *J Mass Spectrom* 2005, **40**:464-474.
93. Castanha ER, Fox A, Fox KF: **Rapid discrimination of Bacillus anthracis from other members of the B. cereus group by mass and sequence of "intact" small acid soluble proteins (SASPs) using mass spectrometry.** *J Microbiol Methods* 2006, **67**:230-240.
94. Dickinson DN, La Duc MT, Haskins WE, Gornushkin I, Winefordner JD, Powell DH, Venkateswaran K: **Species differentiation of a diverse suite of Bacillus spores by mass spectrometry-based protein profiling.** *Appl Environ Microbiol* 2004, **70**:475-482.
95. Fergenson DP, Pitesky ME, Frank M, Horn JM, Gard EE: **Distinguishing Seven Species of Bacillus Spores Using BioAerosol Mass Spectrometry.** Lawrence Livermore National Laboratory (LLNL) L, CA: USDOE 2005.
96. Krebs MD, Zapata AM, Nazarov EG, Miller RA, Costa IS, Sonenshein AL, Davis CE: **Detection of biological and chemical agents using differential mobility spectrometry (DMS) technology.** *Ieee Sensors Journal* 2005, **5**:696-703.
97. Demirev PA, Feldman AB, Kowalski P, Lin JS: **Top-down proteomics for rapid identification of intact microorganisms.** *Anal Chem* 2005, **77**:7455-7461.
98. Delvecchio VG, Connolly JP, Alefantis TG, Walz A, Quan MA, Patra G, Ashton JM, Whittington JT, Chafin RD, Liang X, Grewal P, Khan AS, Mujer CV: **Proteomic profiling and identification of immunodominant spore antigens of Bacillus anthracis, Bacillus cereus, and Bacillus thuringiensis.** *Appl Environ Microbiol* 2006, **72**:6355-6363.
99. Min J, Lee J, Deininger RA: **Simple and rapid method for detection of bacterial spores in powder useful for first responders.** *J Environ Health* 2006, **68**:34-37, 44, 46.
100. Branch D, Brozik S: **Low level detection of a Bacillus anthracis simulant using a love-wave biosensors.**Edited by: Technology MSa. Sandia National Laboratories; 2003:33.
101. Helfinstine SL, Lavrentovich OD, Woolverton CJ: **Lyotropic liquid crystal as a real-time detector of microbial immune complexes.** *Lett Appl Microbiol* 2006, **43**:27-32.

102. Stephens JR: **Flourescence cross section meaurements of biological agent simulants.** *Conference on Obscuration and Aerosol Research* Los Alamos National Lab 1996.
103. Sainathrao S, Mohan KV, Atreya C: **Gamma-phage lysin *PlyG* sequence-based synthetic peptides coupled with Qdot-nanocrystals are useful for developing detection methods for *Bacillus anthracis* by using its surrogates, *B. anthracis*-Sterne and *B. cereus*-4342.** *BMC Biotechnol* 2009, **9**:67.
104. Stephans JR: **Measurements of the Ultraviolet Fluorescence Cross Sections and Spectra of Bacillus Anthracis Simulants.**Edited by: Lab LAN 1998.
105. Stephens JR: **Identification of BW agents simulants on building surfaces by infrared reflectance spectroscopy.** *CBW Protection Symposium; May 10-13; Stockholm, Sweden* 1998, 11.
106. Utrup LJ, Werner K, Frey AH: **Minimizing pathogenic bacteria, including spores, in indoor air.** *J Environ Health* 2003, **66**:19-26, 29.
107. Lee SA, Willeke K, Mainelis G, Adhikari A, Wang HX, Reponen T, Grinshpun SA: **Assessment of electrical charge on airborne microorganisms by a new bioaerosol sampling method.** *Journal Of Occupational And Environmental Hygiene* 2004, **1**:127-138.
108. Clark Burton N, Adhikari A, Grinshpun SA, Hornung R, Reponen T: **The effect of filter material on bioaerosol collection of Bacillus subtilis spores used as a Bacillus anthracis simulant.** *J Environ Monit* 2005, **7**:475-480.
109. Lighthart B, Prier K, Bromenshenk J: **Detection of aerosolized bacterial spores (Bacillus atrophaeus) using free-flying honey bees (Hymenoptera apidae) as collectors.** *Aerobiologica* 2004, **20**:191-195.
110. Teschke K, Chow Y, Bartlett K, Ross A, van Netten C: **Spatial and temporal distribution of airborne Bacillus thuringiensis var. kurstaki during an aerial spray program for gypsy moth eradication.** *Environmental Health Perspectives* 2001, **109**:47-54.
111. Valadares De Amorim G, Whittome B, Shore B, Levin DB: **Identification of Bacillus thuringiensis subsp. kurstaki strain HD1-Like bacteria from environmental and human samples after aerial spraying of Victoria, British Columbia, Canada, with Foray 48B.** *Appl Environ Microbiol* 2001, **67**:1035-1043.
112. Krauter P, Biermann A, Larsen L: **Transport efficiency and deposition velocity of fluidized spores in ventilation ducts.** *Aerobiologia* 2005, **21**:155-172.
113. Dougherty GM, Hadley DR, o'Conner PR: **Engineered aerosol production for laboratory scale chemical/biological test and evaluation.**Edited by: Energy Do. Lawrence Livermore National Laboratory; 2007:28.
114. Krauter P, Biermann A: **Reaerosolization of Fluidized Spores in Ventilation Systems.** *Appl Environ Microbiol* 2007.
115. Scott Duncan EJ, Kournikakis B, Ho J, Hill I: **Pulmonary deposition of aerosolized *Bacillus atrophaeus* in a swine model due to exposure from a simulated anthrax letter incident.** *Inhalation Toxicology* 2009, **21**:141-152.
116. Drobniewski FA: **Bacillus-Cereus And Related Species.** *Clinical Microbiology Reviews* 1993, **6**:324-338.
117. Helgason E, Caugant DA, Olsen I, Kolsto AB: **Genetic structure of population of Bacillus cereus and B. thuringiensis isolates associated with periodontitis and other human infections.** *J Clin Microbiol* 2000, **38**:1615-1622.
118. Green M, Heumann M, Sokolow R, Foster LR, Bryant R, Skeels M: **Public health implications of the microbial pesticide Bacillus thuringiensis: an epidemiological study, Oregon, 1985-86.** *Am J Public Health* 1990, **80**:848-852.
119. McClintock JT, Schaffer CR, Sjoblad RD: **A Comparative Review Of The Mammalian Toxicity Of Bacillus Thuringiensis-Based Pesticides.** *Pesticide Science* 1995, **45**:95-105.
120. Samples JR, Buettner H: **Ocular Infection Caused By A Biological Insecticide.** *Journal Of Infectious Diseases* 1983, **148**:614-614.
121. Hernandez E, Ramisse F, Ducoureau JP, Cruel T, Cavallo JD: **Bacillus thuringiensis subsp. konkukian (serotype H34) superinfection: case report and experimental evidence of pathogenicity in immunosuppressed mice.** *J Clin Microbiol* 1998, **36**:2138-2139.
122. Jackson SG, Goodbrand RB, Ahmed R, Kasatiya S: ***Bacillus cereus* and *Bacillus thuringiensis* isolated in a gastroenteritis outbreak investigation.** *Letters in Applied Microbiology* 1995, **21**:103-105.
123. Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P: **Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments.** *Microbiol Mol Biol Rev* 2000, **64**:548-572.
124. Ash C, Farrow JAE, Wallbanks S, Collins MD: **Phylogenetic Heterogeneity Of The Genus Bacillus Revealed By Comparative-Analysis Of Small-Subunit-Ribosomal Rna Sequences.** *Letters In Applied Microbiology* 1991, **13**:202-206.
125. Brumlick MJ, Bielawska-Drozd A, Zakowska D, Liang X, Spalletta RA, Patra G, DelVecchio VG: **Genetic diversity among *Bacillus anthracis, Bacillus cereus and Bacilus thuringiensis* strains using repetative element polymorphisms-PCR.** *Polish Journal of Microbiology* 2004, **53**:215-225.
126. Burton JE, Oshota OJ, Silman NJ: **Differential identification of Bacillus anthracis from environmental Bacillus species using microarray analysis.** *Journal Of Applied Microbiology* 2006, **101**:754-763.
127. Fritze D: **Taxonomy of the Genus *Bacillus* and related genera: The aerobic endospore-fromiung bacteria.** *Phytopathology* 2004, **94**:1245-1248.
128. Gohar M, Gilois N, Graveline R, Garreau C, Sanchis V, Lereclus D: **A comparative study of Bacillus cereus, Bacillus thuringiensis and Bacillus anthracis extracellular proteomes.** *Proteomics* 2005, **5**:3696-3711.
129. Han CS, Xie G, Challacombe JF, Altherr MR, Bhotika SS, Bruce D, Campbell CS, Campbell ML, Chen J, Chertkov O, Cleland C, Dimitrijevic M, Doggett NA, Fawcett JJ, Glavina T, Goodwin LA, Hill KK, Hitchcock P, Jackson PJ, Keim P, Kewalramani AR, Longmire J, Lucas S, Malfatti S, McMurry K, Meincke LJ, Misra M, Moseman BL, Mundt M, Munk AC, *et al*: **Pathogenomic sequence analysis of Bacillus cereus and Bacillus thuringiensis isolates closely related to Bacillus anthracis.** *J Bacteriol* 2006, **188**:3382-3390.
130. Helgason E, Okstad OA, Caugant DA, Johansen HA, Fouet A, Mock M, Hegna I, Kolsto : **Bacillus anthracis, Bacillus cereus, and Bacillus thuringiensis–one species on the basis of genetic evidence.** *Appl Environ Microbiol* 2000, **66**:2627-2630.
131. Helgason E, Tourasse NJ, Meisal R, Caugant DA, Kolsto AB: **Multilocus sequence typing scheme for bacteria of the Bacillus cereus group.** *Appl Environ Microbiol* 2004, **70**:191-201.
132. Hill KK, Ticknor LO, Okinaka RT, Asay M, Blair H, Bliss KA, Laker M, Pardington PE, Richardson AP, Tonks M, Beecher DJ, Kemp JD, Kolsto AB, Wong AC, Keim P, Jackson PJ: **Fluorescent amplified fragment length polymorphism analysis of Bacillus anthracis, Bacillus cereus, and Bacillus thuringiensis isolates.** *Appl Environ Microbiol* 2004, **70**:1068-1080.
133. Ivanova N, Sorokin A, Anderson I, Galleron N, Candelon B, Kapatral V, Bhattacharyya A, Reznik G, Mikhailova N, Lapidus A, Chu L, Mazur M, Goltsman E, Larsen N, D'Souza M, Walunas T, Grechkin Y, Pusch G, Haselkorn R, Fonstein M, Ehrlich SD, Overbeek R, Kyrpides N: **Genome sequence of Bacillus cereus and comparative analysis with Bacillus anthracis.** *Nature* 2003, **423**:87-91.
134. Jackson PJ, Hill KK, Laker MT, Ticknor LO, Keim P: **Genetic comparison of Bacillus anthracis and its close relatives using amplified fragment length polymorphism and polymerase chain reaction analysis.** *J Appl Microbiol* 1999, **87**:263-269.
135. Keim P, Kalif A, Schupp J, Hill K, Travis SE, Richmond K, Adair DM, Hugh-Jones M, Kuske CR, Jackson P: **Molecular evolution and diversity in Bacillus anthracis as detected by amplified fragment length polymorphism markers.** *J Bacteriol* 1997, **179**:818-824.
136. Keim P, Klevytska AM, Price LB, Schupp JM, Zinser G, Smith KL, Hugh-Jones ME, Okinaka R, Hill KK, Jackson PJ: **Molecular diversity in Bacillus anthracis.** *J Appl Microbiol* 1999, **87**:215-217.
137. Priest FG, Barker M, Baillie LW, Holmes EC, Maiden MC: **Population structure and evolution of the Bacillus cereus group.** *J Bacteriol* 2004, **186**:7959-7970.
138. Rasko DA, Ravel J, Okstad OA, Helgason E, Cer RZ, Jiang L, Shores KA, Fouts DE, Tourasse NJ, Angiuoli SV, Kolonay J, Nelson WC, Kolsto AB, Fraser CM, Read TD: **The genome sequence of Bacillus cereus ATCC 10987 reveals metabolic adaptations and a large plasmid related to Bacillus anthracis pXO1.** *Nucleic Acids Res* 2004, **32**:977-988.
139. Read TD, Peterson SN, Tourasse N, Baillie LW, Paulsen IT, Nelson KE, Tettelin H, Fouts DE, Eisen JA, Gill SR, Holtzapple EK, Okstad OA, Helgason E, Rilstone J, Wu M, Kolonay JF, Beanan MJ, Dodson RJ, Brinkac LM, Gwinn M, DeBoy RT, Madpu R, Daugherty SC, Durkin AS, Haft DH, Nelson WC, Peterson JD, Pop M, Khouri HM, Radune D, *et al*: **The genome sequence of Bacillus anthracis Ames and comparison to closely related bacteria.** *Nature* 2003, **423**:81-86.
140. Todd SJ, Moir AJ, Johnson MJ, Moir A: **Genes of Bacillus cereus and Bacillus anthracis encoding proteins of the exosporium.** *J Bacteriol* 2003, **185**:3373-3378.

141. Turnbull PC: **Definitive identification of Bacillus anthracis–a review.** *J Appl Microbiol* 1999, **87**:237-240.
142. Valjevac S, Hilaire V, Lisanti O, Ramisse F, Hernandez E, Cavallo JD, Pourcel C, Vergnaud G: **Comparison of minisatellite polymorphisms in the Bacillus cereus complex: a simple assay for large-scale screening and identification of strains most closely related to Bacillus anthracis.** *Appl Environ Microbiol* 2005, **71**:6613-6623.
143. Xu D, Cote JC: **Phylogenetic relationships between Bacillus species and related genera inferred from comparison of 3' end 16 S rDNA and 5' end 16S-23 S ITS nucleotide sequences.** *Int J Syst Evol Microbiol* 2003, **53**:695-704.
144. Easterday WR, Van Ert MN, Simonson TS, Wagner DM, Kenefic LJ, Allender CJ, Keim P: **Use of single nucleotide polymorphisms in the *plcR* gene for specific Identification of *Bacillus anthracis*.** *Journal of Clinical Microbiology* 2005, **43**:1995-1997.
145. Easterday WR, Van Ert MN, Zanecki S, Keim P: **Specific detection of bacillus anthracis using a TaqMan mismatch amplification mutation assay.** *BioTechniques* 2005, **38**:731-735.
146. Sylvestre P, Couture-Tosi E, Mock M: **A collagen-like surface glycoprotein is a structural component of the Bacillus anthracis exosporium.** *Molecular Microbiology* 2002, **45**:169-178.
147. Rety S, Salamitou S, Garcia-Verdugo I, Hulmes DJ, Le Hegarat F, Chaby R, Lewit-Bentley A: **The crystal structure of the Bacillus anthracis spore surface protein BclA shows remarkable similarity to mammalian proteins.** *J Biol Chem* 2005, **280**:43073-43078.
148. Knaysi G: **The Endospore Of Bacteria.** *Bacteriol Rev* 1948, **12**:19-77.
149. Warth AD, Ohye DF, Murrell WG: **The composition and structure of bacterial spores.** *J Cell Biol* 1963, **16**:579-592.
150. Gerhardt P, Ribi E: **Ultrastructure Of The Exosporium Enveloping Spores Of Bacillus Cereus.** *J Bacteriol* 1964, **88**:1774-1789.
151. Knaysi G: **Further Observations On The Spodogram Of Bacillus Cereus Endospore.** *J Bacteriol* 1965, **90**:453-455.
152. Hachisuka Y, Kozuka S, Tsujikawa M: **Exosporia and appendages of spores of Bacillus species.** *Microbiol Immunol* 1984, **28**:619-624.
153. Charlton S, Moir AJG, Baillie L, Moir A: **Characterization of the exosporium of Bacillus cereus.** *Journal Of Applied Microbiology* 1999, **87**:241-245.
154. Peng JS, Tsai WC, Chou CC: **Surface characteristics of Bacillus cereus and its adhesion to stainless steel.** *Int J Food Microbiol* 2001, **65**:105-111.
155. Beaman TC, Pankratz HS, Gerhardt P: **Ultrastructure of the Exosporium and Underlying Inclusions in Spores of Bacillus megaterium Strains.** *J Bacteriol* 1972, **109**:1198-1209.
156. Koshikawa T, Yamazaki M, Yoshimi M, Ogawa S, Yamada A, Watabe K, Torii M: **Surface hydrophobicity of spores of *Bacillus* spp.** *Journal of General Microbiology* 1989, **135**:2717-2722.
157. Sousa JCF, Silva MT, Balassa G: **An exosporium-like outer layer in Bacillus subtilis spores.** *Nature* 1976, **263**:53-54.
158. Kramer MJ, Roth IL: **Ultrastructural Differences In Exosporium Of Sterne And Vollum Strains Of Bacillus Anthracis.** *Canadian Journal Of Microbiology* 1968, **14**:1297.
159. Knaysi G: **On the structure and nature of the endospore in strain C3 of *Bacillus cereus*.** *J Bacteriol* 1955, **69**:130-138.
160. Kojima K, Sato T: **Fine Filaments On Outside Of Exosporium Of Bacillus Anthracis Spores.** *Journal Of Bacteriology* 1966, **91**:2382.
161. Hachisuka Y, Kuno T: **Filamentous appendages of Bacillus cereus spores.** *Jpn J Microbiol* 1976, **20**:555-558.
162. Beaman TC, Pankratz HS, Gerhardt P: **Ultrastructure of the exosporium and underlying inclusions in spores of Bacillus megaterium strains.** *J Bacteriol* 1972, **109**:1198-1209.
163. Sousa JC, Silva MT, Balassa G: **An exosporium-like outer layer in Bacillus subtilis spores.** *Nature* 1976, **263**:53-54.
164. Driks A: **Bacillus subtilis spore coat.** *Microbiol Mol Biol Rev* 1999, **63**:1-20.
165. Takamatsu H, Watabe K: **Assembly and genetics of spore protective structures.** *Cell Mol Life Sci* 2002, **59**:434-444.
166. Bechtel DB, Bulla LA Jr: **Electron Microscope Study of Sporulation and Parasporal Crystal Formation in Bacillus thuringiensis.** *J Bacteriol* 1976, **127**:1472-1481.
167. Gerhardt P, Pankratz HS, Scherrer R: **Fine Structure of the Bacillus thuringiensis Spore.** *Appl Environ Microbiol* 1976, **32**:438-440.
168. Smirnova TA, Mikhailov AM, Tyurin VS, Azizbekyan RR: **The fine structure of spores and crystals in various Bacillus thuringiensis serotypes.** *MIKROBIOLOGIYA* 1984, **53**:455-462.
169. Doyle R, Nedjat-Haiem F, Singh J: **Hydrophobic characteristics of *Bacillus* spores.** *Current Microbiology* 1984, **10**:329-332.
170. Zandomeni RO, Fitzgibbon JE, Carrera M, Steubing E, Rogers JE, Sagripanti J-L: **Spore Size Comparison Between Several Bacillus Species.** Edited by: MD G-CIAPG 2005.
171. Carrera M, Zandomeni RO, Fitzgibbon J, Sagripanti JL: **Difference between the spore sizes of Bacillus anthracis and other Bacillus species.** *J Appl Microbiol* 2007, **102**:303-312.
172. Lighthart B: **Physics of microbial bioaerosols.** In *Atmosphere Microbial Aerosols.* Edited by: Lighthart B, Mohr AJ. New York: Chapman and Hall; 1994:5-27.
173. Cox CS: **Physical aspects of bioaerosols.** In *Bioaerosols handbook.* Edited by: Cox CS, Wathes CM. London: Lewis; 1995:15-25.
174. Carrera M, Zandomeni RO, Sagripanti JL: **Wet and dry density of Bacillus anthracis and other Bacillus species.** *J Appl Microbiol* 2008, **105**:68-77.
175. Jaax NK, Davis KJ, Geisbert TJ, Vogel P, Jaax GP, Topper M, Jahrling PB: **Lethal experimental infection of rhesus monkeys with Ebola-Zaire (Mayinga) virus by the oral and conjunctival route of exposure.** *Arch Pathol Lab Med* 1996, **120**:140-155.
176. Baweja RB, Zaman MS, Mattoo AR, Sharma K, Tripathi V, Aggarwal A, Dubey GP, Kurupati RK, Ganguli M, Chaudhury NK, Sen S, Das TK, Gade WN, Singh Y: **Properties of *Bacillus anthracis* spores prepared under various environmental conditions.** *Arch Microbiol* 2008, **189**:71-79.
177. Sirena S, Scagliosi G: **Lavori E Lezioni Originali.** *Riforma medicia* 1894, **2**:340-343.
178. Szekely Av: **Beitrag zur Lebensdauer der Milzbrandsporen.** *Zeit Hygiene Infectionskrankheiten* 1903, **44**:359-363.
179. Busson B: **Ein beitrag zur Kenntnis der Lebensdauer von Bacterium coli und Milzbrandsporen.** *Centralbl Bakteriol, Parasitenkd Infektionskr* 1911, **58**:505-509.
180. Graham-Smith GS: **The longevity of dry spores of *B. anthracis*.** *Journal of Hygiene* 1930, **30**:213-215.
181. Minett FC, Dhanda MR: **Multiplication of *B. anthracis* and *Cl. chauvei* in soil and water.** *Indian Journal of Veterinary Science and Animal Husbandry* 1941, **11**:308-328.
182. Novel R, Reh T: **De la longevite des spores du *Bacillus anthracis* et de la conservation des pouvoirs pathogcne *ei* antigene.** *Schweizerische Zeitschrift fu¨r Pathologie und Bakteriologie* 1947, **10**:180-192.
183. Dearmon IA Jr, Lively DH, Roth NG: **Survival time as a rapid method of determining virulence with *Bacillus anthracis*.** *J Bacteriol* 1956, **72**:666-672.
184. Dearmon IA Jr, Orlando MD, Rosenwald AJ, Klein F, Fernelius AL, Lincoln RE, Middaugh PR: **Viability and estimation of shelf-life of bacterial populations.** *Appl Microbiol* 1962, **10**:422-427.
185. Wilson JB, Russell KE: **Isolation Of Bacillus Anthracis From Soil Stored 60 Years.** *J Bacteriol* 1964, **87**:237-238.
186. Lewis JC: **Dormancy.** In *The Bacterial Spore.* Edited by: Hurst A, Gould GW. London: Academia Press; 1969:**1**:301-358.
187. Roberts TA, Hitchins AD: **Resistance of spores.** In *The Bacterial Spore.* Edited by: Gould GW, Hurst A. London: Academic Press; 1969:**1**:611-670.
188. Van Ness GB: **Ecology of anthrax.** *Science* 1971, **172**:1303-1307.
189. Manchee RJ, Broster MG, Melling J, Henstridge RM, Stagg AJ: **Bacillus anthracis on Gruinard Island.** *Nature* 1981, **294**:254-255.
190. Dragon DC, Rennie RP: **The ecology of anthrax spores: tough but not invincible.** *Can Vet J* 1995, **36**:295-301.
191. Pinnock DE, Brand RJ, Milstead JE: **The field persistence of Bacillus thuringiensis spores.** *Journal of Invertebrate Pathology* 1971, **18**:405.
192. Reynolds CM, Ringelberg DB: **Non-indigenous endospore persistence following release in a snow - soil system.** *Cold Regions Science and Technology* 2008, **52**:146-154.
193. Pinnock DE, Brand RJ, Jackson KL, Milstead JE: **The field persistence of Bacillus thuringiensis spores on Cercis occidentalis leaves.** *Journal of Invertebrate Pathology* 1974, **23**:341.
194. Pruett CJH, Burges HD, Wyborn CH: **Effect of exposure to soil on potency and spore viability of Bacillus thuringiensis.** *Journal of Invertebrate Pathology* 1980, **35**:168.
195. West AW, Burges HD, Wyborn CH: **Effect of incubation in natural and autoclaved soil upon potency and viability of Bacillus thuringiensis.** *Journal of Invertebrate Pathology* 1984, **44**:121.
196. Petras SF, Casida LE: **Survival of Bacillus thuringiensis Spores in Soil.** *Appl Environ Microbiol* 1985, **50**:1496-1501.

197. West AW, Burges HD, Dixon TJ, Wyborn CH: **Effect Of Incubation In Non-Sterilized And Autoclaved Arable Soil On Survival Of Bacillus-Thuringiensis And Bacillus-Cereus Spore Inocula.** *New Zealand Journal Of Agricultural Research* 1985, **28**:559-566.
198. West AW, Burges HD, Dixon TJ, Wyborn CH: **Survival Of Bacillus-Thuringiensis And Bacillus-Cereus Spore Inocula In Soil - Effects Of Ph, Moisture, Nutrient Availability And Indigenous Microorganisms.** *Soil Biology & Biochemistry* 1985, **17**:657-665.
199. Smith RA, Barry JW: **Environmental persistence of Bacillus thuringiensis spores following aerial application.** *J Invertebr Pathol* 1998, **71**:263-267.
200. Murray TJ: **Thermal death point II. Spores of *Bacillus anthracis*.** *Journal Of Infectious Diseases* 1931, **48**:457-467.
201. Curran HR, Evans FR: **The Viability of Heat-activatable Spores in Nutrient and Nonnutrient Substrates as Influenced by Prestorage or Poststorage Heating and Other Factors.** *J Bacteriol* 1947, **53**:103-113.
202. Stein CB: **Some observations on the tenacity of *Bacillus anthracis*.** *Veterinary Medicine* 1947, **42**:13-22.
203. Evans FR, Curran HR: **Influence of preheating, pH, and holding temperature upon viability of bacterial spores stored for long periods in buffer substrates.** *J Bacteriol* 1960, **79**:361-368.
204. Walker HW, Matches JR, Ayres JC: **Chemical composition and heat resistance of some aerobic bacterial spores.** *J Bacteriol* 1961, **82**:960-966.
205. Marquis RE, Bender GR: **Mineralization and heat resistance of bacterial spores.** *J Bacteriol* 1985, **161**:789-791.
206. Moussa-Boudjemaa B, Gonzalez J, Lopez M: **Heat resistance of Bacillus cereus spores in carrot extract acidified with different acidulants.** *Food Control* 2006, **17**:819.
207. Xu S, Labuza TP, Diez-Gonzalez F: **Thermal inactivation of Bacillus anthracis spores in cow's milk.** *Appl Environ Microbiol* 2006, **72**:4479-4483.
208. Fernelius AL, Wilkes CE, Dearmon IA Jr, Lincoln RE: **A probit method to interpret thermal inactivation of bacterial spores.** *J Bacteriol* 1958, **75**:300-304.
209. Lemieux P, Wood J, Lee C, Serre S: **Thermal destruction of CB contaminants bound on building materials experiements and modeling.** *Scientific Conference on Chemical and Biological Defense Research; Timonium, MD* 2005, 1-9.
210. Islam MS, Inoue A, Igura N, Shimoda M, Hayakawa I: **Inactivation of Bacillus spores by the combination of moderate heat and low hydrostatic pressure in ketchup and potage.** *Int J Food Microbiol* 2006, **107**:124-130.
211. van Asselt ED, Zwietering MH: **A systematic approach to determine global thermal inactivation parameters for various food pathogens.** *Int J Food Microbiol* 2006, **107**:73-82.
212. Mitscherlich E, Marth EH: *Microbial Survival in the Environment* Berlin: Springer 1984.
213. Hilgren J, Swanson KM, Diez-Gonzalez F, Cords B: **Susceptibilities of *Bacillus subtilis*, *Bacillus cereus*, and avirulent *Bacillus anthracis* spores to liquid biocides.** *J Food Prot* 2009, **72**:360-364.
214. Sokurova EN, Meisel MN: **The combined action of ultra-violet and x-rays on the spores of *Bacillus anthracis*.** *Biophysics (USSR)(English Translation)* 1958, **4**:474-477.
215. Kenar L, Ortatatli M, Yaren H, Karayilanoglu T, Aydogan H: **Comparative sporicidal effects of disinfectants after release of a biological agent.** *Military Medicine* 2007, **172**:616-621.
216. Pribil W, Gehringer P, Eschweiler H, Cabaj A, Haider T, Sommer R: **Assessment of *Bacillus subtilis* spores as a possible bioindicator for evaluation of the microbicidal efficacy of radiation processing of water.** *Water Environ Res* 2007, **79**:720-724.
217. Van Ert MN, Easterday WR, Simonson TS, U'Ren JM, Pearson T, Kenefic LJ, Busch JD, Huynh LY, Dukerich M, Trim CB, Beaudry J, Welty-Bernard A, Read T, Fraser CM, Ravel J, Keim P: **Strain-Specific Single-Nucleotide Polymorphism Assays for the Bacillus anthracis Ames Strain.** *J Clin Microbiol* 2007, **45**:47-53.
218. Vogler AJ, Driebe EM, Lee J, Auerbach RK, Allender CJ, Kubota K, Andersen GL, Radnedge L, Worsham PL, Keim P, Wagner DM: **Rapid and specific identification of North American *Yersinia pestis* and the common laboratory strain, CO92.** *BioTechniques* 2008, **44**:201-207.
219. Agoston R, Soni KA, McElhany K, Cepeda ML, Zuckerman U, Tzipori S, Mohacsi-Farkas C, Pillai SD: **Rapid concentration of *Bacillus* and *Clostridium* spores from large volumes of milk, using continuous flow centrifugation.** *J Food Prot* 2009, **72**:666-668.
220. Carey LF, St Amant DC, Guelta MA: **Production of Bacillus Spores as a Simulant for Biological Warfare Agents.**Edited by: Army. EDGEWOOD CHEMICAL BIOLOGICAL CENTER ABERDEEN PROVING GROUND MD; 2004:40.
221. Burton JE, Oshota OJ, North E, Hudson MJ, Polyanskaya N, Brehm J, Lloyd G, Silman NJ: **Development of a multi-pathogen oligonucleotide microarray for detection of Bacillus anthracis.** *Mol Cell Probes* 2005, **19**:349-357.
222. Farnsworth JE, Goyal SM, Kim SW, Kuehn TH, Raynor PC, Ramakrishnan MA, Anantharaman S, Tang WH: **Development of a method for bacteria and virus recovery from heating, ventilation, and air conditioning (HVAC) filters.** *Journal Of Environmental Monitoring* 2006, **8**:1006-1013.
223. Knudson GB: **Photoreactivation of ultraviolet-irradiated, plasmid-bearing, and plasmid-free strains of Bacillus anthracis.** *Appl Environ Microbiol* 1986, **52**:444-449.
224. Galeano B, Korff E, Nicholson WL: **Inactivation of vegetative cells, but not spores, of Bacillus anthracis, B-cereus, and B-subtilis on stainless steel surfaces coated with an antimicrobial silver- and zinc-containing zeolite formulation.** *Applied And Environmental Microbiology* 2003, **69**:4329-4331.
225. Montville TJ: **Thermal Resistance of Bacillus anthracis Spores and Surrogates.** *Proceedings of The Institute of Food Technologists' First Annual Food Protection and Defense Research Conference* Atlanta, Georgia 2005.
226. De Siano T, Padhi S, Schaffner DW, Montville TJ: **Growth characteristics of virulent Bacillus anthracis and potential surrogate strains.** *J Food Prot* 2006, **69**:1720-1723.
227. Levin DB, Valadares de Amorim G: **Potential for aerosol dissemination of biological weapons: lessons from biological control of insects.** *Biosecur Bioterror* 2003, **1**:37-42.
228. Levin D: **Monitering human exposure to Bacillus thuringiensis after spray.** 2004.
229. Perez A, Hohn C, Higgins J: **Filtration methods for recovery of Bacillus anthracis spores spiked into source and finished water.** *Water Res* 2005, **39**:5199-5211.

doi:10.1186/2041-2223-1-4
Cite this article as: Greenberg *et al.*: Identifying experimental surrogates for *Bacillus anthracis* spores: a review. *Investigative Genetics* 2010 **1**:4.