DECONTAMINATION OF BACTERIAL SPORES BY A PEPTIDE-MIMIC

R. Nagarajan, Wayne S. Muller, Rebekah Ashley and Charlene M. Mello
US Army Natick Soldier Center, Kansas Street, Natick, MA 01760

ABSTRACT

The threat of bioterrorism involving pathogenic microorganisms, most importantly *Bacillus anthracis*, has called urgent attention to detailed studies of bacterial spores, especially from the point of view of their decontamination. Bacterial spores are the most resistant life forms known. The spores are dormant species and they have to undergo the process of germination first before they can be subjected to deactivation. In this work, we demonstrate that a peptide-mimic (cationic, amphiphilic) chemical agent, dodecylamine is capable of performing the dual functions of germinating the dormant spore as well as deactivating it. We have followed the kinetics of germination by dodecylamine to show that germination is a relatively rapid process. We show that dodecylamine exhibits a broad spectrum germinating activity against various *Bacillus* spores, although there is some species variability. We demonstrate that dodecylamine is capable of efficiently deactivating the spores in suspension medium providing a 5 to 6 log-kill. Further, we have produced hydrophobic polymer nanofibers integrating dodecylamine, to facilitate their use as protective coatings on various surfaces. We demonstrate significant anti-sporal activity of 2 log-kill (99% of the spores) for these nanofibers even without the optimization of the nanofiber characteristics.

1. INTRODUCTION

1.1 Bacterial Spores

Under conditions of nutrient deprivation, vegetative cells of *Bacillus* species undergo sporulation, a process recognized nearly 130 years ago (see for a recent review, Logan 2005). Dormant spores are formed which are able to survive for years. In this state, there is no metabolic activity and consequently, no production of ATP. The unique structure and morphology of the spores allows them to withstand extremes of environmental conditions such as heat, UV radiation, and many common chemical agents. In contrast, under the same environmental conditions, the vegetative cells would have been entirely deactivated. Most bactericidal chemical agents have little or no sporicidal activity or require much higher concentrations and long contact times for any sporicidal action (Bloomfield and Arthur, 1994). In spite of their dormancy and resistance to environmental factors, the spores possess a sensing mechanism to respond quickly to changes in their environment. On encountering specific chemical stimuli, the spores respond by the process of germination in which they lose their structural properties contributing to dormancy and resistance. Germination is followed by a process referred to as outgrowth when biosynthetic activity is resumed, eventually leading to metabolically active vegetative state (Setlow, 1994; Atrih and Foster, 2002; Setlow, 2003; Moir et al, 2002).

1.2 Spore Structure

The unique structure and morphology of the spores is responsible for their dormancy and resistance. The basic spore structure is composed of multiple compartments as shown schematically in Figure 1.

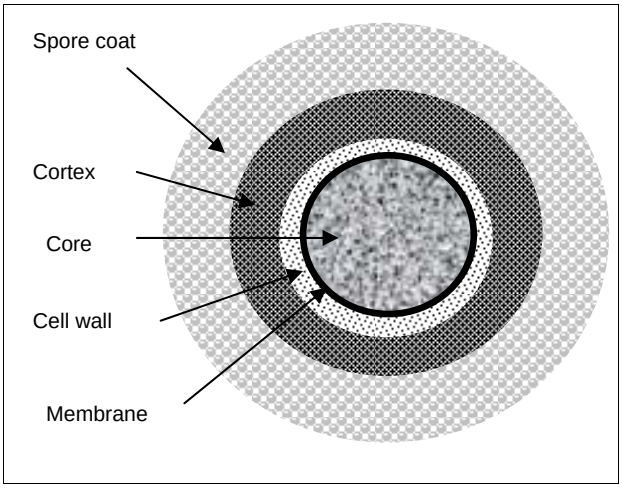

Fig. 1 Schematic representation of a bacterial endospore showing the multi-compartmental structure composed of the spore coat, cortex and core.

The spore coat is a multilayered structure composed of many cross-linked proteins which acts as a permeability barrier. Beneath the coat is a peptidoglycan layer consisting of a thin cell wall and the outer cortex. The cell wall guarantees the maintenance of cellular integrity after germination. Lytic-enzymes which hydrolyze the peptidoglycan layer during germination are present in the cortex. The cortex is essential for maintaining the heat resistance of the spore. DNA and all metabolic constituents are contained in the spore core. In the dormant state, the core is dehydrated with only 10 to 30 percent of the water content of the vegetative cell. The enzymes contained in the core become active on germination. All minerals (mainly Ca^{2+}, Mn^{2+} and Mg^{2+}) are stored as chelates with dipicolinic acid (DPA) in the spore core. Further, the core also contains many small

acid soluble proteins that are associated with the spore DNA and are essential for the UV resistance of spores.

1.3 Spore Germination and DPA Release

Conversion of the dormant spore to vegetative cell takes place via the processes of germination and outgrowth (Moir et al, 2002; Setlow, 2003). When the spore encounters conditions that trigger germination, the cortex is hydrolyzed, the small acid soluble proteins are quickly degraded and the refractility of the spores is rapidly lost (lowering of optical density). Dipicolinic acid stored in the core is then released. The germinated spore then outgrows, involving swelling because of the uptake of water and recommencement of metabolic activity.

Germination may be induced by exposure to nutrients such as amino acids and sugars, by enzymes, by high hydrostatic pressure and by some non-nutrient chemicals such as dodecylamine (see next section). The mechanism of germination is still not adequately understood although it is widely being studied (Lefebvre and Antippa, 1982; Moir et al, 2002; Setlow, 2003). The presence of receptors on spores that can recognize nutrient germinants is a necessity for the amino acid induced germination. However, for dodecylamine induced germination, no such specificity has been identified.

Germination of spores has been monitored most commonly either by optical density changes (an early reference is, Powell, 1950) or by measuring the amount of dipicolinic acid released from the spores (an early reference is, Woese and Morowitz, 1958). Whether both of these measures represent the same biological process is not clear since multiple steps may be involved in the germination process and the DPA release and loss of refractility may correspond to different steps in the germination process (Lefebvre and Antippa, 1982).

1.4 Use of Dodecylamine

Two studies available in the current literature have used dodecylamine for spore germination. Rode and Foster (1961) studied dodecylamine induced germination of *Bacillus megaterium* by following optical density changes in spore suspensions. They reported that for short contact times (3 minutes in their experiment), dodecylamine treated spores were viable while for longer contact times, the spores were killed rapidly. Setlow et al., (2003) studied the germination of *Bacillus subtilis* using wild type spores and mutants lacking germinant receptors. They concluded that dodecylamine may trigger spore germination by a mechanism involving the activation of neither the nutrient germinant receptors on the spore nor the cortex-lytic enzymes. They also reported that dodecylamine was effective in spore killing, although no data were presented.

1.4 Electrospun Nanofibers

Polymer nanofibers, intended for use as protective coating materials, have been prepared in this work by the electrospinning process. Electrospinning generates fibers in the size range of 10 nm to 10 μm using electrostatic forces (Doshi and Renecker, 1995). The process was invented about 70 years ago by Formhals (1934). A schematic of how electrospinning is carried out is shown in Figure 2.

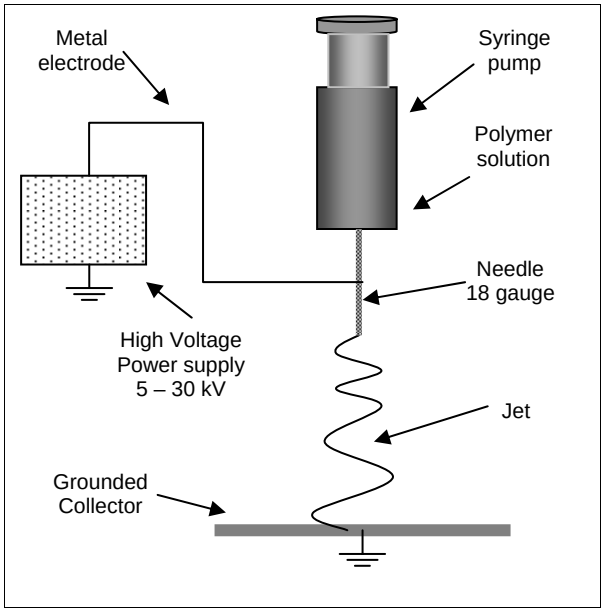

Fig. 2 Schematic of electrospinning process

A solution of the polymer is placed in a syringe pump with a needle attached to the end. As the pump displaces the fluid, a droplet of the solution becomes suspended from the tip of the needle where it is held by surface tension forces. An electrode from a high voltage power supply is placed in contact with the needle tip, thus applying an electrical potential, which induces free charges in the polymer solution. These charged ions move to the electrode of opposite polarity (in this case the collector) in response to the applied electric field. This introduces a tensile force in the polymer solution. When the tensile force overcomes the surface tension force associated with the pendent drop of the liquid at the capillary tip, a jet of liquid is ejected from the tip. The solvent evaporates as the jet travels from the tip to the collector. The evaporation of the solvent leaves dry nanofibers at the collector surface. The stability of the jet depends on the viscous and viscoelastic properties of the polymer solution and unstable jets result in breakup into droplets and the formation of beaded structures.

Electrospun nanofiber matrices display high permeability and small pore sizes, making them attractive barrier materials (Gibson et al, 1998) They can be used

for creating nanocomposites (Huang et al, 2003). The possibility of depositing nanofibers on a variety of substrates, the possibility of surface modifications of fibers and incorporation of stimuli-sensitivity, and the possibility of incorporating nanofibers on fabric materials because of their flexibility and conformability, add to their potential for chemical biological defense applications (Schreuder-Gibson et al, 2002, 2003).

2. EXPERIMENTAL METHODS

2.1 Preparation of Spores.

Spores of *B. cereus* and *B. atrophaeus* were prepared by the solid phase method of growth on plates, with a general sporulation medium consisting of 8 g nutrient broth, 4 g yeast extract, 0.01 g $MnCl_2.4H_2O$, 20 g agar and 5 g peptone, per litre of solution. The plates were inoculated with the bacillus species from a glycerol stock and then incubated at 37°C for five days. The spores were collected from the plates using a cell scraper and were then subjected to a purification process. This involved centrifugation at 12,000 rpm for 10 to 12 minutes at 4°C, followed by washing with ice cold water, performed at least 8 times. After each centrifugation, the solid pellet consisted of two regions, a dense inner pellet comprised of the spores and a softer outer layer comprised of vegetative cells and cell debris. This softer portion was removed in the washing stage. Typical spore suspensions having a spore number density of about 10^7 cfu/ml (cfu-colony forming unit) were prepared and refrigerated for use over a two-month period.

Spores from four species of *Bacillus*, *B. megatarium*, *B. atrophaeus*, *B. subtilis* and *B. stearothermophilus* were also prepared by the solid phase method described above, but using modified Schaeffer's sporulation medium. The medium consisted of (per litre) 16 g Difco nutrient broth, 2 g KCl, 0.25 g $MgSO_4.7H_2O$, 17 g agar, 1 ml of 1 M $Ca(NO_3)_2$, 1 ml of 0.1 M $MnCl_2.4H_2O$, 1 ml of 1 mM $FeSO_4$ and 2 ml of 50%(w/v) glucose.

2.2 Germination of Spores.

The experiment involved the addition of a solution of dodecylamine with specified concentration to a spore suspension and then following the DPA release with time. DPA release was quantitatively monitored either by UV absorbance or by time-resolved fluorescence of released DPA as a complex with terbium, using a microplate method optimized in our laboratory.

In the experiments designed to compare the germination behavior of the four *Bacillus* organisms, optical density measurements using UV spectroscopy was used to follow spore germination. In these experiments, the initial spore suspension was adjusted to an OD_{600} = 1.0, centrifuged and pellet suspended in 10mM Tris HCl buffer solution. This buffer suspension contained 0.6 mM dodecylamine. The suspended spores were incubated at four different temperatures, 25°, 37°, 50° and 70°C and the DPA released was determined by OD readings at 270 nm at times of 0, 30, 60, 120, 180 and 240 minutes. To determine the total DPA content of these spores, the spore suspensions in the buffer (without the presence of dodecylamine) were boiled for 2.5 hrs and the released DPA was determined by determining the OD at 270 nm. The OD at 270 nm for these samples was taken also at time zero as control. The fractional DPA released is then calculated as

$$f = \frac{[OD_{270}]_t - [OD_{270}]_{t=0}}{[OD_{270}]_{Total} - [OD_{270}]_{t=0}} \quad (1)$$

In the experiments designed to follow the germination kinetics, we used a fluorescence method. The fluorescence method (Rosen et al., 1997; Ponce and Venkateswaran, 2002) uses the photoluminescence of terbium dipicolinate formed by the reaction of terbium chloride with dipicolinic acid. We have optimized the method and have found that using time-resolved fluorescence rather than fluorescence intensity, we can determine DPA concentrations from 100 nM to 1 mM accurately. This is a more sensitive method compared to the UV measurements. In our experiments, DPA determination was done with 200 µl samples in microplate wells with a Gemini XPS Microplate Spectrofluorometer. We initially added 50 µl of $TbCl_3$ solution at appropriate concentration to the wells and then filled the remaining volume of 150 µl with either the spore suspension and buffer or the spore suspension and a solution of dodecylamine at specified concentration. The microwell plate was read at various intervals of time. We also did calibration measurements with DPA in order to calculate the amount of DPA released from the fluorescence measurements.

2.3 Deactivation of Spores

For the spore deactivation experiments, we incubated the spore suspension with dodecylamine for a specified time and temperature. Samples from these systems were then plated on nutrient agar plates, the plates were incubated overnight at 37°C and the viable cells grown on the plates were counted as the number of colony forming units (cfu). In the case of the thermophile, *Bacillus stearothermophilus*, the incubation temperature was 50°C. The plating was done on two types of plates: one was nutrient agar plates prepared from a medium containing 8 g nutrient broth and 15 g agar (per litre). The other was bacterial growth (BG) plates prepared from a medium containing 8 g nutrient broth, 20 g agar, 0.1 g yeast extract, 0.1 g KCl, 5 g glucose, 0.25 g $MgSO_4.7H_2O$, 0.28 g $Fe_2(SO_4)_3.7H_2O$, 2 mg $MnCl_2.4H_2O$ (all per litre).

For the spore deactivation experiments conducted on electrospun nanofibers, 100 μl of the spore suspension was deposited on the substrate surface and allowed to incubate for one hour at ambient conditions. The substrate was then rinsed with water and all of the wash liquid containing spores were collected. This spore wash was then plated to determine the number of surviving spores.

2.4 Electrospinning

Electrospinning experiments were done with an applied voltage of 30 kV. The distance between the tip of the needle and the collector surface was about 15 cm, implying an electric field of 2 kV/cm. The polymer solution contained 6 weight percent polyvinyl chloride (MWt 220,000) with dimethyl formamide as the solvent. The amount of dodecylamine incorporated into the solution was about 10% of the amount of the polymer. A solution flow rate of 240 μl/h was maintained for electrospinning. The nanofibers were collected on an aluminum substrate and also on a glass substrate.

3. RESULTS AND DISCUSSION

3.1 Germination Kinetics

The germination kinetics of *B. atrophaeus* spores was quantitatively followed by time-resolved fluorescence measurements. The fraction f of total DPA in the spores released vs time is plotted in Figure 3. We find that the kinetics of DPA release follows a simple first order release process, namely the rate of release of DPA from the spores depends on their amount present in the core. Denoting by [DPA] the amount of DPA in the spore core,

$$\frac{d[DPA]}{dt} = -k[DPA] \qquad (2)$$

$$\frac{[DPA]_o - [DPA]_t}{[DPA]_o} = f = 1 - \exp(-kt) \qquad (3)$$

The line in Figure 3 is a correlation of the experimental data based on this first order rate process. Note that both the total amount of DPA in the spores and the rate constant k were determined by fitting the experimental data. This total amount of DPA along with the experimentally determined amount of DPA released is used to calculate the fraction of DPA released.

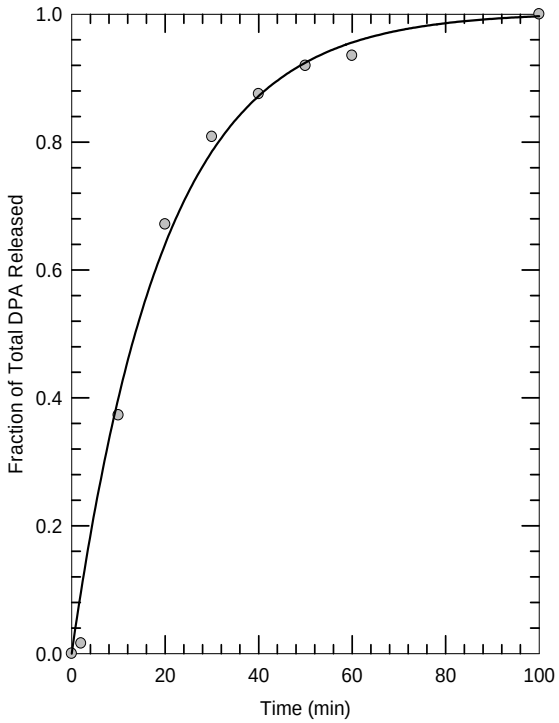

Fig. 3 Fraction of total DPA released from *Bacillus atrophaeus* spores on germination at 37°C induced by 0.25 mM dodecylamine. The DPA was determined by time-resolved fluorescence of terbium-DPA complex.

Nearly half of the DPA is released within 15 minutes of contact and 90% of the DPA within an hour, indicating that DPA release is a relatively rapid process. The apparent first order kinetics need not imply that the germination is such a first order process. Indeed germination monitored by changes in the refractility of the spore suspensions (by OD measurements) show the existence of a lag time and subsequent time dependence describable by one or two exponential processes (McCormick, 1965; Lefebvre and Antippa, 1982; Collado et al, 2006). A fundamental mechanistic and quantitative study of spore germination that will explain the time dependence of all germination markers still remains to be undertaken.

3.2 Variability of *Bacillus* Organisms

The variability in the germination behavior of four species of *Bacillus*, *B. megatarium*, *B. atrophaeus*, *B. subtilis* and *B. stearothermophilus* were tested, at 0.6 mM dodecylamine, using UV measurements of DPA release. The results from these determinations are shown in Figure 4 where the fractional DPA release from the four *Bacillus* organisms is shown at four different temperatures and various times.

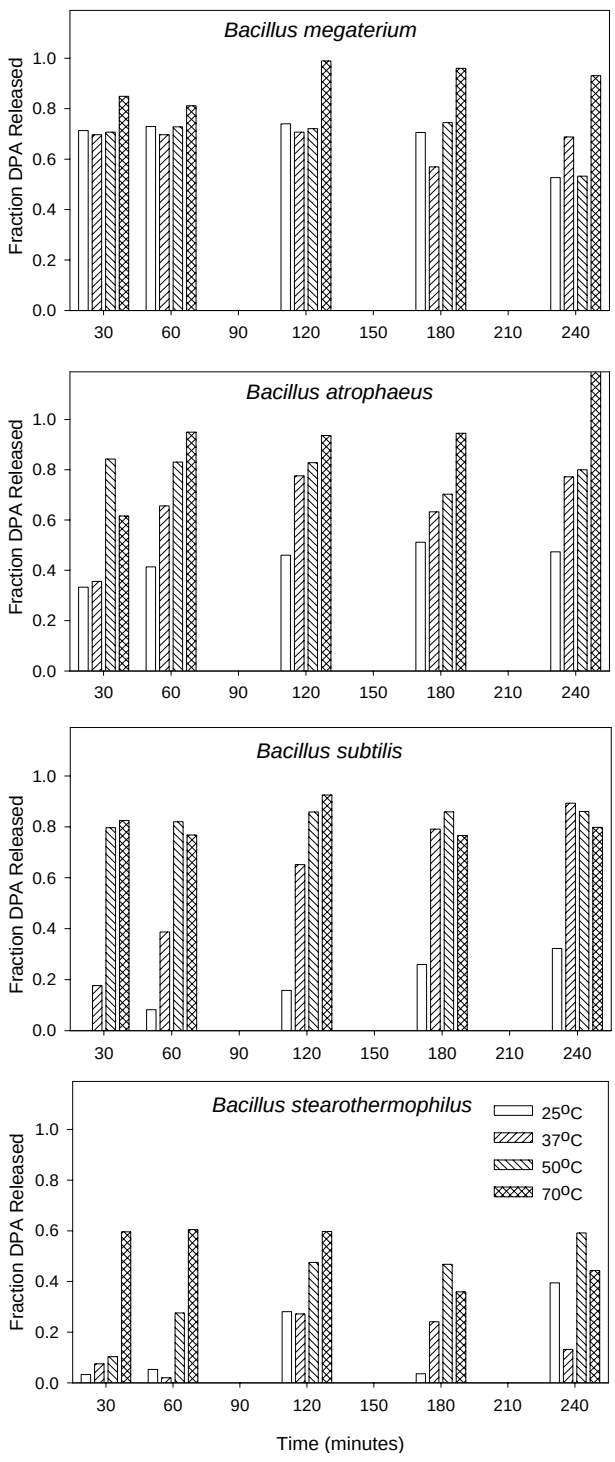

Fig. 4 DPA release measured as a function of time and temperature from four *Bacillus* spores. The DPA released was determined by UV spectroscopy.

Spores of *B. megatarium* were the most sensitive spores to dodecylamine. At 30 minutes, the maximum level of germination appears to take place. Additional time in the presence of dodecylamine, at three of the four temperatures tested, did not appear to enhance germination. The exception is 70ºC for which there is a significant increase in germination at 120 minutes compared to 30 and 60 minutes. However, almost 85% of total DPA release in *B. megaterium* spores occurred before 30 minutes.

The sensitivity of *B. atrophaeus* spores to dodecylamine was significantly less than that of *B. megatarium* spores. The germination differences between these two species were noticeable after 30 minutes of incubation. At this condition, the *B. megaterium* spores had reached their maximum germination at the lower temperatures (25º and 37ºC), while for the *B. atrophaeus* spores the fractional germination was still increasing after 60 minutes at 25ºC and also at 37ºC. However, at the higher temperatures of 50º and 70ºC, dodecylamine produced little variability in the fractional germination of the two species over the four hours.

B. subtilis spores were less sensitive to dodecylamine when compared to both *B. megaterium* and *B. atrophaeus*. The germination differences between *B. subtilis* and the other two species are especially noticeable at the lower temperature of 25ºC. *B. subtilis* spores incubated for 30 minutes at 25ºC did not exhibit any release of DPA. At 25ºC, over the four hour incubation time, *B. subtilis* reached a fractional germination maximum of 0.32 at 4 hrs. *B. megaterium* spores had a fractional germination of 0.71 at 30 minutes and *B. atrophaeus* spores had a fractional germination of 0.46 at 120 minutes. Also at 37ºC, the fractional germination of *B.subtilis* spores was lower compared to the other two species. However after 60 minutes at 37ºC, the fractional release of DPA for *B. subtilis* was similar to *B. atrophaeus* and *B. megaterium*. Interestingly, the fractional germination of all three species exposed to dodecylamine at 50º and 70ºC were very similar over the 4 hour incubation.

B. stearothermophilus spores, the most heat resistant spores known, are the most resistant to dodecylamine. At all temperatures, the *B. stearothermophilus* spores were less sensitive to dodecylamine when compared to the other spore species tested. However at all temperatures dodecylamine produced reasonable amount of DPA release. The *B. stearothermophilus* because of its great heat resistant properties must have very different structural features then the other spores which may contribute to the lower germination effectiveness with dodecylamine.

In treating various spore types with dodecylamine and determining the fractional DPA release, it is evident that dodecylamine has a broad spectrum of germinating activity. However the different *Bacillus* spores display different degrees of sensitivities to dodecylamine, which is most noticeable at lower temperatures. It is interesting that at the higher temperature of 50º and 70ºC these

differences are not persistent. The exception is spores of *B. stearothermophilus* which were the most resistant regardless of the temperature. These results suggest that there are structural differences between spores of various Bacillus species that modulates the germination effectiveness of dodecylamine.

3.3 Deactivation of Spores

The decontamination of spores of *B. atrophaeus* by dodecylamine was measured as a function of the chemical agent concentration. In these experiments, the spores were prepared using a general sporulation medium and the plating experiments were done with the BG plates. The spore suspension was incubated with appropriate amount of dodecylamine for 2 hours at 37°C. The decontamination effectiveness, indicated by the log-kill, is defined as

$$\log-\text{kill} = \log\left(\frac{\text{Original number of spores}}{\text{Surviving number of spores}}\right) \qquad (4)$$

The log-kill (Figure 5) follows a sigmoidal dependence on the concentration of dodecylamine.

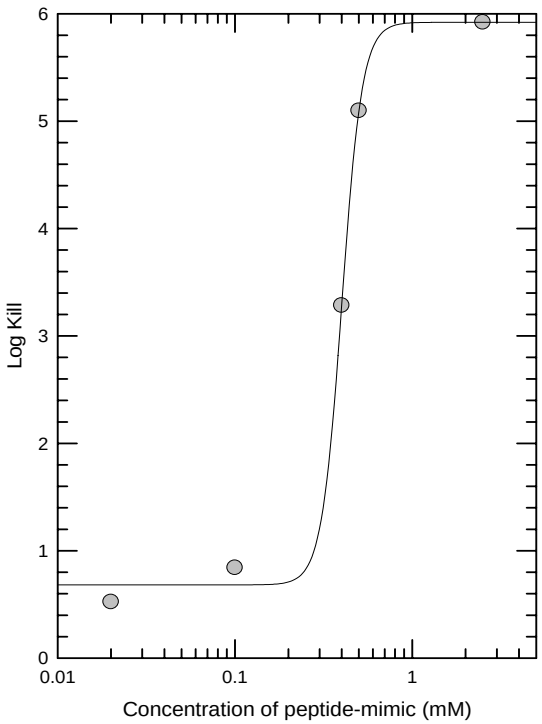

Fig.5 The log-kill for *B. atrophaeus* is plotted against the concentration c of dodecylamine in solution.

$$\log-\text{kill} = a + \frac{bc^n}{d + c^n} \qquad (5)$$

where a,b,d and n are constants. One can identify a critical concentration at which a transition in behavior is obtained in the sense of a dramatic increase in the log-kill.

To obtain some information on the impact of specie variability on decontamination, the deactivation of four *Bacillus* spores, *B. megatarium*, *B. atrophaeus*, *B. subtilis* and *B. stearothermophilus* were also determined after incubating the spore suspensions with 1 mM dodecylamine for 1 hour at 37°C. The incubated samples were plated on plates with nutrient medium (not the BG plates). We found log-kills of 1.3 for *B. megetarium*, 1.8 for *B. atrophaeus*, 1.75 for *B. subtilis* and 0.44 for *B. stearothermophilus*. These smaller estimates for the log-kill may originate from the different spore preparations, different incubation time, and different types of plates used for determining the number of surviving spores. It is well-recognized that spore variability originating from different culture conditions is important, although this problem area has not yet received much attention.

3.4 Preparation of Nanofibers

Nanofibers of polyvinyl chloride (PVC) incorporating the dodecylamine were produced by electrospinning process. A scanning electron micrograph is shown in Figure 6. The resulting fibers have diameters in the range of 100 to 200 nm. The nanofibers were deposited on an aluminum substrate and on a glass substrate and were found to adhere to both surfaces.

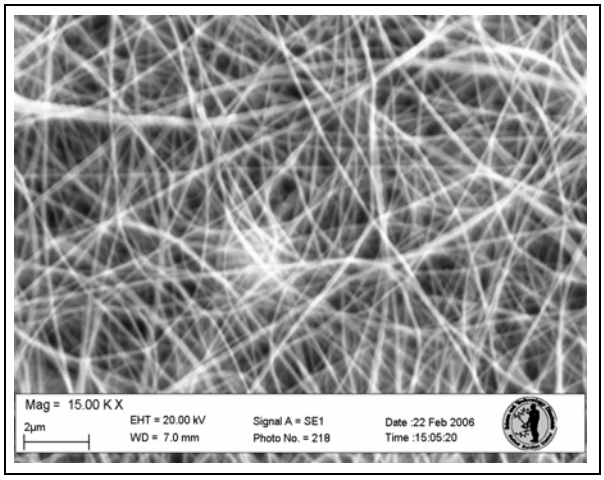

Fig.6 SEM picture of PVC-dodecylamine nanofibers electrospun from a solution in DMF on an aluminum substrate.

The amount of dodecylamine in the fibers is about 10% with the remaining being the carrier polymer. It is possible to vary the proportion of dodecylamine to the polymer. We can also work with other carrier polymers and solvent systems.

3.5 Spore Deactivation with Nanofibers

The nanofibers deposited on the aluminum substrate and on the glass substrate were used to test their decontamination potential against spores of *Bacillus cereus*. The results are tabulated below.

Table 1 Decontamination of *B. cereus* with nanofibers

Spore/Nanofiber/ Substrate system	# of original spores	# of spores surviving
B. cereus PVC-dodecylamine nanofibers Aluminum substrate	1.9×10^6	2.7×10^4
B. cereus PVC- dodecylamine nanofibers Glass substrate	5.5×10^6	3.0×10^4

On both substrates, we observe a log-kill of 2 indicating a 99% killing of spores.

We have made a very crude estimate of the effective concentration of dodecylamine available for decontamination in these nanofiber experiments. From the amount of polymer solution electrospun, we know the total amount of dodecylamine present in nanofibers on the substrate surface. Considering that only the dodecylamine in the surface region of nanofibers are accessible and taking the system volume to be the volume of the spore suspension we deposited on the substrate, we estimate the effective concentration of dodecylamine to be about 0.23 mM. From the sigmoidal plot shown in Figure 5, this concentration is below the critical concentration associated with the sharp increase in log-kill. Therefore, optimization of the nanofiber loading on the substrate and the concentration of dodecylamine in the polymer can lead to an increase in the effective concentration of the available dodecylamine and enhance the log-kill to larger values attained in solution. Further, depending upon the substrate, a variety of polymers can be used in place of PVC to improve compatibility with the substrate, for protective coating applications.

CONCLUSION

We demonstrate that dodecylamine is an effective chemical agent for germinating as well as decontaminating a number of *Bacillus* spores. We show that this chemical agent can be combined with a polymer to produce electrospun nanofibers which exhibit effective spore decontamination properties. These nanofibers can potentially be used as coatings on a variety of substrate surfaces including textiles.

Self-decontaminating surfaces and coatings for individual protection and decontamination represents our primary objective. Research efforts to understand the mechanism of dodecylamine germination and killing; maintenance of activity in a polymeric carrier; and covalent attachment of the alkylamines to surfaces are currently being pursued to bring us closer to the realization of protective and decontaminating surfaces for biological agents.

ACKNOWLEDGEMENTS

The work was supported by a DARPA grant (CMM) and an ILIR grant (WSM). One of the authors (RN) was supported by a National Research Council Senior Research Associateship and another author (RA) by NSC as an intern.

REFERENCES

Atrih, A., and S.J. Foster, 2002: Bacterial endospores the ultimate survivors, *Int. Dairy J*., **12**, 217-223.

Bloomfield, S.F., and M. Arthur, 1994: Mechanisms of inactivation and resistance of spores to chemical biocides, *J. Appl. Bacterial. Symp. Suppl*. 76, 91S-104S.

Collado, J., A. Fernandez, M. Rodrigo, and A. Martinez, 2006: Modelling the effect of a heat shock and germinant concentration on spore germination of a wild strain of *Bacilllus cereus*, *Int. J. Food Microbiol*., **106**, 85-89.

Doshi, J., and D.H. Reneker, 1995: Electrospinning process and applications of electrospun fibers, *J. Electrostatics*, **35**, 151-160.

Formhals, A., 1934: process and apparatus for preparing artificial threads, U S Patent 1,975,504.

Gibson, P.W., H.L. Schreuder-Gibson, C. Pentheny, 1998: Electrospinning technology: direct application of tailorable ultrathin membranes, *J. Coated Fabrics*, **28**, 63-72.

Huang, Z.M., Y.Z. Zhang, M. Kotaki, and S. Ramakrishna, 2003: A review on polymer nanofibers by electrospinning and their applications in nanocomposites, *Composites Sci. Technol*., **63**, 2223-2253.

Lefebvre, G.M., and A.F. Antippa, 1982: The kinetics of germination in bacterial spores, *J. Theor. Biol*., **95**, 489-515.

Logan, N.A., 2005: *Bacillus anthracis*, *Bacillus cereus*, and other aerobic endospore forming bacteria. *Topley and Wilson's Microbiology and Microbial Interactions*, P.R. Murray et al (Eds), 10th ed., Bacteriology. Arnold, London . pp.

McCormick, N.G., 1965: Kinetics of spore germination, *J. Bacteriol.*, **89**, 1180-1185.

Moir, A., B.M. Corfe, and J. Behravan, 2002 : Spore germination, *Cell. Mol. Life Sci*., **59**, 403-409.

Ponce, A., and K. Venkateswaran, 2002: Improved technique for detecting endospores via luminescence, *NASA Technical Report*, **26**, No.7.

Powell, J.F., 1950: Factors affecting the germination of thick suspensions of *Bacillus subtilis* spores in L-alanine solutions, *J. Gen. Microbiol*., **4**, 330-338.

Rode, L.J., and J.W. Foster, 1961: Germination of bacterial spores with alkyl primary amines, *J. Bacteriol*., **81**, 768-779.

Rosen, D.L., C. Sharpless, and L.B. McGown, 1997: Bacterial spore detection and determination by use of terbium dipicolinate photoluminescence, *Anal. Chem*., **69**, 1082-1085

Schreuder-Gibson, H.L., P.H. Gibson, K. Senecal, M. Sennett, J. Walker, W. Yeomans, D. Ziegler, and P.P. Tsai , 2002: Protective textile materials based on electrospun nanofibers, *J. Adv. Materials*, **34**, 44-55.

Schreuder-Gibson, H.L., Q. Truong, J.E. Walker, J.R. Owens, J.D. Wander, and W.E. Jones, Jr., 2003: Chemical and biological protection and detection in fabrics for protective clothing, *MRS Bulletin*, August, 574-578.

Setlow, P., 1994: Mechanisms which contribute to the long-term survival of spores of *Bacillus* species, *J. Appl. Bacterial. Symp. Suppl.* 76, 49S-60S.

Setlow. P., 2003: Spore germination, *Curr. Opin. Microbiol*., **6**, 550–556

Setlow, B., A.E. Cowan, and P. Setlow, 2003: Germination of spores of *Bacillus subtilis* with dodecylamine, *J. Appl. Microbiol*., **95**, 637-648.

Woese, C., and H.J. Morowitz, 1958: Kinetics of the release of dipicolinic acid from spores of *Bacillus subtilis*, *J. Bacteriol*., **76**, 81-83.