An apparatus and method for automated monitoring of airborne bacterial spores. The apparatus is provided with an air sampler, a surface for capturing airborne spores, a thermal lysis unit to release DPA from bacterial spores, a source of lanthanide ions, and a spectrometer for excitation and detection of the characteristic fluorescence of the aromatic molecules in bacterial spores complexed with lanthanide ions. In accordance with the method, computer-programmed steps allow for automation of the apparatus for the monitoring of airborne bacterial spores.

23 Claims, 3 Drawing Sheets
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Koehler, T.M., Bacillus anthracis Genetics and Virulence Gene Regulation, Current Topics in Microbiology & Immunology, vol. 271, pp. 143-164, 2002.


Sorasaenee, K. et al, Cooperative Binding of Tb(III) Supramolecular Complexes with Dipicolinic Acid: Improved Sensitivity of Metal-Containing Lumophores in Biomedical Applications, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California, 1 page total (2003).


FIGURE 1
S1: Instrument Initialization
   1 min

S2: Define Parameters
    e.g. threshold

S3: Air Sampling
    15 min

S4: Thermal Lysis Unit
    15 sec, 200°C

S5: Lanthanide Solution Addition (TbCl₃)

S6: Spectrophotometer Measurement (UV light, fluorescence detection)

S7: Data Storage

S8: Above Threshold?
    Yes → Alarm On
    No → S9

S9: Sampling Time End?
    Yes → Sampling Completed
    No → S8
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APPARATUS AND METHOD FOR
AUTOMATED MONITORING OF AIRBORNE
BACTERIAL SPORES

CROSS REFERENCE TO RELATED
APPLICATIONS

This application claims priority to U.S. Provisional Ser. No. 60/671,918 for “Automated Monitoring of Airborne Bacterial Spores” filed on Apr. 15, 2005, incorporated herein by reference in its entirety. This application may also be related to U.S. Ser. No. 10/306,331 filed on Nov. 27, 2002 and U.S. Ser. No. 10/355,462 filed on Jan. 31, 2003, both of which are incorporated herein by reference in their entirety. The present application may also be related to U.S. Ser. No. 11/810,005 filed on Jun. 4, 2007, to U.S. Ser. No. 11/453,296, filed on June 13, 2006, and to U.S. Ser. No. 11/332,788 filed on Jan. 12, 2006.

STATEMENT OF GOVERNMENT SUPPORT

The present invention was made with support from the United States Government under Grant number NAS7-1407 awarded by NASA. The United States Government has certain rights in the invention.

BACKGROUND

Bacterial endospores are highly resistant, thick walled structures formed by vegetative cells during a process called sporulation, and they are quite possibly the most resistant forms of life (Hindle and Hall, 1999 Analyst, 124, 1599-1604). They are highly resistant to radiation, chemical agents, dessication, and other normally harmful environments (Nicholson, W. L. et al. 2000. Microbiol Molecular Biol. Rev., 64, 548). Several bacterial genera are capable of producing endospores; Bacillus and Clostridium are the two most common endospore-producing genera. Within the Bacillus genus is the species Bacillus anthracis the causative agent for anthrax.

Current methods for rapidly detecting airborne bacterial spores that can carry toxins, such as those that cause anthrax, are prohibitively costly to implement and maintain. Additionally, the present monitoring methods are limited by the use of species-specific antigens and DNA probes, which would not detect a seemingly benign species that is modified to carry the anthrax toxin (Koehler, T. M. 2002. Current Topics in Microbiology & Immunology, 271, 143-164; Uchida, I. 1991. J. Bacteriol. 175, 5329-5338).

Therefore, what is needed is an automated apparatus for rapidly detecting bacterial spores that are less costly and not limited to a particular species or DNA sequence.

SUMMARY

According to a first aspect of the present disclosure, a method for the apparatus as disclosed above is provided, the method comprising the steps of: (a) operating the air sampler to deposit sampled air onto the surface; (b) moving the surface from the air sampler to a position proximal to the thermal lysis unit; (c) positioning the thermal lysis unit onto the surface, thereby allowing for thermal lysis of any spores on the surface, thus releasing DPA from any lysed spores; (d) positioning the thermal lysis unit off of the surface to a position proximal to the source of lanthanide ions; (e) operating the source of lanthanide ions to provide lanthanide ions to the surface, thereby putting the lanthanide ions in contact with any DPA on the surface, resulting in said DPA-lanthanide complex; (f) moving the surface to a position away from the source of lanthanide ions to a position proximal to the spectrometer comprising a UV source; (g) operating the spectrometer to expose the surface to the UV source, thereby exciting said DPA-lanthanide complex and producing fluorescence; (h) operating the spectrometer to detect the fluorescence resulting from the excited DPA-lanthanide complex of step (g), and (i) quantifying the fluorescence intensity and/or imaging the fluorescence of step (h).

According to a second aspect of the present disclosure, a method for the apparatus as disclosed above is provided, the method comprising the steps of: (a) operating the air sampler to deposit sampled air onto the surface; (b) moving the surface from the air sampler to a position proximal to the source of lanthanide ions; (c) operating the source of lanthanide ions to provide lanthanide ions to the surface; (d) moving the surface to a position away from the source of lanthanide ions to a position proximal to the thermal lysis unit; (e) moving the thermal lysis unit onto the surface, thereby allowing for thermal lysis of any spores on the surface, thus releasing DPA from any lysed spores, and putting DPA in contact with the lanthanide ions provided to the surface in step (c) to form said DPA-lanthanide complex; (f) moving the thermal lysis unit off of the surface to a position proximal to a spectrometer comprising a UV source; (g) operating the spectrometer to expose the surface to the UV source, thereby exciting said DPA-lanthanide complex and producing fluorescence; (h) operating the spectrometer to detect the fluorescence resulting from the excited DPA-lanthanide complex of step (g); and (i) quantifying the fluorescence intensity and/or imaging the fluorescence of step (h).

One advantage of a bacterial monitor of the present disclosure, is that the chemical marker used for detection—DPA—is essential to the viability of the bacterial spore yet is present whether the spores are alive or dead. Furthermore, since approximately 10^9 molecules of DPA are present in each bacterial spore as compared to 1 copy of DNA, expensive sample amplification schemes are not required, which significantly lowers the complexity and cost of the monitoring apparatus. Furthermore, DPA binding to terbium exhibits a million-fold greater binding constant than amino acids or nucleic acids.

Another advantage of the present disclosure is that it is not susceptible to false positives due to natural fluctuations in bacterial spore concentrations because these fluctuations occur in a concentration regime many orders of magnitude lower (natural concentrations range from 0.01-0.5 bacterial spores per liter of air) than those, for example, present during an anthrax attack (Pastuszka, J. S. et al., 2000, Atmospheric Environment, 34, 3833-3842).

A further advantage of the present disclosure is that unlike other fluorescence detection methods, the DPA triggered lanthanide luminescence method for bacterial spore detection is not mired by varying background fluorescence from interfer-
providing terbium or europium ions to the DPA released from rescence lifetimes from impurities are generally on the order of nanoseconds. Thus, after excitation of the lanthanide-DPA complex with UV light (from a Xenon flash lamp, laser or UV LED), the fluorescence is not detected for several microseconds, and with the detector programmed to capture after several microseconds, the background luminescence having occurred after nanoseconds, will not interfere.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1 shows an air sampler with quartz fiber tape on spools in accordance with a first embodiment of the present disclosure.

FIG. 2 shows a computer-operated, automated apparatus for the unattended monitoring of bacterial spores in accordance with a second embodiment of the present disclosure.

FIG. 3 shows a flow chart of steps of a computer program of the present disclosure for the automated detection of bacterial spores in the air in accordance with the present disclosure.

**DETAILED DESCRIPTION**

Rapid detection of bacterial spores of the present disclosure is based on dipicolinic acid (DPA)-triggered lanthanide ion (terbium, europium) luminescence (Hindle and Hall, 1999 Analyst, 124, 1599-1604). The core of bacterial spores contains up to 1 molar (M) DPA that can be released into bulk solution by lysis of the spore (Murell, 1969, Bact. Spore 1, 216). The released DPA binds the lanthanide ions with high affinity and triggers intense green luminescence under UV excitation.

An apparatus according to the present disclosure herein, enables for automated monitoring of aerosolized bacterial spores by implementing a lanthanide (terbium or europium ions) luminescence assay (U.S. patent application Ser. No. 10/306,331; U.S. patent application Ser. No. 10/355,462) in conjunction with a computer-operated apparatus comprising: an aerosol capture device (air sampler) (1) (FIGS. 1, 2) a surface (2) for adherence of the spores captured by the air sampler (e.g. quartz fiber tape), a thermal lysis unit (3) to induce bacterial spore lysis, a source of lanthanide ions (4) for providing terbium or europium ions to the DPA released from the lysed spores, a lifetime-gated luminescence spectrometer (5) with UV source for excitation and subsequent detection of the DPA-lanthanide complex, an alarm programmed to sound at a specific fluorescence threshold, as well as a computer programmed to control the apparatus (FIGS. 2, 3).

In one embodiment of the present disclosure, an automated apparatus is provided for the detection of airborne bacterial spores, wherein the apparatus comprises an air sampler, and a surface connectable to the air sampler suitable for capturing spores from the air.

In a second embodiment of the present disclosure, an apparatus is provided for the detection of airborne bacterial spores, wherein the apparatus comprises an air sampler, a surface connectable to the air sampler suitable for capturing spores from the air, a thermal lysis unit for lysing the spores on the surface resulting in fluorescence and detection of the fluorescence, and an alarm programmed to sound at a specific fluorescence threshold.

In another embodiment, the apparatus provided in the present disclosure is an automated apparatus that does not require outside intervention apart from establishing the program parameters and fluorescence threshold. This automated apparatus further comprises a computer program as outlined in FIG. 3.

The air sampler (1) of the present disclosure can be of any type so long as the rate at which it samples the air (usually by way of a vacuum) is conducive to capturing spores from the air onto the surface with a high efficiency (Example 1, FIGS. 1, 2).

A surface connectable to the air sampler (2) can be of any type so long as it allows for the capture of bacterial spores from the air. Commonly this will be a type of filter tape that is directly attached to the air sampler (Example 2). Examples of surfaces for capturing the spores in the sampled air are, but are not limited to, one of the following: quartz fiber tape, electrically charged tape and polymer tape. The term “tape” herein refers to any surface that allows for the passage of gases in the air, and will capture microscopic species such as bacterial spores.

The glass fiber tape used in the UDT Model 7001 (Example 1, FIG. 1) allows for the option of integrating air sampling onto one spot for an extended period and/or sampling the air onto a pristine spot after a cycle of air sampling. This option is regulated by the movement of the glass fiber tape on at least two spools (6) which when stabilized, keep the tape in one place at the opposite end of the intake of the air sampler (FIG. 1).

Alternatively, after a sample of air is captured onto the tape, the tape can be moved by rotation of the spools, allowing for interaction of the tape and its contents with the thermal lysis unit. Thermal lysis is then followed by the administration of the lanthanide ions in solution, followed by excitation and finally detection.

A thermal lysis unit (3) can be of any type so long as its interaction with the surface results in the lysis of any spores on the surface (Example 3). The purpose of the thermal lysis is to release the DPA from any spores on the surface, thereby allowing for detection of the spore. Thus, thermal lysis using an easily customized lysis implement (e.g. cautery, soldering iron) is incorporated into the apparatus of the present disclosure for administering high temperatures (~200° C) for the lysis of the bacterial spore(s) and subsequent release of DPA onto the surface.

A source of lanthanide ions (4) (terbium or europium) provided to the surface can be of various configurations so long as the source provides the lanthanide ions to the surface. Any lysed spores on the surface will release DPA onto the surface. The mixture of lanthanide ions and DPA molecules will form high affinity complexes on the surface that will fluoresce upon excitation by ultraviolet (UV) light (Example 4).

A lifetime-gated fluorescence spectrometer (5) with a UV source of the present disclosure (Example 5) allows for the excitation of the lanthanide-DPA complex. Accordingly, the spectrometer of the present disclosure is positioned such that the UV source (light) is directed toward the surface through which the sampled air was filtered. In this way, the UV light will be directed at any lanthanide-DPA complexes on the surface. The spectrometer of the present disclosure further contains a fluorescence spectrometer for detection of the fluorescence resulting from an excited lanthanide-DPA complex (FIG. 2).
EXAMPLE 1

Air Sampler

For capture of aerosolized bacterial spores according to the present disclosure, a Model 7001 air sampler (Universal Detection Technology, Beverly Hills, Calif.) is shown in FIG. 1. This Model 7001 air sampler has a high transfer efficiency (>95%) for microbe-containing aerosols, and it uses a glass fiber filter tape. The air sampler shown in FIG. 2 was customized for the automated apparatus as shown. The top half of the assembly leads to the air inlet, and the bottom connects to the vacuum pump. A motorized nozzle (8) makes an airtight connection during air sampling, and it is retracted after sampling to allow the tape to move forward. Similar air samplers can be used.

EXAMPLE 2

Surface for Capture of Spores

The surface (2) for capturing spores from the air sampled and taken in by the air sampler was a quartz glass fiber filter tape (Whatman) custom made for the Model 7001 air sampler (FIG. 1). Quartz fiber tape collects micron-sized particles as air is pulled through the filter. The same quartz fiber tape is used in the automated apparatus shown in FIG. 2.

EXAMPLE 3

Implement for Thermal Lysis of Spores

The thermal lysis unit (3) in FIG. 2 (consists of a soldering iron with a flat tip mounted on a motorized stage. To lyse (pop) spores the thermal lysis unit makes contact with the surface at high temps (~200°C).

EXAMPLE 4

Source of Lanthanide Ions

The source of lanthanide ions (4) used in the present disclosure consisted of a dropper (10) providing 25 µl from a reservoir of 10-µM TbCl₃ (terbium chloride hexahydrate, 99.999%, Aldrich, Milwaukee, Wis.) (FIG. 2). In FIG. 2, the source of lanthanide ions is connected to the surface of the apparatus, adjacent to the lanthanide ion dropper (10).

EXAMPLE 5

Lifetime-Gated Fluorescence Spectrometer

The lifetime-gated fluorescence spectrometer (5) used in the present disclosure is a miniature spectrometer with fiber-optic probe (Ocean Optics, USB2000-FL-2048-element fluorescence spectrometer and PX-2—Pulsed Xenon Flash Lamp, Dunedin Fla.). The fiber optic probe contains both fibers for excitation and detection. For background, the luminescence spectrum of 25 µl of 10-µM TbCl₃ on the fiber tape was subtracted from all luminescence spectra (FIG. 2).

EXAMPLE 6

Computer for Operation of Detector

The computer for operating the automated detector of the present disclosure was programmed according to the outline...
proximal to the source of lanthanide ions; (e) operating the
providing lanthanide ions to the surface.

face, thereby allowing for thermal lysis of any spores on the
surface, and a second condition where the air
plated, and can be made without departing from the scope of
the invention as defined in the appended claims.

The invention claimed is:

1. An apparatus for the detection of airborne bacterial spores, wherein the apparatus comprises:
an air sampler comprising an intake;
a surface connectable to the air sampler, the surface suit-
able for spore adhesion and on-surface spore detection;
a thermal lysis unit for lysing the spores on the surface
resulting in release of dipicolinic acid (DPA);
a source of lanthanide ions for providing lanthanide ions to
the released DPA on the surface to form a DPA-lan-
thanide complex; and

2. The apparatus of claim 1, wherein the air sampler is
moveable between a first condition where the air sampler is
proximal to the surface, and a second condition where the air
sampler forms an airtight seal with the surface.

3. The apparatus of claim 1, further comprising at least two
spools, the surface connectable to the intake of the air sampler
being located on said spools.

4. The apparatus of claim 1, wherein the surface connect-
able to the air sampler is selected from the group consisting of
quartz fiber tape, electrostatically charged tape and polymer
tape.

5. The apparatus of claim 1, wherein the thermal lysis unit
is selected from the group consisting of a canuterizer and a
soldering iron.

6. The apparatus of claim 1, wherein the source of lan-
thanide ions comprises terbium ions.

7. The apparatus of claim 1 wherein the source of lan-
thanide ions comprises europium ions.

8. The apparatus of claim 1, wherein the source of lan-
thanide ions comprises a reservoir comprising a dropper for
providing lanthanide ions to the surface.

9. The apparatus of claim 8 wherein the dropper adminis-
ters 25 μl drops of 10 µM terbium chloride.

10. The apparatus of claim 1, wherein the UV source is
selected from the group consisting of a Xenon flash lamp, a
laser, and a UV LED.

11. A method for the operation of the apparatus of claim 1,
the method comprising steps of: (a) operating the air sampler
to deposit sampled air onto the surface; (b) moving the sur-
face from the air sampler to a position proximal to the thermal
lysis unit; (c) positioning the thermal lysis unit onto the sur-
face, thereby allowing for thermal lysis of any spores on the
surface, thus releasing DPA from any lysed spores; (d) posi-
tioning the thermal lysis unit off of the surface to a position proximal to the source of lanthanide ions; (e) operating the
source of lanthanide ions to provide lanthanide ions to the
surface, thereby putting the lanthanide ions in contact with
the released DPA on the surface to form a DPA-lan-
thanide complex; and

12. A method for the operation of the apparatus of claim 1,
the method comprising steps of: (a) operating the air sampler
to deposit sampled air onto the surface; (b) moving the sur-
face from the air sampler to a position proximal to a source of
lanthanide ions; (c) operating the source of lanthanide ions to
provide lanthanide ions to the surface; (d) moving the surface
to a position away from the source of lanthanide ions to a
position proximal to the thermal lysis unit; (e) moving the
thermal lysis unit onto the surface, thereby allowing for ther-
mal lysis of any spores on the surface, thus releasing DPA
from any lysed spores, and putting DPA in contact with the
lanthanide ions provided to the surface in step (c) to form said
DPA-lanthanide complex; (f) moving the thermal lysis unit
off of the surface to a position proximal to the spectrometer
comprising a UV source; (g) operating the spectrometer to
expose the surface to the UV source, thereby exciting said
DPA-lanthanide complex and producing fluorescence; (h) detecting fluorescence resulting from the excited DPA-lanthanide complex of step (g); and (i) quantifying the fluorescence intensity and/or imaging the fluorescence of step (h).

13. The apparatus of claim 1, further comprising a com-
puter and/or an alarm programmed to sound at a specific
fluorescence threshold, the computer comprising a computer
program to control automation of said apparatus.

14. The apparatus of claim 1, wherein the surface connect-
able to the air sampler suitable for spore adhesion and on-
surface spore detection is a filter tape that can be directly
attached to the air sampler.

15. The apparatus of claim 1, wherein the apparatus is
adapted for multiple detections each detection occurring on a
different portion of the surface, through a relative movement
between the surface and the air sampler.

16. The apparatus of claim 1, further comprising an
arrangement for relative movement between the surface and
the air sampler to adapt the apparatus to multiple detections,
each detection occurring on a different portion of the surface.

17. An apparatus for the detection of airborne bacterial spores, wherein the apparatus comprises:
an air sampler comprising an intake;
a surface connectable to the air sampler, the surface suit-
able for spore adhesion and on-surface spore detection;
a thermal lysis unit.

18. The apparatus of claim 17, wherein the apparatus is
adapted for multiple detections each detection occurring on a
different portion of the surface, through a relative movement
between the surface and the thermal lysis unit.

19. The apparatus of claim 17, further comprising an
arrangement for relative movement between the surface and
and
the thermal lysis unit to adapt the apparatus to multiple detections, each detection occurring on a different portion of the surface.

20. An apparatus for the detection of airborne bacterial spores, wherein the apparatus comprises:
- an air sampler comprising an intake;
- a surface connectable to the air sampler, the surface suitable for spore adhesion and on-surface spore detection;
- a thermal lysis unit for lysing the spores on the surface resulting in release of dipicolinic acid (DPA);
- a source of lanthanide ions for providing lanthanide ions to the released DPA on the surface to form a DPA-lanthanide complex; and
- a lifetime-gated fluorescence spectrometer having a UV source for on-surface excitation of the DPA-lanthanide complex resulting in fluorescence and subsequent detection of the fluorescence from the surface, wherein the surface is connectable to the source of lanthanide ions or the lifetime-gated fluorescence spectrometer through a relative movement of the surface with respect to the source of lanthanide ions or the lifetime-gated fluorescence spectrometer.

21. The apparatus of claim 20, wherein the apparatus is adapted for multiple detections each detection occurring on a different portion of the surface, through a relative movement between the surface and source of lanthanide ions or the lifetime-gated fluorescence spectrometer.

22. The apparatus of claim 20, further comprising an arrangement for relative movement between the surface and the source of lanthanide ions or the lifetime-gated fluorescence spectrometer to adapt the apparatus to multiple detections, each detection occurring on a different portion of the surface.

23. A method for the operation of the apparatus of claim 13, the method comprising steps of:
- (a) operating the air sampler to deposit sampled air onto the surface;
- (b) moving the surface from the air sampler to a position proximal to the thermal lysis unit;
- (c) positioning the thermal lysis unit onto the surface, thereby allowing for thermal lysis of any spores on the surface, thus releasing DPA from any lysed spores;
- (d) positioning the thermal lysis unit off of the surface to a position proximal to the source of lanthanide ions;
- (e) operating the source of lanthanide ions to provide lanthanide ions to the surface, thereby putting the lanthanide ions in contact with any DPA on the surface, resulting in said DPA-lanthanide complex;
- (f) moving the surface to a position away from the source of lanthanide ions to a position proximal to the spectrometer comprising a UV source;
- (g) operating the spectrometer to expose the surface to the UV source, thereby exciting said DPA-lanthanide complex and producing fluorescence;
- (h) operating the spectrometer to detect the fluorescence resulting from the excited DPA-lanthanide complex of step (g); and
- (i) quantifying the fluorescence intensity and/or imaging the fluorescence of step (h); (j) comparing the fluorescence of step (h) with a programmed fluorescence threshold; (k) sounding the alarm if the fluorescence of step (h) exceeds and/or meets the programmed fluorescence threshold.

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