

**UNIQUE CHROMOSOMAL SEQUENCE OF
BACILLUS ANTHRACIS AND METHODS OF
MAKING AND USING THEREOF
INCLUDING REAL-TIME PCR ASSAYS**

CROSS REFERENCE TO RELATED
APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Nos. 60/556,045 filed 24 Mar. 2004, and 60/564,639, filed 23 Apr. 2004, which names Elizabeth A. Bode, William J. Hurtle, and David A. Norwood, Jr. as inventors, both of which are herein incorporated by reference in their entirety.

ACKNOWLEDGMENT OF GOVERNMENT
SUPPORT

Employees of the United States Army made this invention. The government has rights in the invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention generally relates to nucleic acid molecules of *Bacillus anthracis*. The nucleic acid molecules may be used in nucleic acid assays.

2. Description of the Related Art

Bacillus anthracis is a spore-forming gram-positive bacterium well known for its recent use as a bioterrorist agent. Identification of *B. anthracis* can be done clinically utilizing gram stain, colony morphology, and various biochemical tests. See Logan & Turnbull (2003) In Manual of Clinical Microbiology. American Society of Microbiology, Washington D.C. However, these methods are time consuming and more rapid tests, such as polymerase chain reaction (PCR), have been employed to detect *B. anthracis* in clinical samples. See Oggioni, et al. (2002) J. Clin. Microbiol. 40:3956-3963.

Real-time PCR is preferred over conventional PCR methods for the identification of organisms because it is fast, less labor intensive, and adds the specificity of a probe. Real-time PCR assays have been used to identify *anthracis* based on virulence genes associated with the toxin-encoding plasmid (pX01) and capsule-encoding plasmid (pX02). See Higgins, et al. (2003) Appl. Environ. Microbiol. 69:593-599; Oggioni, et al. (2002) J. Clin. Microbiol. 40:3956-3963; and Patra, et al. (2002) Ann. N.Y. Acad. Sci. 969:106-111. While the presence of both pX01 and pX02 is needed to give *B. anthracis* its virulence, it is conceivable that these plasmids could be passed to its genetic neighbors with unknown implications. Thus, a chromosomal marker for use in nucleic acid based assays such as real-time PCR assays is more desirable.

Unfortunately, past attempts at developing a chromosomal real-time PCR assay have failed due to the close genetic relationship of *Bacillus* species. *B. anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis* have very little variability and are genetically indistinguishable using multilocus enzyme electrophoresis. See Helgason, et al. (2000) Appl. Environ. Microbiol. 66:2627-2630. Recent work using rep-PCR has shown that the previously listed species of *Bacillus* as well as *Bacillus mycoides*, *Bacillus pseudomycoides*, and *Bacillus weihenstephanensis* do have some genetic differences. See Cherif, et al. (2003) J. Appl. Microbiol. 94:1108-1119. A real-time PCR assay based on the chromosomal rpoB gene has been developed and used,

however, it targets a region that is variable between *Bacillus* species, therefore, the specificity of the assay is dependent on PCR conditions and specific primers and probes. See Drago, et al. (2002) J. Clin. Microbiol. 40:4399; and Qi, et al. (2001) Appl. Environ. Microbiol. 67:3720-3727.

Thus, a need still exists for a unique chromosomal nucleotide sequence in *B. anthracis* for use in nucleic acid based assays such as real-time PCR assays.

SUMMARY OF THE INVENTION

The present invention generally relates to nucleic acid molecules that are specific for *Bacillus anthracis*.

In some embodiments, the present invention provides an isolated nucleic acid molecule comprising at least about 11 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement. In some embodiments, the nucleic acid molecule comprises

- (a) at least about 22 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (b) at least about 30 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (c) at least about 40 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (d) at least about 50 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (e) at least about 60 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (f) at least about 70 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (g) at least about 80 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (h) at least about 90 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (i) at least about 100 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement; or
- (j) the sequence set forth in SEQ ID NO:1 or its complement.

In some embodiments, the present invention provides a nucleic acid molecule of which consists essentially of at least about 11 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement. In some embodiments, the nucleic acid molecule consists essentially of

- (a) at least about 22 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (b) at least about 30 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (c) at least about 40 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (d) at least about 50 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (e) at least about 60 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (f) at least about 70 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (g) at least about 80 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (h) at least about 90 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement;
- (i) at least about 100 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement; or
- (j) the sequence set forth in SEQ ID NO:1 or its complement.

In some embodiments, the present invention provides a nucleic acid molecule which consists of at least about 11 consecutive nucleotides of the sequence set forth in SEQ ID NO:1 or its complement. In some embodiments, the nucleic acid molecule consists of