Some *Bacillus thuringiensis* Strains Share *rpoB* Nucleotide Polymorphisms Also Present in *Bacillus anthracis*

Identification of *Bacillus anthracis* is considerably difficult because of its very high phenotypic and genotypic similarity to other members of the *Bacillus cereus* group. Differentiation methods based on morphological and phenotypic characteristics are time-consuming, and false results may be obtained for atypical strains. On the other hand molecular discrimination based on the presence of two *B. anthracis* virulence plasmids, pXO1 and pXO2, is not sufficient because plasmids can be lost or transferred to other bacilli. Therefore, several chromosomal markers have been investigated and applied (1, 8). In 2001 Qi et al. (7) described single nucleotide polymorphisms (SNPs) in the *rpoB* gene and their usefulness for *B. anthracis* identification. Since then, several articles describing various molecular methods for *rpoB* sequence-based detection of *B. anthracis* have been published (for example see references 2 and 9).

We conducted studies of single-strand conformation polymorphisms (SSCPs) of the *rpoB* gene in a large collection of *B. cereus* group strains. Surprisingly, we found that the nucleotide sequence of the *rpoB* gene fragment containing the marker SNPs of two reference strains of *Bacillus thuringiensis* was identical to that of the homologous region in *B. anthracis*. Therefore, *rpoB* gene-based tools could not distinguish these strains from *B. anthracis*, thus resulting in false-positive anthrax identification.

We tested 95 samples of total genomic DNA of *Bacillus* species belonging to the *B. cereus* group (*B. anthracis*, *B. thuringiensis*, *B. cereus*, *B. weihenstephanensis*, *B. mycoides*, and *B. pseudomycoides*) using the multitemperature-SSCP technique, which has an extended capacity to detect SNPs (6). Primers *rpoB1f* (5′-GGTGATAACGAATACTTAAGC-3′) and *rpoB1r* (5′-AATTCGATCAAGTGATCCAGC-3′) were used to amplify a 321-bp fragment of the *rpoB* gene encompassing marker SNPs described by Qi et al. (7). Amplification was performed using AmpliTaq Gold DNA polymerase (Applied Biosystems). PCR products were denatured as previously described (4) and loaded onto an 8% polyacrylamide gel containing 3.75% glycerol. The multitemperature-SSCP assay was carried out in Tris-buffered EDTA in DNA Pointer (Kucharczyk TE, Poland) with 40 W of constant power. The gel temperatures were 35°C, 20°C, and 5°C, each stage lasting 45 min. Gels were silver stained, scanned, and analyzed using GelScan software version 1.3 (Kucharczyk TE).

The multitemperature-SSCP analysis of the *rpoB* marker revealed indistinguishable profiles for all *B. anthracis* strains tested whereas most of the nonanthrax bacilli generated apparently different profiles. However, two *B. thuringiensis* strains tested, HD146 (serovar *dermstadiensis*) and HD868 (serovar *tochigiensis*) (10), exhibited the same profile as *B. anthracis* did (Fig. 1). We performed DNA sequencing of these amplicons using an automated fluorescent 377 DNA sequencer and BigDye Terminator v3.1 (Applied Biosystems) in accordance with the manufacturer's instructions. Each strand of the amplified amplicons was sequenced separately using primer *rpoB1f* or *rpoB1r*. The final sequence of the *rpoB* core region covering the marker SNPs was determined by comparison of data from the two strands. Results obtained indicated that *rpoB* core regions of *B. thuringiensis* HD146 and HD868 were indistinguishable from each other as well from the sequence reported by Qi et al. (7) for *B. anthracis*. To confirm that PCR templates HD146 and HD868 were obtained from nonanthrax bacilli, we conducted the SG-749 PCR-restriction fragment length polymorphism (RFLP) assay described by Daffonchio et al. (1). The RFLP patterns of HD146 and HD868 were evidently dissimilar to that of the reference strain Sterne 34F2, indicating that these samples contained non-*B. anthracis* DNA.

Taken together, results obtained by DNA sequencing and the PCR-RFLP assay indicate that we found rarely occurring *B. thuringiensis* strains possessing *B. anthracis*-specific SNPs in the *rpoB* gene. To our knowledge, such strains have not been reported to date.

Interestingly, in 2002, Ellerbrok et al. (2) found that some strains of *B. cereus* can give positive signals in *rpoB* targeted real-time PCR over 10 cycles later than *B. anthracis* can. Their results showed that *B. cereus* may possess an *rpoB* gene having a sequence closely resembling the sequence present in *B. anthracis*. However, the *rpoB* sequence of the aforementioned *B. cereus* strains was not determined. In 2005 Elzi et al. (3) described a *B. cereus* isolate possessing three of the four *B. anthracis* characteristic SNPs in *rpoB*. We found two *B. thuringiensis* strains with nucleotide polymorphisms in the *rpoB* gene identical to those of *B. anthracis*. The findings support the concept that *B. anthracis*, *B. cereus*, and *B. thuringiensis* belong to the same species and that what distinguishes them functionally are mostly genes carried on plasmids (5). Nevertheless, plasmids might be lost or transferred to other bacilli. Keeping in mind the risk of bioterrorism as well as the need for monitoring the occurrence of *B. anthracis* in the environment, identification methods independent of the presence of plasmids are essential. But these results raise the question of whether *rpoB* can be considered a reliable marker for specific anthrax identification.

![FIG. 1. A. Multitemperature-SSCP analysis of the rpoB gene. Lane 1, B. anthracis Sterne 34F2; lane 2, B. thuringiensis HD146; lane 3, B. thuringiensis HD868; lanes 4 to 12, other B. thuringiensis strains tested. B. SG-749 RFLP patterns of DNA separated on a polyacrylamide gel and silver stained. Lane 1, 100-bp ladder; lanes 2 and 5, B. anthracis Sterne 34F2; lane 3, B. thuringiensis HD146; lane 4, B. thuringiensis HD868.](https://doi.org/10.1128/JCM.44.4.1606–1607.2006)
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REFERENCES

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