GENOME WATCH

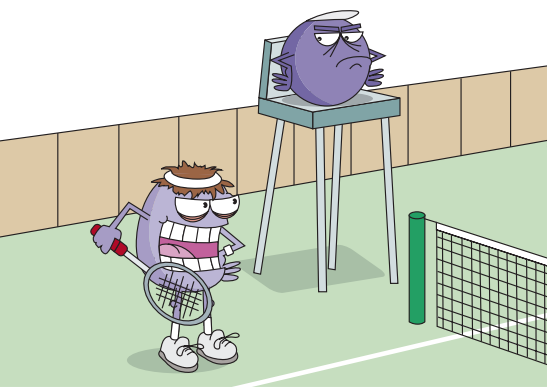
You cannot *B. cereus*

Susannah J. Salter

This month's Genome Watch looks at the different *Bacillus* species that can cause anthrax.

Anthrax is a very serious disease that is of particular public health interest owing to its potential for use in a bioterrorist attack. It is caused by the Gram-positive spore-forming bacterium *Bacillus anthracis* and affects a wide range of domestic and wild animals, as well as humans. Infection normally occurs through skin contact with infected animals or animal products, but can also occur through ingestion or inhalation of spores. In humans, infection results in lesions at the points of contact and oedema, followed in some cases by meningitis or sepsis. The septic phase progresses rapidly, with coma and death occurring within a few hours[1]. The stability of *Bacillus* spp. spores makes *B. anthracis* a concern for its potential use in a bioterrorist attack, as happened in Japan in 1993 and the United States in 2001.

The genus *Bacillus* includes several closely related species that are phenotypically and pathogenically divergent, in some cases owing to the presence of virulence plasmids rather than chromosomal differences. Several closely related *Bacillus* spp. isolates were collected from deceased chimpanzees and a gorilla between 2001 and 2004 in the Taï National Park in Côte d'Ivoire and the Dja Reserve in Cameroon. Examination of one of the chimpanzees from the Taï National Park revealed severe haemorrhage in the lungs, intestines and other internal organs, and characteristic oedema and damage to the lungs, indicative of an anthrax-like acute bacterial infection. Gram-positive, rod-shaped bacteria were found in all tissues, and real-time PCR confirmed the presence of *B. anthracis* markers. However, these isolates differed phenotypically from *B. anthracis*.



When Klee *et al.* sequenced the genome of strain CI, which was isolated from this chimpanzee, they found that it was closely related to *Bacillus cereus* but contained the two *B. anthracis* virulence plasmids[2]. Strikingly, ten motility-associated and chemotaxis-associated genes that are disrupted by frameshifts in *B. anthracis* are intact in strain CI, and the flagellum biosynthesis cluster is fully functional, explaining the differences in motility between this strain and *B. anthracis*. However, strain CI contains several features associated with *B. anthracis*, including similarly organized genes at the *secA2* locus, which is responsible for the secretion system of proteins associated with pathogenicity, and an inactive PlcR, which regulates 45 genes in *B. cereus*.

The finding that a *Bacillus* species other than *B. anthracis* can cause anthrax-like disease demonstrates the transformative effect of the natural acquisition of both anthrax virulence plasmids on the pathogenicity of *B. cereus*. Bacilli other than *B. anthracis* that had previously been found to be atypically virulent, such as *Bacillus thuringiensis* serovar konkukian (which causes tissue necrosis) and *B. cereus* str. E33L (which causes anthrax-like disease in zebras), did not possess both plasmids.

During an anthrax outbreak, forensic analysis of the strain responsible is particularly important for disease control and for ascertaining its origin. However, identifying a strain quickly and accurately in the event of an outbreak of anthrax is challenging. *B. anthracis* strains can be typed by multiple-loci variable-number tandem repeat analysis (MLVA), but this can be technically challenging and can give variable results in different laboratories or when using different equipment. Kuroda *et al.* compared the genomes of 27 *B. anthracis*, 22 *B. cereus* and seven *B. thuringiensis* isolates, and *Bacillus weihenstephanensis* str. KBAB4, with two Japanese *Bacillus anthracis* isolates that they sequenced[3]. Nearly 3,000 single-nucleotide polymorphisms (SNPs) from the chromosome and plasmids were validated by BLASTN (nucleotide basic local alignment search tool) analysis of >10,000 candidate SNPs, from which 80 were chosen to differentiate the strains.

The authors tested a PCR assay based on these SNPs and found that *B. anthracis* and its virulence plasmids were easily identified. The assay also identified other species carrying virulence genes or divergent plasmids. Although full-genome sequencing is the gold standard for forensic identification of strains, a PCR assay that demonstrates the similarity of an anthrax outbreak isolate to a set of known strains will be useful for early stages of containment and investigation, and would also quickly identify an atypical *Bacillus* sp. isolate such as *B. cereus* biovar *anthracis* str. CI.

Susannah J. Salter is at the Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK.
e-mail: microbes@sanger.ac.uk

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Competing interests statement
The author declares no competing financial interests.