

media and Congress. Contact with those letters led to 22 cases of anthrax, including five deaths, along with cleanup measures that, for example, cost the U.S. Postal service \$1.2 billion to decontaminate several of its facilities, according to Jason Bannan of the FBI Chemical-Biological Sciences Unit in Quantico, Va., and a participant in the ASM plenary session. "FDA never had a case like this before," he says.

No spore-containing letter was recovered from the first attack that led to the death of a photojournalist in Boca Raton, Fla. However, investigators recovered spores as part of a granular, white powdery material from an envelope involved in the second incident. Bannan describes it as a "crude prep," in part because it also contained *Bacillus subtilis*. Additional material from other letters to then-Senator Tom Daschle (D-SD) and to Senator Patrick Leahy (D-VT) in October 2001 appeared "more refined," was "beige" instead of white, and contained no spores other than *B. anthracis*.

The FBI quickly requested outside microbiologists to help in analyzing those materials. The available "research assays . . . didn't meet forensic standards," says Keim who, with his collaborators at NAU, worked closely with the FBI, as did other outside groups of microbiologists and investigators with other expertise. Moreover, efforts to develop such assays were complicated by the strictly clonal biology that *B. anthracis* follows during replication.

Those facts soon led microbial and molecular forensics investigators into conducting genomics-level analyses, according to Jacques Ravel, now at the University of Maryland School of Medicine and Institute for Genome Sciences in Baltimore, Md. A more conventional phenotypic analysis supplemented those genomic-level efforts, leading another group of microbiologists at USAMRIID, who were working with the FBI and others on

Sludge Bacteria Prove Apt at Degrading Cholesterol

Gordonia cholesterolivorans, a bacterial species isolated from sewage sludge, can actively degrade cholesterol in such settings but might be used to make or modify novel types of cholesterol derivatives for medical applications, according to Oliver Drzyzga and his collaborators at the Universidad Complutense de Madrid, Spain. Because some *Gordonia* species are human pathogens, it is unlikely that these bacteria will ever be used directly to control cholesterol levels in humans, Drzyzga points out. "We are trying to work out exactly how *G. cholesterolivorans* metabolizes cholesterol so that we can identify and construct metabolically engineered strains that are more rapid and effective in breaking down cholesterol." Details appear in the May *International Journal of Systematic and Evolutionary Microbiology*.

the anthrax investigation, to take advantage of distinct *B. anthracis* "morphotypes" that could be observed on growth plates. Those morphotypes vary not only by colony appearance but also in sporulation efficiencies and in telltale mutations at a rare "hot spot" within the otherwise stable genome of this species.

That information became the basis for a PCR screening assay for *B. anthracis* specimens that then was validated at Commonwealth Biotechnologies (CB) in Richmond, Va., and the Midwest Research Institute in Palm Bay, Fla., to ensure that such testing could meet forensics standards applied by U.S. courts. By 2007, the "highly specific" PCR assay identified several samples during a "blinded" analysis that included "seized materials," Bannan says. Ultimately, the PCR-based analysis along with other information from the criminal side of the investigation indicated that the anthrax-causing specimens from the 2001 letters derived from stocks produced several years earlier at USAMRIID for an aerosol challenge in anthrax vaccine studies, he says.

Based on that and other information from more conventional lines of evidence, FBI investigators concluded that Ivins, who died following a drug overdose in July 2008, produced

spores from those stocks for the 2001 anthrax attacks.

Despite that painstaking analysis and the unequivocal conclusions put forth by FBI officials, doubts linger over some matters that are mainly scientific as well as others that intersect with the broader thrust of the investigation. For instance, none of the microbiologists, including Bannan and similar specialists at FBI, was privy to other evidence, including lab records from USAMRIID, that their FBI colleagues collected. "I know nothing of that information," he says. "I'm a microbiologist, and was not involved in the seizure of evidence."

Other lingering questions focus on more purely scientific issues, some of them pertaining to how the lethal bacteria were handled. For example, USAMRIID held *B. anthracis* in aqueous suspensions, not as spores. Presumably, the spores sent via letters were produced in at least two separate batches, contaminated with *B. subtilis* at least once, but when and how remain unknown. "We don't know the process used," Bannan says. "We never found the equivalent *B. subtilis* at USAMRIID in any of the evidence that we had." Efforts to trace the source of that bacterial contaminant "didn't lead anywhere," adds Keim.

Early reports suggested that the