past 30 years is that, while some antigens worked well individually, they interfered with one another when combined, according to Pier. “We therefore decided to adopt the live, attenuated vaccine approach, as it would make production easier, and would provide a large array of antigens, and induce multiple types of immunity,” he says.

The main issue for the combination vaccine is not cost, as the multiple components are relatively inexpensive, but safety. Indeed, unlike the experience with live-attenuated viral vaccines, the success record for live-attenuated bacterial vaccines is not very impressive. Only one of them, the Ty21a strain of *Salmonella enterica* serovar Typhi, which protects against typhoid fever, is used with any regularity. Although the side effects of that vaccine are mild, there is little other experience to illuminate safety issues with this broad class of bacterial vaccines.

Additionally, in the case of an attenuated vaccine to protect against lung infections from *P. aeruginosa*, delivery could prove a challenge, particularly if delivering it by inhalation proves necessary for inducing protective immunity, according to Pier. That approach “presents larger safety issues,” including, potentially, “an unacceptable level of reactivity—inflammatory responses leading to local pain, discomfort, and more systemic reactions, such as aches, pains, and fever,” he says. “We just don’t have much experience with this approach in humans.”

However, the need for a vaccine to protect against *P. aeruginosa* is acute—and growing, in terms of the numbers of elderly, who are at risk for hospital-acquired infections, and in terms of the frequency of such infections being resistant to available antibiotics, Pier says. “*P. aeruginosa* is one of the most notorious bacteria manifesting this resistance.”

Individuals with cystic fibrosis are also especially vulnerable to lung infections caused by this pathogen, points out Gerd Doering of the Institute for Medicine, Microbiology, and Hygiene in Tübingen, Germany. “There is an unmet need for prevention of *P. aeruginosa* infection in cystic fibrosis and other patients [such as those with severe burns] at risk for *P. aeruginosa* infection, and Pier’s [research] addresses this superbly,” he says. “This paper demonstrates conclusively the feasibility of eliciting broad-based immunity against unrelated strains of *P. aeruginosa* using a live attenuated vaccine,” adds Reuben Ramphal of the University of Florida in Gainesville.

**David C. Holzman**

David C. Holzman is the *Microbe* Journal Highlights Editor

**Manganese-Oxidizing Microbes Form Giant Stromatolites in Caves**

Manganese-oxidizing microorganisms produce gigantic stromatolites that extend more than 2 km inside the Spanish caves in which they continue to grow, according to a team of Spanish and Australian researchers, led by Carlos Rossi from the University of Madrid in Madrid, Spain. Although other investigators previously reported finding manganese-oxide crusts in caves, the structures they found lacked features consistent with having microbial origins. Details from Rossi and his collaborators appear in the December 1, 2010 *Geology* (38:1119–1122).

Stromatolites, sometimes described as fossilized microbial mats, provide evidence for some of the earliest forms of life on Earth. However, not all stromatolites are fossils, and some are found actively growing. For instance, stromatolites in Shark Bay, Australia, are being formed through the ongoing photosynthesis of cyanobacteria (blue-green algae) that trap and bind particles such as silt and sand, forming laminated domes of calcium carbonate, CaCO$_3$, in shallow marine environments.

“Initially we weren’t looking for manganese deposits in this cave,” Rossi says. “Since our first visit to El Soplao, it became apparent that the floor of the main gallery of the cave was covered by thick, black manganese deposits, partly covered by sand and mud.” Moreover, he and his collaborators did not recognize those...
manganese deposits as stromatolite components until they examined several samples using scanning electron microscopy. That analysis revealed an unusual abundance of fossil microbes, while thin sections under a petrographic microscope exposed dendritic structures, very similar to those found in fresh-water carbonate stromatolites. “We returned to the cave to have a closer look at the black layer, realizing its stromatolite nature and its huge extension,” he says. “We were shocked to observe at least three levels of stromatolites, which extend for more than 2 km in the main gallery of the cave.”

Without light to drive photosynthesis, how are these underground stromatolites formed? Electron microprobe analyses and other data support the idea that chemolithotrophic, manganese-oxidizing microbes, growing in a low-gradient water-table stream, are the likely culprits that began this process at least 1 million years ago, according to Rossi and his collaborators. Uranium-series dating of mineral formations lying above the stromatolites is consistent with this minimum age.

“The idea that microbial communities could have thrived in caves during the Precambrian to protect themselves from [ultraviolet irradiation] is exciting but hard to check,” Rossi says. “There are no caves of Precambrian age that we know of. Because of the limitations of normal dating methods, there are very few caves with proved ages older than 1 million years.” (The Precambrian period ended about 590 million years ago.) He and his collaborators are attempting to improve the age dating of the stromatolites and want to determine their growth rates. “We are also mapping the distribution of the different stromatolite levels in this vast cave system, and trying to locate sites in which manganese-iron stromatolites could be growing,” he says.

“The amazing preservation of the bacteria shown in the cave stromatolites is simply not seen in subaerial rock varnishes,” says geographer Ron Dorn of Arizona State University in Tempe, a leading expert on manganese oxide coatings on desert rocks. He considers the findings reported by Rossi and his collaborators “outstanding.”

Barry E. DiGregorio
Barry E. DiGregorio is a science writer in Middleport, N.Y.

Tossing Molecular Wrenches into Viral and Toxin Nanomachines

If antimicrobial molecules could be crafted like metaphors, poetic microbiologists would be strolling golden pathways. Never mind: those microbiologists who now tinker with “nanomachines,” including molecular components of the botulinum and anthrax toxins and from viruses such as Ebola, seem pleased with this new way of framing efforts to develop remedies and preventive agents, as evidenced by several who spoke during the plenary session, “Jamming Nanomachines,” part of the 9th ASM Biodefense and Emerging Diseases Research Meeting, held in Washington, D.C., last February.

The botulinum toxin is a “modular nanomachine,” says Mauricio Montal of the University of California, San Diego, noting that the toxin forms after the full protein is clipped to form one light and one heavy chain that are held together by a disulfide bond. This disulfide-linked pair of polypeptides consists of three “modules,” none of which is toxic by itself, he says. The assemblage is “remarkable when the modules work together, forming the most toxic protein we know.”

In terms of its deadly effects on humans or other vulnerable species, botulinum toxin interferes with nerve-cell transmissions at synaptic junctions by blocking release of acetylcholine mol-