

# Annual Report **2010**



# A global hunt for mari

By Chris Tachibana for CMB

A PhD project goes from old-fashioned exploration biology to state-of-the-art chemical analysis



In 2007, the global research expedition Galathea 3 returned to Copenhagen after a nine-month tour on the ship *Vædderen*. Researchers on board collected hundreds of bacterial samples from oceans around the world, hoping to solve our growing antibiotic resistance problem. In 2011, CMB student Maria Månsson defended her PhD thesis, "Discovery of Bioactive Natural Products from Marine Bacteria", showing this might be possible.

More than 500 of the bacterial strains collected on the Galathea 3 expedition by Professor Lone Gram and co-workers from DTU Food and CMB inhibited pathogenic bacteria. Månsson focused on strains from the genera *Pseudoalteromonas* and *Vibrio*, hoping to identify compounds that might be new antibiotic therapies. First, she needed to evaluate the large collection of bacteria for their potential to produce known and new antibiotics. This required establishing new, efficient methods that bridged biology expertise at DTU Food with chemistry knowledge at CMB. Månsson and her collaborators had to figure out growth conditions that made the bacteria produce antibiotics, and then link that production to a specific chemical profile.

"We found that one way to unlock new chemistry was to mimic the natural conditions of the bacteria," says Månsson. One success was supplementing the growth medium of the marine bacteria with chitin, a carbohydrate found in seawater because it is a major

component of plankton exoskeletons. Other techniques also had to be tweaked. "Marine bacteria need sea salt to grow, and for a chemist, salt is a big challenge," says Månsson. Too much can affect downstream chemical analysis, like the chromatography and mass spectrometry steps. "A lot of my time in the project just dealt with low-tech challenges," she says, such as altering established CMB protocols for extracting compounds from fungi that did not work with the marine bacteria.

CMB expertise, infrastructure, and collaborations, along with Månsson's hard work, has been crucial to the project. Månsson and her collaborators first prioritized the bacterial strains, finding those with the greatest potential to produce antibiotics. The first step in this process was a chemical analysis screen to map all compounds from the different strains, because bacteria often produce multiple bioactive products. Also, unrelated bacteria, or bacteria from different locations can produce the same compounds. One particularly interesting strain from the Galathea expedition was from the surface of a mussel found near the Solomon Islands off Australia, but exotic as that sounds, a soil bacterium from a Danish garden might produce exactly the same antibiotic. "We needed to do careful dereplication," says Månsson, "which is excluding compounds that are already known. People have been screening for antibiotics for a long time so it happens a lot that you find something already known, but it is still worth doing to solve the antibiotic resistance problem."

# ne-derived antibiotics

The project has an edge in discovering new compounds, because few researchers screen for antibiotics from marine bacteria. Filamentous soil bacteria are considered more interesting because they have already yielded antibiotics such as streptomycin. However, Månsson says, "By optimizing all the purification steps for the best possible yield, and with more sensitive measurements, we can look at more of the natural products that bacteria make." The good news for potential drug developers is that marine bacteria may produce especially potent candidate drugs. "When bacteria in the ocean secrete compounds," says Månsson, "they are diluted in the sea, so the compounds usually have a lot of activity in small amounts." These small quantities can be detected and characterized thanks to the highly sensitive analytic equipment at CMB, and access to the powerful magnetic resonance imaging facilities at Carlsberg Research Laboratories.

Månsson named the first two compounds she discovered solonomides. These are small, cyclic peptides that contain unusual amino acids. They do not kill pathogenic bacteria, but quench their virulence. Collaborating with researchers at KU-LIFE, Månsson found that two different solonomides reduced the virulence of USA300, a community-acquired strain of MRSA—methicillin-resistant *Staphylococcus aureus*, that is infamous for infecting young, healthy people. The solonomides are fascinating, but "we don't know how they work," says Månsson. "They resemble molecules used by *S. aureus* to regulate their own virulence so we suspect they are competitive inhibitors that block communication receptors." Virulence factors that might be affected include secreted toxins, or tissue-degrading enzymes. Before solonomides show up in the pharmacy, however, their potential toxicity towards mammals must be explored. Månsson's advisor Associate Professor Thomas Ostfeld Larsen says, "This project has given us an excellent chance for close collaborations with DTU Food, the University of Copenhagen and other partners, and we hope to expand our marine chemistry activities in the coming years."

Månsson hopes the project will launch an academic career in natural product chemistry. "I'm excited about the ecology of these compounds, to try to understand why they are produced, and the biological systems they affect. It's fascinating to dig into a microniche and find the hundreds of bacteria associated with a single algae or marine animal and see how they affect each other with their plethora of chemical substances." Månsson will spend the year of remaining project funding applying her methods to more strains in the Galathea collection, saying, "I focused on 5 strains, but we have 500."

