

Science writer **Meriel Jones** takes a look at some recent papers in SGM journals which highlight new and exciting developments in microbiological research.

## First steps toward new CF treatment

**Sriramulu, D.D., Lünsdorf, H. Lam, J.S. & Römling, U. (2005).** Microcolony formation: a novel biofilm model of *Pseudomonas aeruginosa* for the cystic fibrosis lung. *J Med Microbiol* **54**, 667–676.

Abnormal lung sputum is one of the main problems in cystic fibrosis, leading to bacterial infection and illness. The surface of the lung is covered with a viscous liquid full of material released from cells at a much higher level than normal. Individual components appear to encourage growth of particular forms of the bacterium *Pseudomonas aeruginosa* that go on to damage the lung and can be very difficult to treat with antibiotics. Scientists have worked out that the bacterial cells grow as a biofilm, i.e. as microcolonies surrounded by a carbohydrate matrix. Therefore, one standard experimental system involves growing *P. aeruginosa* cells attached to a solid surface. However, this is not exactly the same as in a lung. One difference is that the bacterial cells attach to each other and the sputum to form a plug, rather than to the surface of lung cells. Another is that the bacteria are very tightly packed so most do not have access to oxygen. A collaboration between researchers at the Karolinska Institutet, Sweden, the GBF at Braunschweig in Germany and the University of Guelph in Canada has now shown that the composition of the sputum is much more important than anyone had guessed.

They created an artificial sputum growth medium (ASM) for *P. aeruginosa* using chemicals to match the composition of the fluid in the lungs of patients with cystic fibrosis as bacterial disease becomes established. Simply growing *P. aeruginosa* in this medium was enough to make the cells form tight clumps visible to the naked eye, rather than attaching to the surface of the culture vessel or living as single cells within the liquid. The cells grew slowly, surrounded by a matrix, just as in real sputum. The researchers discovered that every component of the ASM affected clumping by the simple procedure of leaving out each component in turn. Omitting amino acids had a particularly obvious effect, making the clumps smaller. Low levels of oxygen encouraged the cells to clump, while the fact that *P. aeruginosa* hardly grew at all without the carbohydrate-coated protein mucin suggested that this was the major energy source for the bacteria.

The researchers then started on the other side of the problem, to discover which genes in *P. aeruginosa* were essential for forming tight microcolonies within the artificial sputum. They tested several genes highlighted by other researchers and found that some were indeed important in the ASM. Identifying key gene products could be a first step in developing new treatments, so ASM provides a new way to test therapies against *P. aeruginosa* infections in cystic fibrosis.

## Olive fly symbiosis

**Capuzzo, C., Firrao, G., Mazzon, L., Squartini, A. & Girolami, V. (2005).** 'Candidatus *Erwinia dacicola*', a coevolved symbiotic bacterium of the olive fly *Bactrocera oleae* (Gmelin). *Int J Syst Evol Microbiol* **55**, 1641–1647.

Many insects have essential symbiotic associations with micro-organisms. There are beneficial examples where the microbe provides an essential nutrient missing from the insect's diet, and others where bacteria affect the sex ratio of the insect's offspring. In 1909, an Italian scientist, L. Petri, published a 132-page report about an hereditary symbiosis between bacteria and the olive fly (*Bactrocera oleae*). This fly is the most important pest of olive trees. The insects cannot develop from larvae to adults on

olives without the bacteria and researchers have speculated that the bacteria supply missing nutrients. Adult flies contain the bacteria within a special organ called the oesophageal bulb connected to the pharynx, from which large numbers of bacteria travel to the midgut. Although Petri managed to grow bacteria taken from olive flies, he was never convinced that the numbers he obtained matched the numbers within the insects. He even speculated that the real symbiotic bacteria were viable but non-culturable.

Italian researchers at the Universities of Padua and Udine, led by Vincenzo Girolami, have re-visited this problem using molecular biological methods to detect bacterial DNA within the olive fly without needing to grow the bacteria in pure culture. They had made repeated

attempts to cultivate bacteria from oesophageal bulbs and midguts on many different media without success. However, they found bacterial DNA within these organs that, although similar to DNA from the genus *Erwinia*, did not match any known species. As additional confirmation they obtained the same results for DNA extracted from olive flies collected in Bari, Liguria and Lake Garda, regions of Italy that are up to 800 km apart. The closest relative of the DNA sequence was from bacteria found on fruit and in the intestines of fruit-flies. The researchers therefore feel that it is appropriate to name the symbiotic bacteria from the olive fly 'Candidatus *Erwinia dacicola*' to signify both its unculturable status and that it originates as an inhabitant of a fly from the genus *Dacus*.

## In-patient evolution of the hepatitis C virus

**Brown, R.J.P., Juttla, V.S., Tarr, A.W., Finnis, R., Irving, W.L., Hemsley, S., Flower, D.R., Borrow, P. & Ball, J.K. (2005).** Evolutionary dynamics of hepatitis C virus envelope genes during chronic infection. *J Gen Virol* **86**, 1931–1942.

Hepatitis C virus (HCV) can cause severe liver disease, including cirrhosis and liver cancer. It is present in and transmitted through blood. Around 170 million people worldwide are at risk of disease from HCV and the number is continually increasing. Current treatments are often not effective and it can elude the immune system within the body for years before serious illness is obvious. The European Union has funded research with the aim of identifying better targets for anti-HCV therapy. As part of this, researchers at the University of Nottingham and the Edward Jenner Institute for Vaccine Research in the UK have studied how HCV changes over the years within a patient.

The researchers already knew that proteins on the surface of the virus were the key to how it fools the immune system, so they looked at the genes for these proteins. They wanted to know exactly how these changed over a number of years. To address this question they made use of samples from four patients; two of the patients had mild symptoms, but the other two had a severe and progressive disease. The patients were part of the Trent HCV Study Cohort, which consists of 2,546 people diagnosed with the virus within the 5-12 million inhabitants of the Trent region of eastern central England. The cohort was set up in 1991, when routine identification of HCV in donated blood became possible.

The researchers recorded the gene sequences of two surface proteins, E1 and E2, from HCV within each patient's samples. They cloned a total of 80 sequences, showing that each patient harboured several versions of HCV at any time. The versions of HCV in the patients also changed over the years. The team already knew that this was likely to happen and they wanted to know how the variants had evolved from each other and whether any were correlated with more serious disease. They therefore adopted methods used to work out the times at which each variant had evolved and to identify the most recent common ancestors.

This study showed that many factors affect the diversity of HCV within patients. Each patient had a unique pattern for evolution of HCV variants, without any apparent relationship to the severity of the disease. In three patients, this caused changes in the E2 protein at a location known to interact with antibodies, suggesting that these changes helped the virus to elude the immune system. Changes in further locations gave clues to specific components of the immune system involved in removing HCV and indicated interplay between attachment of the virus to human cells and escape from antibodies.

▲ False-colour SEM of *Aspergillus nidulans* conidiophores. E. Gueho / Science Photo Library

## Fungal sex

**Tsitsigiannis, D.I., Kowieski, T.M., Zarnowski, R. & Keller, N.P. (2005).** Three putative oxylipin biosynthetic genes integrate sexual and asexual development in *Aspergillus nidulans*. *Microbiology* **151**, 1809–1821.

Researchers led by Nancy Keller in the USA have been studying prevention of contamination of food by mycotoxins for some years. Several fungi have a capacity to synthesize these toxic compounds in specific environmental conditions. The research has led the group into the area of signal regulation in fungal sporulation and mycotoxin production. One group of chemical signals common to both processes are oxylipins. These are involved in the switch between vegetative and reproductive growth in filamentous fungi such as *Aspergillus nidulans*. They influence the development of both the cleistothecia that contain the sexual spores and the conidiophores that bear the asexual spores. *A. nidulans* normally develops asexual spores first and can then go on to produce sexual ones. However, researchers have discovered that the amount and level of different oxylipins affect the order of these processes and number of spores produced.

Two genes (*ppoA* and *ppoC*) are involved in the biosynthesis of oxylipins and the team have now identified a third gene, *ppoB*. As well as being involved in the synthesis of one type of oxylipin, the PpoB protein regulates the sporulation process. Without PpoB, the fungus produced many more asexual spores so that the ratio of asexual to sexual spores was eight-times more than normal. After several further experiments the team worked out that the PpoB protein regulated the activity of the other two *ppo* genes, affecting the balance between sexual and asexual spores.

When they combed the databases for similar DNA sequences, they found related ones in many fungi. Oxylipins could determine the timing and balance between sexual and asexual reproduction in many fungi. Some synthesize this lipid-based communication system from the lipids of the plants on which they are growing, linking back to fungal toxins in food where the story began for Nancy Keller's group.