



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

Recent changes in the bird 'flu virus

Campitelli, L., Ciccozzi, M., Salemi, M., Taglia, F., Boros, S., Donatelli, I. & Rezza, G. (2006). H5N1 influenza virus evolution: a comparison of different epidemics in birds and humans (1997–2004). *J Gen Virol* **87**, 955–960.

The bird 'flu virus is often present in the intestines of wild birds and usually does not cause them illness. However, it can be transmitted to domestic poultry, causing illness, and can also infect people. There is currently international concern over the H5N1 version of this virus that, since 1997, has killed millions of birds and has also infected around 100 people, killing about half of them. Like all viruses, bird 'flu has a small number of genes and mechanisms for them to change. The versions of each gene in different viral strains can recombine to create new varieties of the virus. Subtle changes also happen as the virus replicates within its host's cells. Understanding the way that the virus changes over time is essential to plan the development of new vaccines and drugs ahead of disease epidemics.

Researchers at the Istituto Superiore di Sanità in Rome, Italy, in collaboration with the University of Florida, USA, have recently focused on small changes to the virus. They used computer programs to examine the complete genome sequence of 684 strains of bird 'flu that had infected domestic poultry or humans in 1997 or 2004. The programmes were designed to test several

possibilities for evolution of the virus. The researchers were especially keen to know if there was selection for any particular changes in the virus and whether there was anything distinctive about the sequences obtained from infections in people.

Their analysis indicated that there was very little evidence for positive selection. The strains from humans were either intermingled with those from domestic birds, or were grouped by geographical region. This matched the fact that all the people had caught the disease by direct contact with birds, reinforcing the idea that the virus has not adapted for transmission from person to person. All but three of the changes in the virus appeared to happen at random. Viral sequences from 1997 indicated that the PB2 gene was accumulating changes. Other researchers think that a particular version of this gene results in a virus that is highly pathogenic to mammals, but the Italians' analysis showed that this was not specific to the strains isolated from humans in 1997. In the 2004 strains, the HA gene, which has a major role in virulence, and the NS1 gene, involved in pathogenicity, had both accumulated changes. The implication is that there has been selection for versions of the virus that can evade the immune system, possibly due to the use of poultry vaccines. The simultaneous selection for strains that vary in their interaction with host cells can let the virus infect different hosts. Continuous monitoring of these changes to the virus will be important in designing new vaccines and as an early warning of new strains.

One giant step for algae

Rindi, F., López-Bautista, J.M., Sherwood, A.R. & Guiry, M.D. (2006). Morphology and phylogenetic position of *Spongiocrhysis hawaiiensis* gen. et sp. nov., the first known terrestrial member of the order Cladophorales (Ulvophyceae, Chlorophyta). *Int J Syst Evol Microbiol* **56**, 913–922.

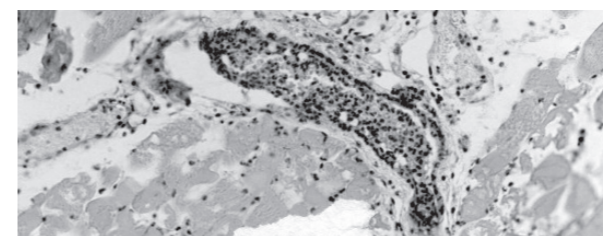
A multinational collaboration between the National University of Ireland at Galway and the Universities of Alabama and Hawaii has brought to light a new algal species with a special evolutionary significance. The land algae of the Hawaiian Islands have been recorded

since 1876, but only haphazardly. The researchers were therefore not surprised to come across unidentified species, but did not at first realize the significance of the bright golden-yellow coating on the bark of many trees on beaches along the windward coast of O'ahu. Microscopic examination showed that it was a green alga with a very unusual budding-like mechanism of cell division. This had only been recorded in two genera, but other features of the cells indicated that it did not belong to either. The researchers decided to call it *Spongiocrhysis hawaiiensis* after the fact that it formed a golden layer, crispy when dry and spongy when wet, on trees in Hawaii.

Molecular methods have been used for decades to study relationships among the green algae. They fall into two major

evolutionary groups, one of which led to the land plants. The other contains most living green algae, and is divided into four main classes. Two of these include most of the terrestrial green algae. When the researchers applied molecular analysis to their Hawaiian sample, they discovered that it belonged to an order of green algae which was so far known only from marine and freshwater habitats, included in a third class. The discovery of *S. hawaiiensis* is therefore much more significant than the discovery of just another new algal species.

It has long been realized that the Hawaiian Archipelago is home to a large variety of microbes, and may be a biodiversity hot-spot. Modern systematic surveys of the larger algal flora are likely to reveal even more surprises.



New ways to tackle gas gangrene

Bryant, A.E., Bayer, C.R., Aldape, M.J., Wallace, R.J., Titball, R.W. & Stevens, D.L. (2006). *Clostridium perfringens* phospholipase C-induced platelet/leukocyte interactions impede neutrophil diapedesis. *J Med Microbiol* **55**, 495–504.

Gas gangrene is one of the most rapid and destructive infections of damaged human tissue. Feared as a consequence of agricultural, industrial and battle-field injury, the infection becomes established in less than 8 hours and can advance, destroying healthy muscle, at several inches per hour. This rapid progress is caused by toxins released by bacteria. Among the consequences is shut-down in blood flow to the infection site, preventing an effective immune response and the supply of oxygen to the human tissues. The bacterium *Clostridium perfringens* thrives in the absence of oxygen. Shock and organ failure occur and many patients die, even with antibiotics and intensive care. Amputation is still the single best treatment. It is not surprising that researchers want to find new treatments.

The alpha toxin is an enzyme, phospholipase C. It contributes significantly to the decline in blood flow by causing blood cells to aggregate and block the blood vessels. It also causes a reduction in the tissue's immune response by preventing a specific type of blood cell, the neutrophil, from moving into the infected tissues. Researchers in the USA and UK have been working out exactly how the toxin causes these effects, with the hope that drugs used to treat similar conditions of blood flow disorders might also be rapidly effective in gas gangrene.

The toxin promotes aggregation of platelets and also of platelets with neutrophils. The neutrophils are the cells that should migrate from the blood vessels into the body's tissues as part of the inflammatory response to infection. However, the toxin traps most of the neutrophils in large aggregates, preventing this migration. The researchers worked out which molecules on the surfaces of the blood cells were involved in sticking them together. The toxin activates the protein gpIIb/IIIa on the surface of the platelets, making them adhere to each other. A second protein, CD62P, is also stimulated by the toxin, attaching the platelets to neutrophils. New treatments would have to affect both of these processes to ensure a continued supply of oxygen and an immune response in tissues infected by *C. perfringens*. Possible treatments are now being evaluated.

▲ *C. perfringens* gas gangrene is characterized by widespread muscle necrosis, marked intravascular leukostasis and the lack of a tissue inflammatory response. These features are reproduced here in an experimental model of wild-type *C. perfringens* infection. Amy Bryant

Responding to the environment

Budde, I., Steil, L., Scharf, C., Völker, U. & Bremer, E. (2006). Adaptation of *Bacillus subtilis* to growth at low temperature: a combined transcriptomic and proteomic appraisal. *Microbiology* **152**, 831–853.

Microbiologists have been gradually working out how bacteria respond to changes in their environment, using new methods as they become available. Until recently they have had to study changes in the expression of bacterial genes one by one, piecing small bits of information together. However, now that many bacterial genomes have been sequenced, researchers can view how every single gene in the genome is affected by an environmental change. This almost gives them the opposite problem of too much information to comprehend.

German scientists have now completed a study of the changes in both the protein complement and gene expression in *Bacillus subtilis* caused by growth at low temperature. In the lab, the bacteria are normally grown at 37 °C. To see what happened at a lower temperature, cells were grown at 15 °C for at least three generations and their nucleic acids and proteins were then extracted. Measurement of the transcription of the entire 4,107 gene complement of *B. subtilis* is now so routine that the researchers could use commercial arrays carrying duplicate spots of every gene for their experiments.

They discovered that about 14 % of all the *B. subtilis* genes were affected. Expression of 279 genes was increased at 15 °C, while that of another 301 was reduced at the lower temperature. The role of some of these genes was previously entirely unknown, so the researchers now have at least one fact about them to guide further research. Changes to other gene products made sense in terms of what was already known about them. For example, the bacterial cells grow more slowly at the lower temperature and reductions in expression of genes involved with bacterial growth fitted with this. Others that were already known to be controlled by SigB, which switches on a large set of genes in response to stress, were induced, again fitting with current knowledge.

However, changed expression of genes is not the whole story. Gene products are used to make proteins, and there can be many independent changes to proteins to optimize survival in a changing environment. Separating and identifying proteins is more difficult than analysing changes in gene expression. The researchers used a technique that allowed them to see if there were any effects on 1,085 of the bacterial proteins, and to identify many of them. There were massive changes in the protein profile, with almost half of them affected. Although some were the proteins encoded by genes whose expression was affected by the cold, many others were not. This graphically illustrates that bacteria respond at many levels to environmental change, emphasizing the complexity of life within even single-celled organisms.