Microbiology Today Editor Meriel Jones takes a look at some papers in current issues of the Society's journals which highlight new and exciting developments in microbiological research.

It's good to talk

Cystic fibrosis is the most common inherited lethal disease among Caucasians. It impairs the transport of chloride, with the symptom of sticky, dehydrated mucus in the lungs. This lets bacterial pathogens colonize these airways, producing chronic, and often fatal infections. One pathogen, *Pseudomonas aeruginosa*, has the unpleasant characteristic of being able to conceal its presence from the immune system until it has sufficient numbers to overwhelm the host defences. To do this it uses a cell-to-cell communication system called quorum sensing, based around signal molecules called *N*-acylhomoserine lactones (AHLs). The system requires two proteins, one to synthesize the AHL and another to detect its presence once there is a critical amount in the surroundings. The sensor then switches on a battery of virulence factors in the form of lytic enzymes, toxins and other secondary metabolites, amounting to perhaps 4 % of the genes in *P. aeruginosa*. Bacteria that are defective in quorum sensing are substantially less virulent. This has obviously made researchers very interested in the process, since interfering with it could aid treatment of bacterial infections.

Burkholderia cepacia has emerged as another important pathogen of patients with cystic fibrosis and it also has an AHL-dependent quorum-sensing system. Patients who are suffering from *P. aeruginosa* infection can become infected with *B. cepacia* as well. This can result in no obvious symptoms or a slow and continuous decline in lung function, but for 20 % of patients the consequence is rapid and fatal pneumonia. Researchers in Germany and Denmark have been investigating whether the two bacteria can communicate with each other using isolates of *P. aeruginosa* that were isolated from a patient before, during and after *B. cepacia* arrived. One isolate initially produced six different AHL molecules, but this dropped to trace amounts of only one type during the time that *B. cepacia* was also infecting the lungs. The *B. cepacia* isolate produced two types of AHL, one of them the same as one produced by *P. aeruginosa*. Both of these AHLs were able to stimulate the production of lytic enzymes by mutants of *B. cepacia* that had lost the ability to synthesize AHLs themselves, although they did not do the same for mutants of *P. aeruginosa*.

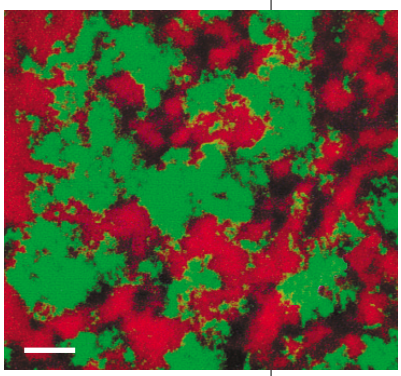
To check that this was not simply a coincidence, the researchers inserted the gene for a fluorescent green protein from the jellyfish *Aequorea victoria* into the bacteria in such a way that it would only be synthesized after the cells detected a quorum-sensing signal. They designed a series of these reporter systems for each AHL, and both species of bacteria, using a version of the protein that was unstable so that they could easily repeat measurements, or see the effect of changing conditions on exactly the same cells. The glowing green colour was easy to detect, even in individual cells, and gave results that matched the earlier ones. They showed very clearly that communication between the bacteria was one-way: *B. cepacia* could detect the presence of *P. aeruginosa* through some of the AHL molecules, but not vice versa.

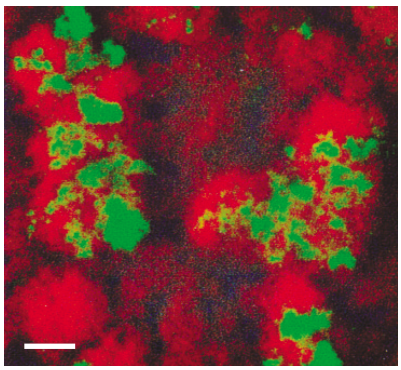
Although the researchers are still investigating the consequences of this interspecies communication, they have managed to find a novel compound to block it, inspired by an Australian seaweed called *Delisea pulchra*. Most bacteria have some sort of quorum-sensing system, so it is not surprising that other organisms have evolved ways to interfere with it. The seaweed produces a number of halogenated furanones that have antifouling and antibacterial properties. The compounds are similar to AHLs and the researchers have been using their reporter systems to watch the effect of synthetic compounds. One, called furanone 56, turns out to inhibit production of the glowing green protein and some virulence factors by *P. aeruginosa* at concentrations well below any effects on the growth of the bacterial cells. This has the attraction that the furanone could be developed into a drug that decreased the virulence of bacteria without creating a selection pressure for resistance.

The researchers also grew *P. aeruginosa* cells containing the fluorescent sensor in a growth medium that flowed continuously, part of the time in channels less than a millimetre wide. The bacteria settled onto this surface and began to grow as a biofilm, which is their normal form of growth and makes them more resistant to antibiotics and other biocides. As the biofilm matured over 2 weeks, clumps of cells started to glow once they became large enough to synthesize enough AHL to trigger their own quorum-sensing system. The semi-solid nature of the biofilm itself meant that the signal molecules were less likely to be carried away. When furanone 56 was in the growth medium the biofilm was much thinner, and the cells glowed a paler green, although the researchers could not add enough furanone 56 to block signalling entirely. The researchers are continuing to work out the details of exactly how furanone 56 affects the bacteria, and whether a different furanone would be more effective, but they are certain that this is one way to develop new non-antibiotic anti-pathogenic agents that will make bacteria less virulent and more sensitive to biocides.

OPPOSITE PAGE:
Scanning electron micrograph of mature cysts of *Helicosporidium* sp. produced in *Helicoverpa zea* larvae. Cysts are approximately 6·5 µm in diameter.
COURTESY DRION G. BOUCIAS & AURÉLIEN TARTAR, UNIVERSITY OF FLORIDA, USA

BELOW:
Mixed biofilms of *B. cepacia* strain H111-I and *P. aeruginosa* strain SH1. The perception of AHL molecules by *B. cepacia* is indicated by the appearance of green-fluorescent cells; the distribution of *P. aeruginosa* cells is visualized by their red fluorescence. The white arrow indicates a microcolony of *B. cepacia*. Bars, 20 µm.
COURTESY K. RIEDEL, TUM, FREISING, GERMANY





Riedel, K., Hentzer, M., Geisenberger, O., Huber, B., Steidle, A., Wu, H., Høiby, N., Givskov, M., Molin, S. & Eberl, L. (2001). *N*-Acylhomoserine-lactone-mediated communication between *Pseudomonas aeruginosa* and *Burkholderia cepacia* in mixed biofilms. *Microbiology* **147**, 3249–3262.

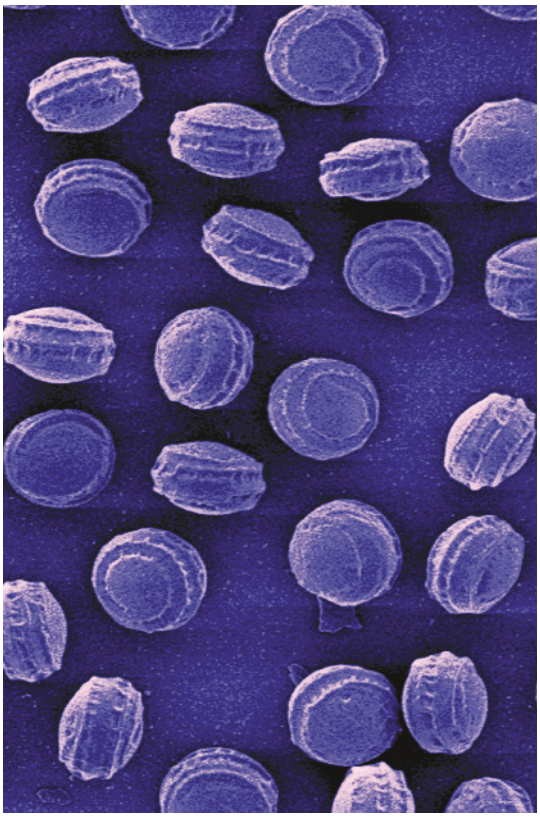
Hentzer, M., Riedel, K., Rasmussen, T.B., Heydorn, A., Andersen, J.B., Parsek, M.R., Rice, S.A., Eberl, L., Molin, S., Høiby, N., Kjelleberg, S. & Givskov, M. (2002). Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology* **148**, 87–102.

An ugly duckling

In 1921 David Keilin described a parasite of flies that he called *Helicosporidium parasiticum*. He went on to be a pioneer in the study of cellular respiration, and one of the founders of biochemistry, as well as keeping up his interest in parasitology.

However, in *H. parasiticum* he left a mystery for later researchers. Pathogens like it turned up in all sorts of invertebrates, including mites, scarabs, mosquitoes and even pond water. Their characteristic feature is cysts within their host, containing a core of three ovoid cells and one filamentous one. They are certainly not bacteria, but it has never been obvious whether they are animals, plants or fungi. The first attempt to classify them, in the 1930s, gave them their own order within the protozoa, the grouping of single-celled animals. In the 1970s some researchers argued that helicosporidia should be reclassified as fungi because of the way they infected their hosts.

One of the big difficulties with parasites is that it is often impossible to separate them from their host, and the hosts themselves are difficult to keep in the laboratory. Researchers at the University of Florida and US Department of Agriculture have now managed to grow *Helicosporidium* within a laboratory strain of moth, rather than its normal blackfly host. This let them collect enough cysts to isolate pure DNA from the parasite, and then apply



the techniques of molecular taxonomy to reveal finally its true relatives.

Because they wanted to answer such a fundamental question as whether or not *Helicosporidium* was an animal, the researchers examined genes that are known to have changed very little during evolution. They compared genes from *Helicosporidium* with those from a wide selection of other protozoa, animals, fungi and plants, including humans, baker's yeast and maize. To their surprise, the greatest similarity was to green algae. However, they did the comparisons and the answer always came up that the closest relatives of *Helicosporidium* were primitive green plants, particularly the species *Prototheca zopfii*. This was interesting for two reasons. First, the genus *Prototheca*, like *Helicosporidium*, lacks the green pigment chlorophyll that gives plants their colour. Second, several species of *Prototheca* are pathogens of animals, including humans. Researchers have finally given *Helicosporidium* a home with its own sort.

Tartar, A., Boucias, D., Adams, B.J. & Becnel, J.J. (2002). Phylogenetic analysis identifies the invertebrate pathogen *Helicosporidium* sp. as a green alga (*Chlorophyta*). *Int J Syst Evol Microbiol* **52**, 273–279.

Hitting the target

One of the difficulties in cancer chemotherapy is directing the cytotoxic drugs only to cancer cells and not to all the others in the body. Researchers are slowly devising new approaches to do this better. One idea is to add a new enzyme to the tumour, and then give the patient a chemical, called a prodrug, which is harmless until it meets the enzyme. The enzyme then catalyses the conversion of the prodrug into a cytotoxic chemical. This should kill the tumour cells and others in their immediate vicinity, but leave all other cells unharmed. Although it is obviously an attractive strategy, there are a number of problems to be solved before it becomes an effective cancer therapy.

Researchers at the Centre for Applied Microbiology and Research in the UK have been investigating one problem, namely finding suitable enzymes to use with a prodrug. A good way to confine the cytotoxic effects to tumour cells is to use an enzyme from bacteria to catalyse a reaction that does not happen in mammalian cells. The prodrug 5-aziridinyl-2,4-dinitrobenzamide (CB 1954) is converted into a cytotoxic compound by a type of nitroreductase enzyme that is not present in normal human cells. Some enzymes do not catalyse the reaction very effectively, and can produce harmless, rather than cytotoxic products. However, many bacteria contain this sort of enzyme, and the researchers have been investigating one from *Bacillus amyloliquefaciens* that looks particularly suitable.

They isolated minute amounts of the enzyme from the bacteria, and then used the sequence of this protein to search for its gene. It turned out that similar genes had been found in other bacteria, but without anyone knowing their function. With the gene in their hands, the researchers could synthesize enough of the enzyme to investigate its properties in detail. It turned out that it had a higher affinity for the prodrug than other enzymes, and also converted it all into a cytotoxic product. When they tested its effectiveness at killing mammalian cells in the laboratory it worked, but not as well as another bacterial enzyme. The researchers conclude from this that other properties may be important for an effective therapy, and they are in the process of investigating more bacterial enzymes.

Anlezark, G.M., Vaughan, T., Fashola-Stone, E., Michael, N.P., Murdoch, H., Sims, M.A., Stubbs, S., Wigley, S. & Minton, N.P. (2002). *Bacillus amyloliquefaciens* orthologue of *Bacillus subtilis yurO* encodes a nitroreductase enzyme which activates the prodrug CB 1954. *Microbiology* **148**, 297–306.

Fighting fire with fire

About 15 % of human cancers are caused by viruses, so it is ironic that scientists are now trying to use viruses to combat cancer. There have been attempts to treat cancer with viruses since the early years of the 20th century, although these very seldom resulted in complete remission. However, the combination of much better understanding of cancer and viruses, along with the tools of molecular biology, is giving researchers the opportunity to create viruses that use their natural biology to destroy cancer without harming the normal cells within the body. Some are already effective enough for clinical trials. Christopher Ring, from Glaxo SmithKline Research and Development in the UK, has taken stock of the current situation in a recent issue of *Journal of General Virology*.

Viruses multiply as intracellular parasites, taking over the cell machinery to manufacture more virus. This exploitation often kills the cell, even if the virus itself does not deliberately rupture the cell to release the new viral particles. Researchers are now realizing that this natural cytolytic effect can be harnessed to add to the attack on tumour cells. The trick is to target the virus to the correct cells. The diversity of cancers and viruses means that researchers are investigating several strategies. Some viruses naturally infect tumour cells more efficiently than normal cells, although the reasons are not known. Extracts of tumours infected with one of these, Newcastle disease virus, have been used since the mid-1960s to augment other anti-tumour therapies.

Some viruses only infect cells that are in the process of proliferation, and others can force cells into the growth cycle. Proliferation is, of course, an essential feature of cancer cells, and researchers are exploiting this to target viruses to tumour cells. Different methods are required for different types of cancer. For example, the brain is unusual in that normal brain tissue is non-proliferating. Some mutants of herpes simplex virus can only replicate in proliferating neural cells, and so have potential against brain tumours. However, most tumours are surrounded by normal cells that are also proliferating, so more subtle direction is needed.

One idea is to modify a viral protein so that it will bind to a molecule unique to the surface of cancer cells, rather than normal host cells. Although this is difficult, researchers are making progress in re-directing viruses to infect only specific sorts of cells. Adenoviruses infect cells after two stages of recognition and then entry. Careful changes to the viral proteins involved are throwing up modifications that re-target the virus to different types of cells. Researchers have discovered that once they had made some changes to the surface of measles virus, it was able to infect previously uninfectable cell types, as well as still being able to infect its normal host cells. These results have convinced researchers that somewhere among the many viruses, one will form the basis of a tumour-selective anti-cancer agent in the future.

An alternative approach is to ensure that the virus requires factors present only in cancer cells before it can replicate.

For example, one adenovirus, called ONYX-015, seems to require a protein that is mutated in many types of cancer cells and infects tumour cells more efficiently than normal ones. In early clinical trials in patients with advanced head and neck cancer, the virus confined its replication to tumour tissue. There was significant regression in the tumour in 21 % of patients who could be evaluated. Combining the virus with cytotoxic drugs in another trial increased the regression to 63 % of patients.

Another way to ensure that viruses can replicate only in cancer cells is by altering the control regions of key viral genes so that they are switched on by proteins only present in cancer cells. Adenoviruses have been created in this way that are specific to prostate, colon or breast cancer cells, and some of these are currently in clinical trials.

A final strategy is suicide gene therapy. This uses a virus to deliver a new gene to cells that converts a non-toxic compound into a cytotoxic drug. This allows chemotherapy to be delivered more effectively to cancer cells alone, rather than affecting all the cells in the body. When, for example, the mammalian *CYP2B1* gene is expressed, the cells convert a precursor compound to the anti-cancer metabolite phosphoramide mustard. Researchers have managed to use a virus to deliver this gene to cultures of human glioma cells, and kill them. Other genes that look promising for this sort of therapy are those encoding cytokines that stimulate inflammatory and immune responses. Experiments with these genes in animals have inhibited tumour growth.

The 'ideal' anti-cancer virus would be based on a highly lytic virus that has been modified to replicate only in tumour cells, and would have to be thoroughly evaluated for safety. Current evidence from pre-clinical and clinical studies suggests that combining viruses with standard anti-cancer treatments will result in greater therapeutic benefits. It is obvious that cytolytic virus therapy is at a very early stage of development, but the effectiveness of some approaches already suggests that real benefits in anti-cancer therapy may eventually emerge.

Ring, C.J.A. (2002). Cytolytic viruses as potential anti-cancer agents. *J Gen Virol* **83**, 491–502.

Time to revise the whooping cough vaccine

Bordetella pertussis is the bacterium that causes whooping cough. It used to affect almost all children, and some died, but it has been controlled by vaccination in many countries for about 40 years. Surprisingly, it is now reappearing in places like Australia, Canada, the Netherlands and the USA, which have a good level of vaccination.

The vaccine contains whole *B. pertussis* cells, and immunity is caused by the body's reaction to a number of bacterial antigens, of which pertactin and pertussis toxin have been shown to be particularly important. Staff at clinics in the Netherlands have been collecting strains of *Bordetella* since 1949 and researchers at the National Institute of Public Health and the Environment in Bilthoven, and the University Medical Centre in Utrecht have now examined these strains to see how well they match with the ones used in the vaccine.

Frits Mooi and his colleagues have found three types of pertactin among the Dutch isolates, with all variations occurring in one small area of the protein called region 1. They wanted to know whether these changes altered the way that the immune system recognized pertactin. To do this, they investigated a series of monoclonal antibodies from mice against pertactin. These are antibodies selected to recognize only one small feature of pertactin. They tested these antibodies against the versions of pertactin in clinical Dutch strains of *B. pertussis* and it turned out that this feature was usually region 1. It indicated that this small region was an important source of protection. Antibodies from children who had recently suffered from whooping cough also reacted to region 1. Indeed, immunization of mice with region 1 alone gave them significant protection from infection.

When the researchers tested the vaccine used to protect children within the Netherlands, it was most effective against strains of the bacterium that had the same version of pertactin as that used in the vaccine. Worryingly, it was less effective against strains carrying other versions of pertactin that are now circulating within The Netherlands and other countries. This all points towards a need to revise the whooping cough vaccine, and perhaps to change to one that contains a mixture of individual proteins that all provide protection, rather than one that relies so heavily on pertactin.

King, A.J., Berbers, G., van Oirschot, H.F.L.M., Hoogerhout, P., Knipping, K. & Mooi, F.R. (2001). Role of the polymorphic region 1 of the *Bordetella pertussis* protein pertactin in immunity. *Microbiology* **147**, 2885–2895.

Re-creating our past

What was the first life on Earth like? Over 3,500 million years have passed since the first traces of life were left in this planet's rocks. Even the simplest form of unicellular life has had time for a lot of changes since then. Despite this, all cells retain some similarities in the essential ways that they work. Some of these features are so unvarying among animals, plants and fungi, the eukaryotes, that it was only when researchers started to analyse bacteria that they realized there was more than one way to do these things. It includes some of the precise molecular ways that cells store, copy, repair and make use of their genetic information, as well as structural features. Some bacteria do these in the same way as eukaryotes, while others have subtle, but consistent differences.

One of the consequences of these discoveries has been a debate about bacterial classification and evolution, and the way in which they are related to eukaryotes. As researchers have discovered more species and information about bacteria, ideas have developed and changed. Tom Cavalier-Smith, from the Department of Zoology at the University of Oxford, UK, has made his latest contribution to this debate in a recent paper in IJSEM. He has brought together the latest information from bacterial cell biology and genetics to give a comprehensive revision of the large-scale relationships among the prokaryotes.

He weaves together information from cell biology, the fossil record and comparisons between the same protein in different bacteria. His most startling conclusion is that the ultimate ancestral cell was a photosynthetic green non-sulphur bacterium that first lived about 3,500 million years ago under anaerobic conditions. It had a cell wall similar to that of the modern laboratory workhorse, *Escherichia coli*. Its single-celled descendants ruled the Earth for millennia. He argues that about 850 million years ago, a new type of bacterial cell arose which was surrounded by a single cell membrane, rather than the two of all earlier cells. Other important changes happened to the cell walls, lipid metabolism and to ways of handling genetic information so that there eventually were a total of 19 differences between these neomuran cells and their ancestors. Some of these bacteria became able to live in extremely hot, acid and saline environments and became the group that we now call the archaebacteria. Others rapidly underwent even more substantial changes. They acquired an internal cell skeleton and membrane systems, the ability to engulf other cells and were the origin of the eukaryotes.

The subject of evolution and the origin of life has been a hot topic in biology for well over a century, with ideas changing as biologists have discovered more about the world around them. This hypothesis adds yet another step towards re-creating our past.

Cavalier-Smith, T. (2002). The neomuran origin of the archaebacteria, the negibacterial root of the universal tree and bacterial megaclassification. *Int J Syst Evol Microbiol* **52**, 7–76.