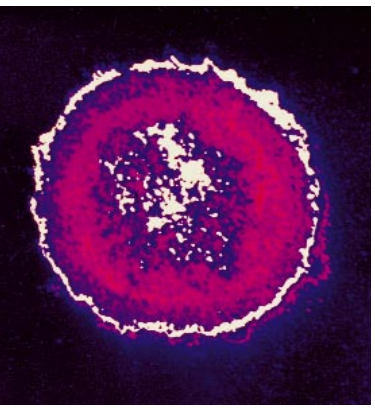
Hot off the Press

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.



ABOVE:
False-colour image of HIV.
PHOTODISC

OPPOSITE PAGE TOP:
Cells lacking sphingosine-1-phosphate lyase have increased resistance to the anticancer drug cisplatin. Sphingosine-1-phosphate lyase null mutants (red line) created by homologous recombination are 25 times more resistant to cisplatin than the parental wild-type cells (yellow line). The sphingosine-1-phosphate lyase mutant also exhibits dramatically altered morphogenesis (right inset) compared to the parent (left inset).
COURTESY GUOCHUN LI, SUPRIYA SRINIVASAN, HANNAH ALEXANDER & STEPHEN ALEXANDER, UNIVERSITY OF MISSOURI, USA

Predicting the progress of AIDS

Among all the scientific work and personal tragedies of AIDS, many aspects are still not understood. One of these is how the infection can take different courses in different individuals. Although the dose and viral strains responsible for human HIV infections are highly variable, there are people known to have been infected through identical routes with the same strain. Even so, the progress of the disease has not been the same. This is even clearer in animal experiments, when there is no question but that all the animals were infected with identical amounts of virus. Simian immuno-deficiency virus (SIV) causes a fatal AIDS-like disease in rhesus macaques and this has been used as a model for HIV and AIDS in humans.

Although the average time until death of a SIV-infected macaque is 1–2 years, some animals die within months while others live for many years. Experiments are carried out with small groups of animals, often four or less, and this natural variation may conceal any effect of a therapeutic trial. Researchers at the University of Pittsburgh School of Medicine, working with staff at the Tulane Regional Primate Research Center, have now reported the result of a decade of work that allows them to know in advance which animals will die rapidly after infection and which survive for a long time.

The key is the way the animal's peripheral blood mononuclear cells (PBMC) do, or do not, allow the virus to multiply. The researchers collected blood from uninfected animals, added virus to PBMC isolated from the blood and recorded how well the virus grew. The macaques were then infected with SIV and the researchers had to care for them as the disease progressed. After bringing together information on 59 macaques from vaccine and therapeutic trials, the scientists are confident that they can now predict which animals will live for a long time with a SIV infection and which ones will die rapidly. Animals with blood cells that are high producers of virus will progress to disease significantly more quickly than if their PBMC are low or intermediate producers of virus. This knowledge can be used in the design of future trials, and since it suggests that virus growth is controlled by innate characteristics of the individuals, it gives an additional factor for understanding, and combating, this lethal disease.

Seman, A. L., Pewen, W. F., Fresh, L. F., Martin, L. N. & Murphey-Corb, M. (2000). The replicative capacity of rhesus macaque peripheral blood mononuclear cells for simian immunodeficiency virus *in vitro* is predictive of the rate of progression to AIDS *in vivo*. *J Gen Virol* 81, 2441–2449.

JGVDirect

On p. 151 of the August issue of *MicrobiologyToday*, an incomplete website address was printed for JGVDirect. The full address is:

http://www.sgm.ac.uk/JGVDirect

The SGM publishes two monthly journals, *Microbiology* and *Journal of General Virology*.

The *International Journal of Systematic and Evolutionary Microbiology* (*IJSEM*, formerly *IJSB*) is published bimonthly on behalf of the IUMS in conjunction with the ICSB.

The three journals are now available online.
For further information visit the journal website:
http://www.sgmjournals.org

Members may purchase SGM journals at concessionary rates. See p. 194 or contact the Membership Office for details. Information on commercial subscriptions is available from the Journals Sales Office.

An early test for cancer?

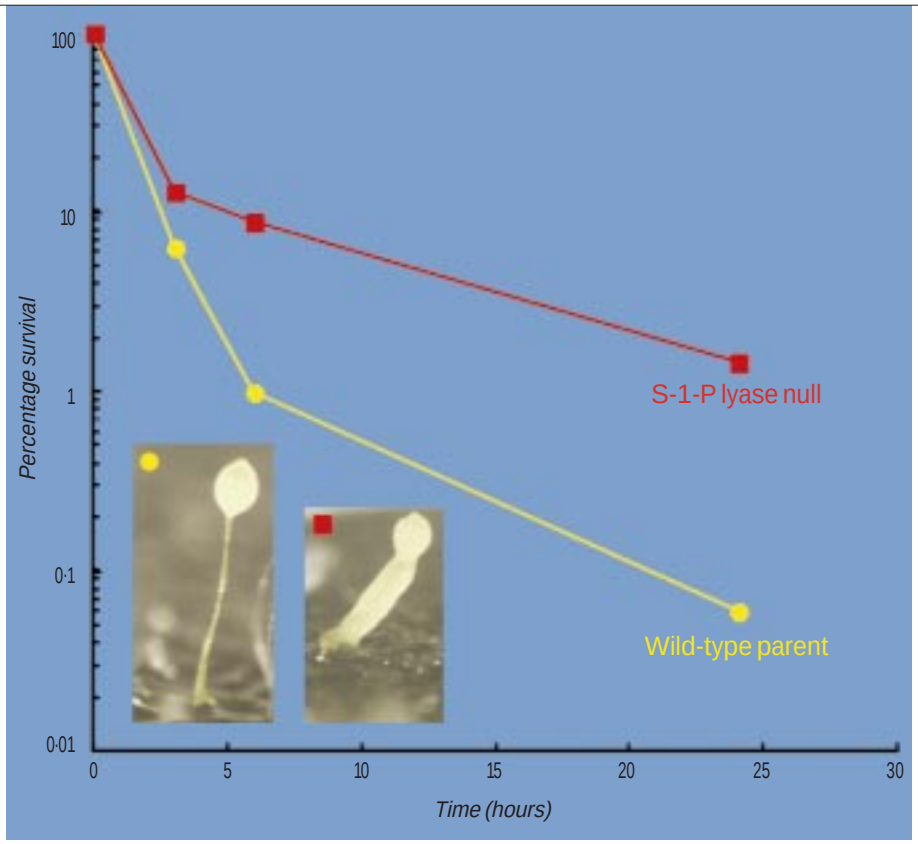
Epstein–Barr virus infects more than 90 % of the human population and has no apparent effect on the vast majority. The virus takes up lifelong harmless residence within one of the types of cell in the immune system, the resting memory B cells. The viral genes stay within the cells in an inactive condition. In a very small number of people, the virus unfortunately re-surfaces in an active state in cancerous tissues, and is considered a factor in the initiation of the disease. One of these is undifferentiated nasopharyngeal carcinoma (NPC). Scientists at the National University of Singapore have been studying the differences between patients with NPC and healthy individuals who nevertheless have antibodies to the virus in their blood.

About half of the people they examined had signs in their blood that the virus was active. The virus requires activity of a number of genes to replicate itself, and the researchers could detect activity of some, but not all of them. It was as if the virus occasionally tried to replicate itself, but was unable to complete the process. When they examined cells taken from the carcinoma itself, or the inflamed throats of some of their otherwise healthy volunteers, the situation was different. The cancer cells had signs of activity of all the viral genes required to start replication, while one gene, *BRLF1*, was inactive in the non-cancerous cells.

The body usually responds to the presence of new proteins by directing the immune system to remove them. A first step in this direction is the synthesis of proteins called antibodies that bind specifically to the new protein and mark it for destruction. When the researchers looked for antibodies to the product of the *BRLF1* gene in the blood of 53 patients with NPC, they could detect it in 83 % of them. In contrast, they could only find one positive reaction among the same number of their control volunteers.

Other scientists have suggested that the product of the *BRLF1* gene may be important in the re-activation of Epstein–Barr virus and the development of cancer. The results of this study indicate that, although this may well be true, it also betrays the presence of the active virus in the form of a unique antibody. The researchers hope that it will be possible to develop this into an early test for the disease.

Feng, P., Ren, E. C., Liu, D., Chan. S. H. & Hu, H. (2000). Expression of Epstein–Barr virus lytic gene *BRLF1* in nasopharyngeal carcinoma: potential use in diagnosis. *J Gen Virol* 81, 2417–2423.



Resistance to anti-cancer drug cisplatin

One bizarre fact about cancer is that damage to DNA can be both its origin and cure. The chemical cisplatin, that joins together adjacent DNA bases, is a widely used treatment for cancer. Unfortunately, its effectiveness is often limited as the cancerous cells develop resistance to it. Scientists are keen to understand the exact nature of this resistance, so that they can improve cancer therapy.

Investigations of cisplatin-resistant tumour cells have spotted a large number of changes that might be the reason for their survival. It can, however, be very difficult to identify the exact molecular details. Researchers at the University of Missouri and the Max-Planck-Institut at Martinsried in Germany decided to take a different approach. They have been studying mutant cells from the slime mould *Dictyostelium discoideum* that can live despite being immersed in the cytotoxic chemical. The researchers say that the advantage of this micro-organism is that they can be confident that any resistant cells contain only a single mutation and this can be identified readily. The biochemistry and cell biology of slime moulds is very similar to that of animals, and they are multicellular for part of their life cycle. Thus, research on this organism is highly applicable to studies of human disease.

So far, the researchers have tested mutations in 10–15 % of the genes in *Dictyostelium* and found seven that individually provide protection against cisplatin. Interestingly, this protection is specific to cisplatin since the *Dictyostelium* cells retained their normal sensitivity to other DNA-damaging chemicals. The function of some of these genes is currently unknown while others immediately suggest how they cause resistance. One of the latter is a mutation in the gene for sphingosine-1-phosphate lyase. This enzyme is required for the breakdown of sphingolipids, in a process that may signal the difference between death or continued life and proliferation in animal cells. The mutation certainly has a very dramatic effect on development in *Dictyostelium*. Instead of developing into an elegant oval of spores on a slender stalk, the mutants have a short, fat stalk and very few spores. Two other mutations, both in genes that might be involved in intracellular communication, also had dramatic effects on development.

The researchers point out that they are combining detailed molecular biology and cell biology in a way that promises further insights into the mechanism of cisplatin resistance. It may also suggest new drugs that could enhance the sensitivity of cancer cells to cisplatin.

Li, G., Alexander, H., Schneider, N. & Alexander, S. (2000). Molecular basis for resistance to the anticancer drug cisplatin in *Dictyostelium*. *Microbiology* 146, 2219–2227.

Any old iron?

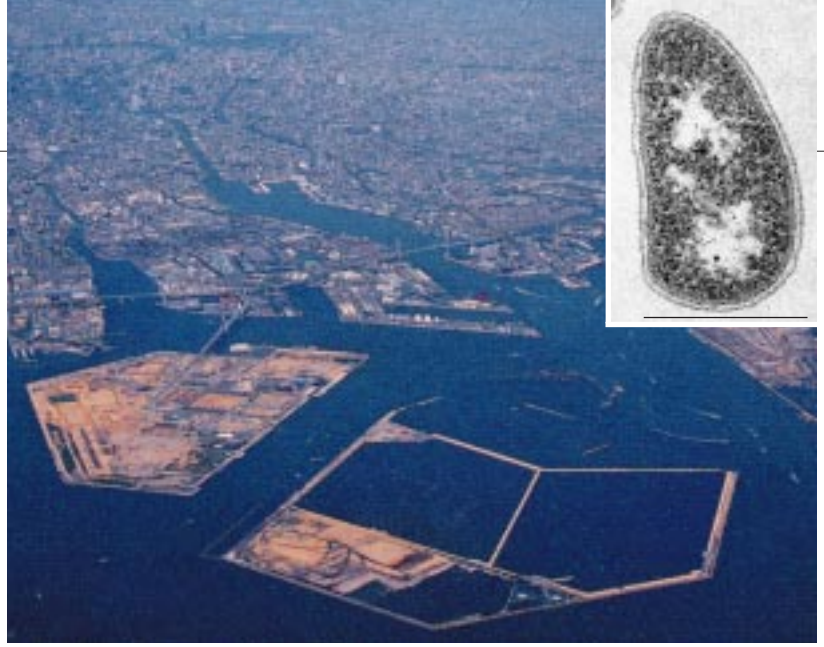
A small amount of iron is an indispensable component of all living cells. However, the very chemistry that makes it essential inside a cell also makes it virtually insoluble in the natural environment. There is around 10 million times less soluble iron in the soil environment than is required to sustain microbial life. The bacteria themselves have solved this conundrum. They synthesize and secrete chemicals, called siderophores, to dissolve and then transport the precious metal back into the cell. As a consequence, several scientists have suggested that bacteria may compete for this essential resource, perhaps through production of ever more efficient siderophores.

Dominique Joyner and Steven Lindow at the University of California in Berkley, USA, have been studying the behaviour of *Pseudomonas syringae*, commonly found on the leaves of plants. To find out if microbes really compete for iron, they wanted a way to measure its availability on the scale experienced by bacteria. To do this they joined the gene for a fluorescent green protein to a small part of one from *Pseudomonas*. This region regulated production of an enzyme required in the biosynthesis of siderophores to ensure that the cell only made them when the environment was low in iron. By putting the two together within the *Pseudomonas* cell, the researchers had a system that should have reflected the external level of iron through the amount of green fluorescence of the cells.

To check that it really worked, cultures of the bacteria were grown in liquid containing known amounts of iron and then their fluorescence was measured. This was inversely proportional to the amount of iron, giving the researchers confidence to move on to measurements of individual bacteria. They used a microscope linked to a computer to assess fluorescence from individual cells and again found a clear relationship between increasing fluorescence and decreasing iron.

When they finally looked at the surface of broad-bean leaves that had been dipped into cultures of the bacteria, things, of course, were more complicated. The leaves already had some bacteria on them, and so the researchers had to pick out the iron-sensing strain from amongst them. About 10 % of these cells had a substantially higher fluorescence than the rest, which implied that some cells sensed much less iron than the majority. Thus, from a bacterium's point of view, although there was adequate iron on the leaf surface for most of them, there were a few cells suffering from a shortage. As the researchers learn more about the very small scale of the microbial environment they hope to eventually understand how such characteristics affect microbial behaviour.

Joyner, D. C. & Lindow, S. E. (2000). Heterogeneity of iron bioavailability on plants assessed with a whole-cell GFP-based bacterial biosensor. *Microbiology* 146, 2435–2445.



Waste not, want not

Waste treatment and disposal sites are good places to discover new species of microbes. They are complex, new habitats, which often contain unusual organic materials and are simply awaiting inhabitants. In addition, there is considerable scientific, commercial and regulatory interest in ensuring that rubbish decays in as harmless a manner as possible. Three recent papers in IJSEM describe new species that have been unearthed in such unsavoury surroundings.

One of the products of microbial life, especially in the oxygen-free depths of a waste site, is methane. Special pipework can be installed to channel this safely away. It is a characteristic waste product of microbes known as methanogens, which are members of the bacterial domain called the *Archaea*. Japanese scientists, studying a disposal site on an artificial island in the sea near Osaka, realized that methane was being produced despite the amount of toxic heavy metals in the waste. Very few of the known methanogens can tolerate heavy metals, so this was a good opportunity to look for them.

The researchers designed conditions that should have been ideal for these unusual organisms, added a small amount of liquid that had leached from the site and watched for growth. It turned out that getting something to grow was fairly easy, but obtaining a pure culture of it was difficult. After many attempts, they succeeded in isolating a roughly spherical organism that produced methane and grew best at 35 °C. Its growth was slowed, but not stopped, by heavy metals. To decide exactly what it was, the researchers compared the sequence of one of its genes against a database of thousands of sequences of the same gene from other bacteria. The closest match was to a member of the genus *Methanocalculus*, but it was sufficiently different to be assigned to a new species, *Methanocalculus pumilus*.

A wastewater treatment plant in Korea has turned out to be the home of a new species of *Janibacter*. This genus, within the family *Intrasporangiaceae*, has been represented by a single species until now. Scientists at the Korea Research Institute of Bioscience and Biotechnology and Sungkyunkwan University have been studying the microbial life in the soil and sludge from this treatment plant, which has to cope with toxic aromatic chemicals. The unusual characteristics of one strain, CS12^T, caught their eyes. The components of its round cells were very similar to *J. limosus*, but sufficiently different to make the researchers examine how well its DNA matched with authentic *J. limosus* DNA. The poorness of the match, along with all the other information, clinched the identity of a new species, which they called *J. terrae*.

A further species of *Janibacter*, *J. brevis*, with the useful ability to degrade trichloroethylene (TCE), a solvent used in dry cleaning and a number of industrial processes, has been reported by Japanese scientists. They isolated it from a sample of groundwater contaminated by leaks of the solvent by providing TCE as the sole source of nourishment. The glistening white bacterial colonies were made up of spherical cells and when the researchers investigated their chemical characteristics, the nearest match was to *Janibacter*.

Mori, K., Yamamoto, H., Kamagata, Y., Hatsu, M. & Takamizawa, K. (2000). *Methanocalculus pumilus* sp. nov., a heavy-metal-tolerant methanogen isolated from a waste-disposal site. *Int J Syst Evol Microbiol* 50, 1723–1729.

Yoon, J.-H., Lee, K.-C., Kang, S.-S., Kho, Y. H., Kang, K. H. & Park, Y.-H. (2000). *Janibacter terrae* sp. nov., a bacterium isolated from soil around a wastewater treatment plant. *Int J Syst Evol Microbiol* 50, 1821–1827.

Imamura, Y., Ikeda, M., Yoshida,, S. & Kuraishi, H. (2000). *Janibacter brevis* sp. nov., a new trichloroethylene-degrading bacterium isolated from polluted environments. *Int J Syst Evol Microbiol* 50, 1899–1903.

Microbiology Announcement!

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Microbiology now has a North American Office, headed by Dr David R. Soll, to which authors in the USA and Canada can submit papers in any subject area.

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TOP LEFT:
An aerial view of downtown Osaka and the Port of Osaka. The two artificial islands in the foreground are the Osaka North Port Sea-Based Solid-Waste-Disposal Site. The pentagonal island is the North Section and the the heptagonal island is the South Section. The land produced by waste-filling can clearly be seen. The organism was isolated from the land produced in the South Section. Inset: Ultrathin section of *Methanocalculus pumilus* MHT-1^T. Bar, 0·5 µm.
COURTESY DR K. TAKAMIZAWA, GIFU UNIVERSITY, JAPAN

ABOVE:
Citrobacter cells loaded with uranium. The left panel shows the cell fairly dark with deposited uranium, but some detail can still be seen, in particular fibrillar material at the cell surface. All this is obscured by the heavy uranium deposit on the cell in the right panel. This cell is loaded with more than its own weight of uranium. The cell appears black and encrusted, but a preciptate of uranyl phopshate can be seen extruding from the cell. Cells in both panels are 1–2 µm in length.
COURTESY LYNNE MACASKIE, UNIVERSITY OF BIRMINGHAM

Full metal jacket

Microbes are the great recyclers of the planet. Much of their work in the decay and dissolution of once-living material goes un-noticed, or is even unwanted. However, their abilities are becoming increasingly appreciated and exploited. Heavy metals are a very difficult type of waste material. Many are toxic and cannot be destroyed but are best concentrated so that the metal can be either re-used or put carefully out of the way. Some micro-organisms are remarkably efficient at crystallizing metals around their cells. This may be for their own protection, but can form part of strategies to clean up metal-contaminated soils.

Researchers at the University of Birmingham, UK, have now reported their most recent study of the way the bacterium *Citrobacter* deals with the heavy metal uranium. This bacterium becomes coated with uranyl phosphate if it is suspended in a solution containing uranium. The crystals on the cell are not simply due to the heavy metal sticking to all available surfaces. The researchers have been gradually building up a picture of how the bacterial cells control the deposition of the toxic metal, allowing them to live despite being covered in several times their own weight of uranium. Through collaboration with the Research and Technology section of BNFL at Preston, UK, they were able to look at the cells using atomic force microscopy, with minimal disturbance to their natural form. This showed the metallic coating particularly well. This ability to accumulate relatively large amounts of the metal is the attraction as part of a system for filtering the pollutant from water.

An enzyme called phosphatase secreted by the cells is an essential component but perhaps surprisingly, it is inhibited by uranyl ions. From detailed measurements of the exact elemental and structural composition of the deposits, the researchers think that the first protective step is association of the uranyl ions with the comparatively few phosphate groups of the lipopolysaccharide coat that always covers *Citrobacter* cells. This traps the toxic metal. The next step involves the phosphatase enzyme which the researchers think is itself associated with the lipopolysaccharide. It releases phosphate ions, which drift away to capture further uranyl ions, imprisoning them away from the vulnerable enzyme and cytoplasm of the cell. Simple chemistry converts the initial complexes into a meshwork of more insoluble sodium uranyl phosphate crystals around the cell.

One particularly intriguing aspect of this research is the level of organization it implies at the cell surface. It is another indication that the enzymes secreted by bacterial cells can be involved in carefully controlled activities, despite being on the furthest fringe of what has traditionally been thought of as a living cell.

Macaskie, L. E., Bonthrone, K. M., Yong, P. & Goddard, D. T. (2000). Enzymically mediated bioprecipitation of uranium by a *Citrobacter* sp.: a concerted role for exocellular lipopolysaccharide and associated phosphatase in biomineral formation. *Microbiology* 146, 1855–1867.

Velvet evolution

Aidan Parte

The International Society for Evolutionary Protistology (ISEP) had its 13th biennial meeting in the Czech Republic at the end of July and beginning of August 2000. ISEP serves people with interests in the taxonomy, phylogeny and evolution of protists – eukaryotes that cannot be placed among the green plants, multicellular animals or mycelial, non-zoosporic fungi.

Hosted by the Czech Academy of Sciences and organized at the local level by Julius Lukeš, the meeting saw more than 100 protistologists from all over the world descend on the ancient town of České Budějovice (Budweis in German), home of the *real* Budweiser beer, in South Bohemia. In 4 days of intense presentations, new theories on early eukaryotic evolution were expounded and accepted ones were lambasted – this was very entertaining at times. Many beautiful micrographs were shown, demonstrating the incredible diversity of protists and the skill and patience of the microscopists who study them.

What has this ISEP meeting got to do with the SGM? The IJSEM has undertaken to publish a number of papers – some invited symposium lectures and other original research papers; we hope to bundle these in a single issue to be published in 2001. Part of the reason for the change of name of the IJSB to IJSEM was to facilitate the journal's expansion into the field of protists, a natural and entirely logical change of scope. Thus, my attendance at the meeting, and publication of papers from it, was intended to give the IJSEM a profile in the evolutionary protistology market.

There was an active social programme at the meeting, too, including one for accompanying persons. There was a reception at the Academy (with local specialities such as carp), an evening in the Masné Krámy pub and an afternoon in the beautiful town of Český Krumlov, followed by a banquet in the magnificent castle gardens. All in all, I found the meeting very interesting and I hope that I learned something about a field which is largely new to me. The hospitality and efforts of Julius and his colleagues at the Academy was really appreciated.

● *Aidan Parte, Managing Editor, IJSEM*

ABOVE:
The 70 m Black Tower in České Budějovice.

BELOW:
The town square in České Budějovice, one of the largest squares in Europe.

PHOTOS AIDAN PARTE