

*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.

## Chlamydial controversy

Chlamydia are a group of bacteria with the unpleasant lifestyle of obligate intracellular parasites. Their activities result in disease in many vertebrate species, ranging from being the leading cause of blindness in humans to causing spontaneous abortion in farm livestock. Their parasitic nature has made it impossible to apply many important bacteriological techniques to them. The sort of plus/minus biological markers normally used to classify bacteria do not work. A small set of biochemical, physiological, morphological, serological and DNA–DNA hybridization methods is used for their identification. With the advent of DNA-based methods there is increasing evidence for chlamydia in animals as diverse as amoebae, bivalves, alligators and chameleons, as well as their long-established hosts of humans and farm animals. As knowledge of their identification, diversity and activities has developed, the number of species, and how to define them, has also changed.

In 1999, Everett *et al.* published in *IJSB* a paper that reclassified the order *Chlamydiales*, with the proposal of two new families, a new genus and five new species. A group of scientists, led by J. Schachter from the Chlamydia Research Laboratory of the University of California, in a Letter to the Editor, question whether this upheaval in chlamydial taxonomy is necessary at this time: as they say, the single genus *Chlamydia* worked, and it has taken years to reach its current status of immediate recognition among the medical profession and public. In reply to this Letter and in a supporting paper, the authors of the 1999 paper have justified and strengthened the basis for the new chlamydial taxonomy.

Bush & Everett (2001) have combined analyses of genes, including one that seems to respond rapidly to evolutionary pressure and others that are important in virulence, with information on the sequences of the ribosomal genes favoured by molecular taxonomists. Their analyses indicated that all these genes were evolving in concert, albeit at very different rates.

Individual species are characterized by a mixture of their typical host animals, molecular details of ribosomal genes, and major rearrangements in the genes on their chromosome. Since the bacteria are obligate parasites, one question about the diversity of chlamydial species is whether it matches and dates from the evolution of their host species. The difficulty here is that there is currently no agreement on the date when some of the host species originated. There is also increasing evidence that some chlamydia live in amoebae and so may be widespread in soil and water. It was found that closely related chlamydial species were no more likely to share a host, or other virulence traits, than distantly related species. Indeed, there is some indication that advantageous genes may have spread laterally among isolates.

However, with any revision to bacterial classification, especially to a group that affects public health, there is always a question of whether this is the right time to change. Whether this change is accepted is in the hands of the microbiologists.

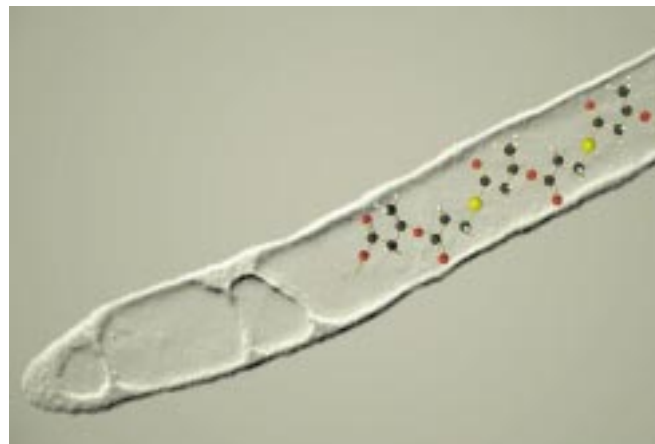
The paper by Bush and Everett is the first in *IJSEM Online* to use the supplementary data system – six sequence alignments can be viewed, printed or downloaded.

This new added-value feature of all SGM's online journals allows authors to append not only supporting data and data that would be unsuitable for the printed journal, but also electronic files such as video. Details are available from the Editorial Offices.

**Bush, R.M. & Everett, K.D.E. (2001).** Molecular evolution of the *Chlamydiae*. *Int JSyst Evol Microbiol* **51**, 203–220.

**Schachter, J. and 31 other authors (2001).** Letter to the Editor: Radical changes to chlamydial taxonomy are not necessary just yet. *Int JSyst Evol Microbiol* **51**, 249.

**Everett, K.D.E. & Andersen, A.A. (2001).** Letter to the Editor: Radical changes to chlamydial taxonomy are not necessary just yet – reply. *Int JSyst Evol Microbiol* **51**, 251–253.



## Microbial alternative to plastics

Cells are rather good at making polymers. Although biologists concentrate on ones like nucleic acids and polysaccharides, a less well known type is attracting considerable industrial attention. These are the polyhydroxyalkanoates (PHAs). Many bacteria make them as stores of carbon and energy, in a similar way to the fat deposits in some animal cells. The feature of PHAs that attracts biotechnologists is that their plastic-like characteristics make them ideal for uses in packaging, medicine and

the food industry. Not only do they come from a renewable resource, and are of course biodegradable, but also bacteria can be induced to synthesize over 130 different forms. Some of these have valuable physical characteristics that would be too expensive to make by conventional chemistry.

The reason for this variety is that the enzyme that makes PHAs is not very fussy about its substrate. Biotechnologists have learnt how to exploit this lack of specificity by adding likely precursors to the growth medium for the bacteria to

### THIS PAGE:

Electron micrograph showing a thin section of a cell of *Ralstonia eutropha* fully packaged with cytoplasmic inclusions of polyhydroxyalkanoic acids with the structure of the new polythioester, consisting of 3-mercaptopropionic acid and 3-hydroxybutyric acid, superimposed.

COURTESY T. LÜTKE-EVERLOH AND A. STEINBÜCHEL, UNIVERSITÄT MÜNSTER, GERMANY

### OPPOSITE PAGE:

Top: Kiba, an 11-year-old Asian elephant who died from systematic haemorrhagic disease, now known to be the work of a newly discovered virus, endotheliotropic elephant herpesvirus (EIHV-1). Below: Kiba's offspring, Plai Kiri, who fortunately shows no sign of the virus.

COURTESY B. EHLERS, ROBERT KOCH-INSTITUT, BERLIN, GERMANY



churn out an ever-growing range of polymers. A new PHA has resulted from a collaboration between polymer chemists and microbiologists at the University of Münster in Germany, and has a very novel chemical feature. It is the first to contain sulfur atoms inserted into the backbone of the polymer. The researchers fed small amounts of 3, 3'-thiodipropionic acid (TDP) to the bacterium *Ralstonia eutropha*, alongside a second carbon source such as fructose. The fructose supplied most of the carbon and energy requirements of the cells, while the TDP ended up in the storage polymer. The researchers found that if they strictly limited the nitrogen in the growth medium, the yield of polymer increased from 9 to 19% of the dry weight of the cells.

Another intriguing aspect is that although this polymer is new to science, it may be a natural product. Some marine algae synthesize sulfur-containing compounds in response to changes in salinity and, since TDP is one of the normal breakdown products, some aquatic bacteria may make the polymer. The researchers now want to investigate whether the physical properties of this new polythioester are as unusual as its chemistry.

**Lütke-Eversloh, T., Bergander, K., Luftmann, H. & Steinbüchel, A. (2001).**

Identification of a new class of biopolymer: bacterial synthesis of a sulfur-containing polymer with thioester linkages. *Microbiology* **147**, 11–19.

## Natural transformation

About 40 bacterial species have so far been shown to take up DNA from their surroundings and incorporate it into their chromosomes. This process, called transformation, occurs naturally during growth, without the intervention of microbiologists. The mechanism evolved a long time ago and the genetic diversity created by this recombination helps the bacteria adapt to environmental changes, or overcome a host's defence mechanisms. Specific proteins are required to recognize suitable DNA, take it into the cell and then fit it into the chromosome. Of course, if things went too far, different species of bacteria would blend into one, and this has certainly not happened. Scientists at the Universities of Bremen and Oldenburg have been looking at the limitations on natural transformation.

Strains of the species *Pseudomonas stutzeri* have very variable traits, including the ability to live on various toxic compounds. They fall into seven groups, called genomovars, each with the same genes, but arranged differently. The ability to live in varied environments seems linked to acquisition of foreign genes and rearrangements in the chromosome. Michael Lorenz and Johannes Sikorski have been examining exactly how efficiently the seven genomovars manage this. They grew strains, mixed with their own purified DNA, on conventional laboratory media and measured how efficiently they took it up. Half of the strains were transformed, but the efficiency varied over a thousand-fold. Adding extra, totally unrelated DNA to the mixtures decreased the uptake of their own DNA to some extent. When the researchers tested the most easily transformed strains to see how well they could incorporate DNA from other genomovars, transformation was usually best with DNA from strains of the same group.

The results indicate that despite the advantage a strain might gain, there are barriers to the free exchange of genes in *P. stutzeri*, thus maintaining diversity within the species. Understanding the exact nature of these barriers is the next step.

**Lorenz, M.G. & Sikorski, J. (2000).** The potential for intraspecific horizontal gene exchange by natural genetic transformation: sexual isolation among genomovars of *Pseudomonas stutzeri*. *Microbiology* **146**, 3081–3090.

## A 'jumbo' virus problem

The Asian elephant is endangered in the wild by habitat destruction. There are now fewer than 50,000 of these animals left. Even when they are brought into the protection of zoos, other dangers can assail them. Researchers in Berlin have been studying the reason for the sudden death of an 11-year-old Asian elephant called Kiba. He died within 24 hours from a systematic haemorrhagic disease, which has caused the death of other elephants in American and European zoos.

The disease is now known to be the work of a newly discovered virus, endotheliotropic elephant herpesvirus (EIHV-1). It was detected using the polymerase chain reaction (PCR) to amplify part of a viral gene from Kiba's blood and tissues. The German researchers have now used electron microscopy to examine thin slices of the elephant's organs. The spherical virus particles in the nuclei of some liver cells looked just like herpesvirus, supporting its identity. To find out how similar this particular virus is to other

herpesviruses, they investigated its other genes using PCR. This revealed that although the virus was related to a particular group called the betaherpesviruses, the relationship was quite distant. It must be the first member of a new genus, or even a new family, of herpesviruses.

One worrying question was whether the virus had also infected the other elephants in Kiba's herd. The researchers designed a test that would detect an EIHV-1 gene with great specificity and sensitivity. To their relief, when they tested blood samples from the herd, including Kiba's offspring Plai Kiri, there was no sign of the virus. The test now offers a way to monitor captive elephants for signs of this lethal disease.

**Ehlers, B., Burkhardt, S., Goltz, M., Bergmann, V., Ochs, A., Weiler, H. & Hentschke, J. (2001).**

Genetic and ultrastructural characterization of a European isolate of the fatal endotheliotropic elephant herpesvirus. *J Gen Virol* **82**, 475–482.

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. 1 or contact the Membership Office for details. Information on commercial subscriptions is available from the Journals Sales Office.

## Collagen look-alike involved in streptococcal infection?

Although the complete sequence of the genome of *Streptococcus pyogenes* was finished in 1999, scientists are still deciphering what it actually means. *S. pyogenes* is an important pathogen of humans. As well as causing superficial skin and throat conditions, it can cause serious invasive infections and can trigger auto-immune attacks, resulting in illnesses such as rheumatic fever. Despite decades of research into the pathogenicity of this

University of Warwick, has picked out a gene, named *sclB*, that encodes a protein with similarity to the mammalian protein collagen. The extensive similarity of the SclB protein to collagen is unprecedented among bacteria. The structure, as deduced from the DNA sequence, indicates that SclB also contains a sequence to direct it to the surface of the bacterial cell. This is probably removed once the protein passes through the cell membrane, leaving a portion to anchor it while the rest protrudes from the surface. An indication of its importance is that this protein seems to be present in most isolates of *S. pyogenes*.

It can be an advantage for a pathogen to continuously change the face it presents to its host. The DNA coding for SclB contains repetitions of the same DNA sequence, and molecular biologists now know that these often regulate the expression of a gene in some way. Mistakes, and thus changes in the number of repeats, are often made during DNA replication, and are an easy way to generate subtle variants of the cell.

Although the function of SclB is unknown, the author speculates that its similarity to collagen, a ubiquitous protein of human skin, tissues and joints, could help trigger an auto-immune response, potentially resulting in damage to joints or the heart valves.

**Whatmore, A.M. (2001).** *Streptococcus pyogenes sclB* encodes a putative hypervariable surface protein with a collagen-like structure. *Microbiology* **147**, 419–429.

## Turning up the heat for BSE

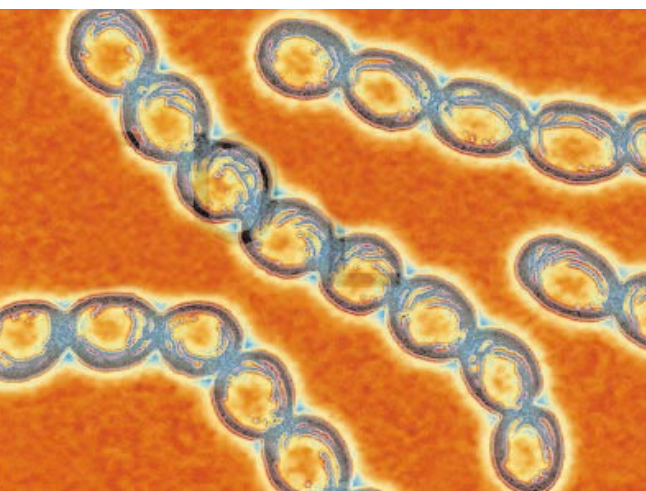
One of the most intriguing features of the BSE epidemic is the stability of the infective prion protein. It is still infectious after exposure to temperatures and chemicals that make most other proteins curl up and disintegrate. One of the reasons seems to be that it forms aggregates in both the brain of infected animals and in tissue extracts. Its chemical structure makes the protein hydrophobic, which means that it dissolves in fats, rather than water, or sticks to itself if nothing else is available. The disturbing aspect of this is that the EU still processes 1.4 million metric tons of bovine fat into soap, detergents, cosmetics and animal feed each year. Although the process usually involves heating the fat in water under pressure for at least 20 minutes at 200 °C, a forthcoming paper in *Journal of General Virology* is the first comprehensive investigation of whether this does indeed remove all detectable prions.

A group of German researchers at Heinrich-Heine University in Düsseldorf investigated how well authentic prion protein survived being heated at temperatures up to 160 °C for 20 minutes. The scientific basis for this method is that the heat initiates chemical reactions between water and protein that will break the protein into its harmless chemical constituents. The researchers used a very sensitive immunological method which could detect even one hundred millionth of a gram of prion protein. After heating the prion in water, or bovine fat, or mixtures of the two, they could estimate how much of it still survived. They could calculate a degradation factor, to indicate how effectively the protein was destroyed in each of their experiments. Lipid definitely protected the protein, reducing the factor by over 100 at the lowest temperature of 100 °C. Its influence, fortunately, disappeared above 160 °C, so that the protein vanished. The researchers wonder if the fat covers the hydrophobic surface of the protein, protecting it from degradation for a time.

The best current estimates of the infectiousness of BSE indicate that it is directly proportional to the amount of prion protein. Public health regulations in Germany assume that autoclaving at 133 °C for 20 minutes will be sufficient to degrade all prions, and there are similar recommendations in the UK and USA. This study gives a way to check that this is really correct, especially with fatty materials, as well as providing the basis for assessing the biological safety of the industrial processes that render beef fat.

**Appel, T.R., Wolff, M., Rheinbaben, F., Heinzl, M. & Reisner, D. (2001).** Heat stability of prion rods and recombinant prion protein in water, lipid and lipid-water mixtures. *J Gen Virol* **82**, 465–473.

Coloured transmission electron micrograph of chains of *Streptococcus pyogenes*. COURTESY ALFRED PASIEKA/SCIENCE PHOTO LIBRARY



organism, the genome sequence has revealed many new, possibly important, proteins.

The bacterium's surface is decorated with a varied collection of proteins, many of which are known, or presumed, to be involved in its pathogenicity. They may help it attach to human cells or evade the immune system. Adrian Whatmore of the Infectious Disease Research Group at the