

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.

THIS PAGE:

The 'face of FMDV', as portrayed on the cover of *Nature* (R. Acharya *et al.*, 1989, *Nature* 337, 709–716). The capsid is viewed down a five-fold axis of the icosahedral virus with the constituent proteins drawn as Calpha traces, colour coded such that VP1 is blue, VP2 is green, VP3 is red and VP4 is yellow. This is the structure of the native virus where the major antigenic loop cannot be visualized owing to its flexibility. Large dotted blue spheres define the approximate position it would occupy on the surface. Subsequent studies of virus treated with DTT (D. Logan *et al.*, 1993, *Nature* 362, 566–568) permitted the visualization of this loop, which also contains the receptor attachment RGD motif. COURTESY DAVID STUART, LIZ FRY AND ROBERT ESNOUF, OXFORD UNIVERSITY

OPPOSITE PAGE:

Cryoultrathin section of *Mycoplasma pneumoniae*. The cytoskeleton is visible in the tip structure. Bar, 100 nm.

PHOTO COURTESY JAN HEGERMANN, INSTITUT FÜR MIKROBIOLOGIE UND GENETIK, GÖTTINGEN, GERMANY

A global view of FMD

The importance of foot-and-mouth disease (FMD) in the UK has coincided with publication in JGV of research by Alan Samuel and Nick Knowles of the Institute for Animal Health at Pirbright in Surrey. They analysed 105 isolates of FMD virus (FMDV) to give the most comprehensive picture so far of its spread and diversity around the planet. They used information held by the OIE/FAO World Reference Laboratory for FMD at the Institute, which has been built up since 1924, and now includes the latest genetic sequence information as well as samples of the virus and reports on outbreaks.

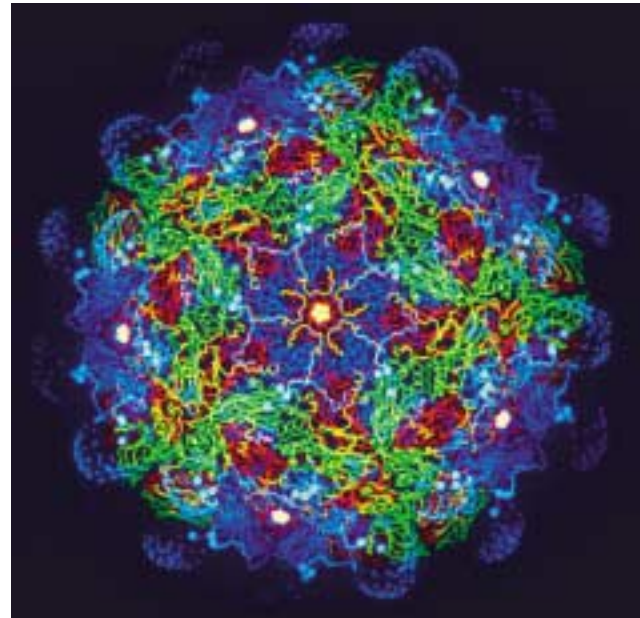
All the isolates were of serotype O, the type involved in the current UK outbreak. This is one of the seven immunologically distinct types of FMDV, although there is considerable variation within each group. To detect this, the researchers now use the sequence of the FMDV genome, which allows the identity of isolates to be checked with great precision. The Reference Laboratory has over 1,000 sequences of part or all of the genome of FMDV serotype O isolates, and the researchers picked ones that would give them a good picture across the whole planet. When they put all the sequence data into a computer program that could cluster the sequences together based on their similarities, it sorted the isolates into eight distinct genetic lineages, called topotypes, each predominantly in particular geographical regions.

When the researchers looked closely at exactly which isolates were in each group, this revealed part of the history of FMD. FMDV belongs to a group of viruses which are very prone to acquire small changes in their genomes quite rapidly. However, some isolates showed surprisingly little variation despite having been isolated years apart. The best explanation for this came from the fact that they were all similar to ones that have been used to make vaccines, with the implication that some of the disease isolates originated from improperly prepared vaccine, giving a new angle on the use of vaccination against FMD.

Other clusters echoed the movements of people around the world. For example, European and South American viruses formed one group. This matches with the first appearance of the disease in Argentina in 1870, in the livestock of European immigrants. They evidently took their disease, as well as their animals, with them. This topotype has probably continued to travel in cattle, because the researchers spotted that an FMDV isolate from Angola was also in this group, and may have come from animals imported from South America in the 1970s.

Some isolates were in unexpected clusters, probably explained by world trade. This might have been the origin of an isolate from Moscow which was most similar to others found half a world away in China, since it came from an outbreak thought to originate from imported pig meat. The disease may also be lasting evidence of clandestine activities, as in an isolate of a topotype usually confined to Ghana, Ivory Coast and Guinea that appeared in Algeria and might have come from illegal movements of Zebu cattle in 1999.

Samuel, A.R. & Knowles, N.J. (2001). Foot-and-mouth disease type O viruses exhibit genetically and geographically distinct evolutionary lineages (topotypes). *J Gen Virol* 82, 609–621.



FMD in pigs

A further report from the Institute for Animal Health concerns FMD in pigs. Infected pigs are powerful emitters of air-borne FMDV and cattle are highly susceptible to infection by inhalation. The pattern of wind-borne spread of FMD over more than 10 km is thus invariably from pigs at source to cattle downwind. This investigation into the early stages of the disease was carried out before the recent outbreak in the UK, in biosecure isolation buildings, with careful precautions to minimize suffering to the animals, as well as to ensure that the virus could not escape.

To start the infection, the researchers injected four pigs with the virus: within 3 days the animals were so unwell that they would not stand up. At this point, eight healthy pigs were brought into their room, left for 2 hours and then returned to their own individual cubicles. The researchers then killed the sick pigs and disinfected the room, their clothing and anything else that had been

in contact with the pigs. Over the next 4 days, they observed the condition of the other pigs and selected two each day to be clinically examined and then killed, keeping blood and other tissues to test later.

One of the difficulties with FMD is to detect its presence, or confirm its absence, in apparently healthy animals. There are several ways to do this, and the basis of the project was to compare the sensitivity of a method based on detecting the viral genome with one that actually measured how infective the virus was by growing it in cultured animal cells. The amount of virus increased very rapidly in some parts of the pigs, reaching as much as 100 million virus particles per gram of tissue. The measurements by both methods matched, with the advantage that figures based on the amount of virus genome were not influenced by the immune response of the pigs to the disease. This tended to decrease the infectiousness



Minimalist microbes

Mycoplasma pneumoniae causes bronchitis in young people that occasionally develops into pneumonia, but usually clears up of its own accord. Although it is not impressive as a pathogen, it has the distinction of pushing the boundaries of a living cell to their lowest limits. Its cells attach themselves as closely as possible to the human cells covering the surface of the respiratory tract and have evolved to exploit this close association to the extent of relying on their involuntary host for many essential compounds. As well as discarding biosynthetic abilities, *M. pneumoniae* has shed its cell wall and replaced it with some sort of internal skeleton. One consequence is that *M. pneumoniae* has one of the smallest genomes of all known bacteria with only 688 genes, all of which were sequenced about 5 years ago.

The stripped-down nature of its cells may make the job of finding out what everything does much easier than in more self-sufficient organisms. One of its unusual components is the presumed internal protein skeleton, which appears as shadowy images in electron microscopic pictures after the rest of the cell's proteins have been gently washed away with salt and detergent. The tip seems to be essential for *M. pneumoniae* to stick to human cells. A group of German microbiologists have been trying to discover what the presumed cytoskeleton is really like, and in particular, what proteins are in it.

After isolating the best protein skeletons that they could, they separated out every individual component as tiny spots within a slab of jelly. Then to identify each one, the researchers delicately picked out the spots and analysed each in a machine that could record at least part of the order of the amino acids in each one. The trick to using this to identify the proteins relied on the fact that they already knew the sequence of all the genes in the bacterium. The sequence of a gene includes the instructions for the order of the amino acids in the protein that is made from it, so from knowing the arrangement of a short length of amino acids, the researchers had a good chance of being able to name the protein.

They found over 100 proteins associated with the fibres from the cells and managed to identify 41 of them. Several of the proteins were ones that had already been fingered as part of the tip of the skeletal structure, along with several others whose role was previously unknown. However, for many of the proteins, it was not clear whether they were really a normal part of the skeleton, or had simply attached to it as the cells broke open. The next step for the German researchers will be to test how some of these proteins are organized within the cell.

Regula, J.T., Boguth, G., Gorg, A., Hegermann, J., Mayer, F., Frank, R & Herrmann, R. (2001). Defining the mycoplasma 'cytoskeleton': the protein composition of the Triton X-100 insoluble fraction of the bacterium *Mycoplasma pneumoniae* determined by 2-D gel electrophoresis and mass spectrometry. *Microbiology* **147**, 1045–1057.

of the virus when its numbers were reaching their highest levels after 3 or 4 days.

When the scientists looked at the pigs' organs, everything seemed normal, apart from the disease lesions on feet, gums, tongues and snout by 3 days after infection. However, the tests for FMDV could detect some virus in almost all tissues tested. There were moderate amounts of FMDV in the pharynx within 24 hours of infection, because this was probably where the virus was first deposited and began to multiply. The highest amounts of virus were in the disease lesions, although even regions of the tongue and skin that looked normal contained large amounts of virus.

Soren Alexandersen, S., Oleksiewicz, M.B. & Donaldson, A.I. (2001). The early pathogenesis of foot-and-mouth disease in pigs infected by contact: a quantitative time-course study using TaqMan RT-PCR. *J Gen Virol* **82**, 747–755.

'FreeTree' taxonomy program

Biologists are very interested in characteristics that make individuals unique and that allow them to be grouped together. Ideas about what defines a species, its relationships to other species, and their evolution, are important to ecologists, epidemiologists and sociobiologists, as well as to taxonomists. The application of molecular methods to taxonomy has solved some problems that were out of reach by traditional methods, but has caused further debate. For example, is it more valid to base taxonomy on the molecular details of a single gene, or on a number of them?

As well as philosophical arguments, there are some technical differences between these two approaches. Many readily available computer programs apply mathematical methods to molecular data from a single gene and include statistical procedures to assess the reliability of the results. In contrast, very few programs can do the same when the data come from a large number of genetic loci. Scientists at Charles University in Prague and the Academy of Sciences of the Czech Republic have been remedying this omission, and a recent paper in *IJSEM* describes the application of their new program, FreeTree, to 731 characteristics, scattered across the genome of ten species of trichomonads. This program gathers similar organisms together according to several criteria, draws diagrams of its results and carries out statistical tests that can be interpreted in terms of confidence in its conclusions. The program

will run under Windows 95/98/NT and is available from the IJSEM's supplementary data facility at <http://ijs.sgmjournals.org/vol51/issue3/>

The trichomonads are a group of flagellate protozoans that infect animals. *Trichomonas vaginalis* is perhaps the best known, because it causes non-gonococcal urethritis in humans, while other species live within mammals, birds and reptiles. The researchers analysed characteristics from 42 trichomonad strains using FreeTree and demonstrated the importance of assessing the reliability of any taxonomy. The results clearly indicated that *Trichomonas vaginalis* had an ancient origin, compared with the trichomonad-like species *Tritrichomonas suis*. However, the program's statistical analysis indicated that no firm conclusions could be drawn from details within the *Trichomonas vaginalis* grouping. In contrast, the statistics indicated that it was safe to infer that the intermixing of harmless isolates of *Tritrichomonas suis* from the intestine of pigs, with pathogenic ones of *Tritrichomonas foetus* from the urogenital system of cattle supported previous suggestions that they are in fact the same species.

Hampl, V., Pavlicek, A. & Flegr, J. (2001). Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with a freeware program FreeTree: application to trichomonad parasites. *Int J Syst Evol Microbiol* **51**, 731–735.

Online submission to SGM journals

Microbiology and *IJSEM* have recently started using the ESPERE online submission and peer-review system. This involves authors submitting a single PDF file of their paper to a secure website. An encrypted URL is forwarded to the Editor who then forwards it to the chosen reviewers. Reports are submitted to the Editor using our online report form.

It is early days yet, but ESPERE should result in a reduction in the time taken to make a decision, thus helping in more rapid publication, and will also result in savings in postage and courier charges. The fact that it is web-based means that it will be easier to get reports rapidly from all over the globe.

We hope that authors, reviewers and Editors will appreciate the benefits of online submission and will embrace this exciting development.

Supplementary data

As mentioned in the February issue of *Microbiology Today*, the supplementary data facility is now in operation. A variety of data types have been attached to papers, including video, figures, tables, large sequence alignments and even a free computer program (FreeTree, see p. 97 of this issue). The system should help contain the size of the printed journals and also provide added value to our online services.

Order! Order!

One of the most obvious things about bacteria is that they all look much the same. This rapidly drove researchers on a quest for other characteristics. Microbiologists have now carefully catalogued around 4,518 species in 998 genera, many of which are defined on features including their patterns of nutrition, the nature of their cell walls, particular types of chemicals in their cells, their typical habitats and, increasingly, information about their genes. John Young, from Landcare Research in New Zealand, has described, in a recent issue of *IJSEM*, the ways that taxonomists use this information to classify bacteria and the challenges that may come from increased knowledge about bacterial genetics.

Classification is the ordering of organisms on the basis of their relationships, e.g. grouping similar strains into species, and species into genera. Phenetic classification is based on overall similarity by equal weighting of all known characters and it can make use of all sorts of information about bacteria, from the shape of cells, or nutritional requirements, to characteristics of the DNA and RNA. Polyphasic classification is based on a consensus of all available methods, phenotypic and genomic.

These two systems can conflict with another goal in systematics, which is to group organisms together based on ancestral relationships (phylogenetic classification), because the possession of a common feature does not necessarily mean that organisms evolved from a recent common ancestor. An obvious example of this among animals is that although both birds and bats have the power of flight, it is almost 250 million years since they shared ancestors. Bacteria have left very little in the fossil record, so all inferences about their ancestry have to come from measurements of differences between the sequences of their genes and assumes that all the changes relate to historical relationships. A problem with phylogenetic classifications is that they sometimes give views on relationships between bacterial isolates that rely on subjective decisions about which gene to investigate. Some recent proposals for new bacterial genera depend on differences in sequence within only one gene, leaving open the question of whether the same degree of difference applies to all the other genes in the two isolates.

One ability of bacteria that would certainly affect the assumptions of phylogenetic classifications is the way they can pick up and retain genes that are beneficial to their survival from unrelated bacteria. The extent of this genetic exchange is still being evaluated, but is certainly much greater than anyone once imagined. For example, it seems as if about 18% of the genome of *Escherichia coli* comes from the stable integration of transferred genes. John Young says there is already speculation that the conserved regions that make one gene particularly valuable for creating phylogenetic classifications, simultaneously make it a prime candidate for transfer between bacteria! As a final thought, he suggests that we might come to view bacterial species and genera as groups of organisms which share a collection of genes, but have access to many others from outside the group. This would certainly be a challenge to some current concepts in bacterial taxonomy.

Young, J.M. (2001). Implications of alternative classifications and horizontal gene transfer for bacterial taxonomy. *Int. J. Syst. Evol. Microbiol.* 51, 945–953.

Surviving acid attack

Although *Escherichia coli*, the well known inhabitant of the human gut, does not like acidic surroundings, it can tolerate even pH 2 for a few hours. Indeed, pathogenic strains have to be able to survive the extremely acidic stomach if they are to reach the intestines and set up an infection. Researchers at the University of South Alabama College of Medicine in the USA have discovered that the cells have at least three distinct ways to protect themselves through this dangerous time in their lives. The researchers have now focused on trying to understand how one system is influenced by the cell's environment. It involves the enzyme glutamate decarboxylase and requires a source of the amino acid glutamate to mop up any acid that enters the cell.

They created strains of *E. coli* that as well as containing the genes encoding the enzyme also had the DNA instructions that switch these genes on or off attached to a so-called reporter gene. Reporter genes encode proteins that are particularly easy to detect. The idea was that the cells would respond to their surroundings as normal, but the reporter gene would make it much simpler to see exactly when glutamate decarboxylase was present. In particular, the researchers wanted to know whether other proteins, called sigma factors, that help recognize the DNA sequence at the start of genes, were involved in switching on production of glutamate decarboxylase. They also looked at what happened when they changed the growth medium to mimic the nutrient-poor stomach, or the nutrient-rich intestine.

They discovered that the enzyme was present when rapidly growing cells experienced mildly acid conditions, but only if they were in a nutrient-poor environment. In contrast, if the cells were about to stop growing, acidity triggered increased enzyme production above a modest level, regardless of the nutrients in the environment, provided the cells also contained a particular sigma factor, called σ^5 . As a further feature, the researchers worked out that another protein, which detected how much of the sugar glucose was in the environment, was also involved in regulating enzyme production. The complexity of the system for detecting and counteracting acidic surroundings makes the researchers certain that it is very important to the survival of *E. coli*.

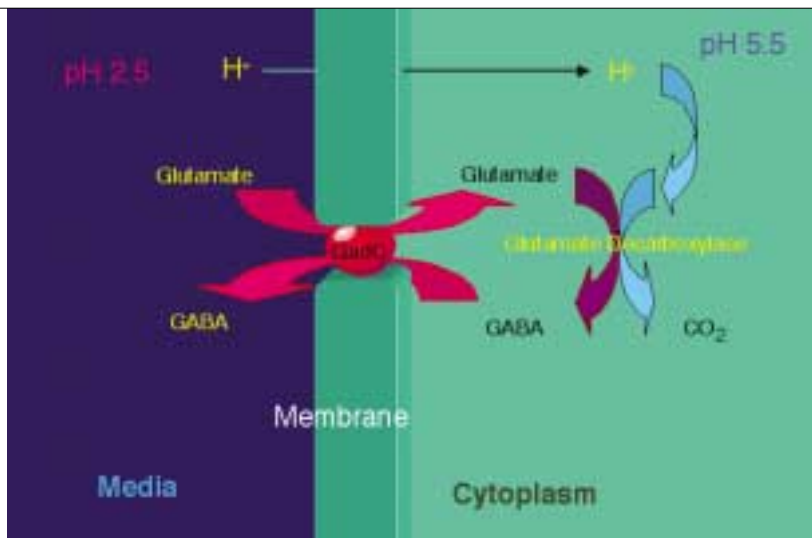
Castanie-Cornett, M.P. & Foster, J.W. (2001). *Escherichia coli* acid resistance: cAMP receptor protein and a 20 bp *cis*-acting sequence control pH and stationary phase expression of the *gadA* and *gadBC* glutamate decarboxylase genes. *Microbiology* 147, 709–715.

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

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

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

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Watching evolution

How can you study evolution and see living creatures really changing over time? Do all changes improve their abilities to live, and reproduce, or are some of them purely accidental? Bacteria offer an almost unique opportunity to study this. Not only are they very small, with short lifespans, but they can be grown in very tightly controlled environments. Even better, their cells can be deep-frozen and then revived to capture the characteristics of any generation.

A group of microbiologists at Michigan State University has tried this out with a strain of soil bacterium from the genus *Ralstonia*. They inoculated twelve flasks of liquid, so the conditions around each cell would be uniform, and also six sets of agar plates where the bacteria would experience gradients of nutrients and their own excreted products. As a way to keep track of the bacteria, they all had resistance to one of two antibiotics. After a thousand generations the researchers compared the appearance and several other properties of each population, as well as the starting, ancestral cells, and were surprised at the extent and variety of the changes.

Almost all the new cells stuck to surfaces more tenaciously than their ancestor and their appearance had also changed, with the cells in one population now 4.7 times longer, while those in three others had shrunk. When the researchers checked whether the cells could use any of 95 different nutrients, no two of the new populations were the same and all differed from their ancestor. The only pattern to the changes was that the cells that had evolved in liquid environments split into two groups, depending upon the antibiotic resistance of the founding population. For other features, like the fats within the cells, ones grown on solid agar retained a spectrum very similar to their parent, while those from the liquid environments had changed.

After the researchers put all the information on the cells together, they could make some guesses at what it meant. One of the themes that showed through was that all the new *Ralstonia* cell populations were better suited to their environments, despite their very different characteristics. Almost all the cells had lost the surface coating, called a capsule, of their ancestor and its absence could account for changes in their nutritional requirements, as well as increased adhesion. Of continuing interest to these workers is the great variability of the changes and the speed at which the changes accumulated. The organism continues to be studied because of this apparent plasticity.

Riley, M.S., Cooper, V.S., Lenski, R.E., Forney, L.J. & Marsh, T.L. (2001). Rapid phenotypic change and diversification of a soil bacterium during 1000 generations of experimental evolution. *Microbiology* **147**, 995–1006.

ABOVE: Glutamate-dependent acid resistance in *E. coli*. The diagram represents a proposed mechanism for glutamate-dependent acid resistance. GadC is a membrane-bound antiporter that exchanges glutamate from the medium for cytoplasmic γ -aminobutyric acid (GABA), the by-product of glutamate decarboxylation. When *E. coli* is present in the extreme acid of the stomach, protons (H^+) pass through the cell membrane and threaten to acidify the cytoplasm to a lethal level. However, the intracellular decarboxylation of glutamate consumes a proton and alkalizes the cytoplasm. This proposed proton sink is maintained by continually bringing more glutamate into the cell via GadC in exchange for GABA which is expelled into the medium. Synthesis of this system increases when cells are exposed to acidic environments or enter into stationary phase.

COURTESY J.W. FOSTER, UNIVERSITY OF SOUTH ALABAMA COLLEGE OF MEDICINE, MOBILE, USA

Are you a keen photographer? Do you have any good images of micro-organisms, scientists at work, food, farming, the environment, industry, biotechnology or academia?

If so, why not enter our photographic competition? Entries may be used in *Microbiology Today*, perhaps even on the cover, or in SGM educational resources.

Entry forms may be downloaded from the SGM website: <http://www.sgm.ac.uk>

● Rules

Entries should be submitted as a labelled transparency (from 35 to 70 mm) and as prints (6 × 4" minimum to 10 × 8" maximum), or, alternatively, they may be emailed to j.meekings@sgm.ac.uk as a high quality TIFF or JPEG file with a file size of no more than 1.5 Mb. Do not send pictures in glass mounts, as these can break in the post. Your name and address must be written on the slide mount of each transparency or on the back of each print. All entries must be accompanied by a signed entry form. The Society does not accept any liability for lost, delayed or incomplete entries.

Entries will be retained by the Society and signature of the form by the entrant acknowledges the right of the Society to use the photographs in Society publications and on the website at <http://www.sgm.ac.uk>

In the event of their use, credits will include the name of the photographer.

Entries will be displayed and judged by a panel at the Autumn 2001 Society meeting in September at the University of East Anglia. The panel's decision will be final. The winner will receive a certificate and cash prize of £250, to be presented at the Society Dinner on Tuesday 11 September.

Send your entries to: Janice Meekings, *Microbiology Today*, Marlborough House, Basingstoke Road, Spencers Wood, Reading RG7 1AG, UK.

The closing date for entries is 31 July 2001.