

Science writer 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.

CENTRE: Virion morphology and ORF1a-1b genomic region of gill-associated virus: an invertebrate nidovirus infecting *Penaeus monodon* prawns.

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BELOW: Clusters of the principal polyphosphate-accumulating organism in a biological phosphate-removing process treating municipal waste water on a technical scale at the Suomenoja waste water treatment plant, Southern Finland. Polyphosphate in fresh activated sludge was stained fluorescently with DAPI. Bar, 10 μ m. PHOTO COURTESY H. MELASNIEMI, UNIVERSITY OF HELSINKI, FINLAND

The mystery of the missing phosphate

All living organisms need a supply of phosphate. So one of the aims in waste water treatment is to remove it and thus prevent polluting blooms of algae in lakes and rivers. The activated sludge process at treatment plants is one method of removal by incorporating the phosphate into micro-organisms. Ever since the 1950s people have known that more phosphate is removed than is needed to sustain the microbes in the sludge. Some of the microbes store it as polyphosphate granules within their cells. Despite considerable work, the identity of these beneficial organisms has been in doubt until recently. Grape-like clusters of large cells containing polyphosphate are usually visible when fresh sewage sludge is viewed under a microscope. Several bacteria are capable of accumulating polyphosphate, but the link between their laboratory performance and the real world has never been clear.

Hannes Melasniemi and Anne Hernesmaa from the University of Helsinki have gone back to basics, looking at real sewage sludge. As they point out, there is no reason why the cells have to be bacteria. Algae, fungi, protozoa and invertebrates, as well as bacteria, are all present in activated sludge. Their microscopic study, involving staining for characteristic features of microbes, clearly shows that the principal polyphosphate accumulator is undoubtedly a yeast. It has a cell surface covered with typical fungal compounds, is unaffected by antibacterial antibiotics, and has large oval cells. It vanishes rapidly once fresh sludge is dropped into conventional laboratory growth media, being replaced by bacteria. This may be one reason why it has taken so long to appreciate that it is even present in activated sludge. Back in 1888 Liebermann identified baker's yeast as the first organism to contain polyphosphate. The beneficial activities of one of its relatives have eventually been revealed over a hundred years later.

Melasniemi, H. & Hernesmaa, A. (2000). Yeast spores seem to be involved in biological phosphate removal: a microscopic *in situ* case study. *Microbiology* **146**, 701–707.

Genes in knots

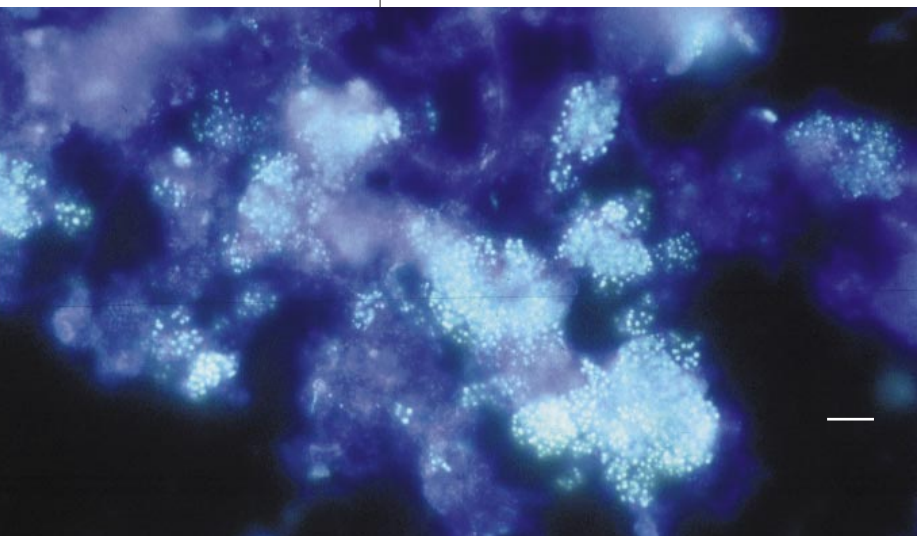
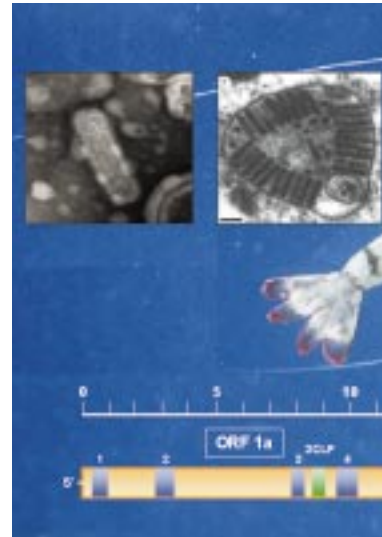
A central tenet of molecular biology maintains that nucleic acids are the genetic material of all living things. Deoxyribonucleic acids (DNA) carry the instructions for most organisms and ribonucleic acids (RNA) are generally used to make everyday working copies. However, some viruses use ribonucleic acid as their information repository. Although philosophers can argue about how well the word 'alive' applies to a virus, for prawn farmers surrounded by dead and dying black tiger prawns, a virus is as much a predator of their prawns as an animal would be.

Gill-associated virus (GAV) has caused massive production losses in farmed prawns in Australia. It replicates in the prawn's lymphoid organ, turning its cephalothorax a characteristic yellow colour. A group of Australian researchers has now reported their analysis of the characteristics of GAV.

The genetic material of GAV is single-stranded RNA. In some ways, this is very convenient for the virus, because the prawn cells that it exploits can immediately carry out the RNA instructions. However, the virus has to ensure that it provides a way to copy its RNA genes, because that is something that cells never do. The researchers examined the viral RNA in great detail and realized that a large part of it consisted of only one gene, which contained instructions for two sets of proteins needed to replicate and transcribe the genome. Since there is a single molecule of genomic RNA, this can only work if the frame for translation makes sense when slipped along a little, as well as in its original format. The experiments showed that the viral RNA forms into an elegant pseudoknot to achieve this slippage. Two very large proteins were produced, which look as if they had to be processed into smaller units to form the replication machinery.

The authors obtained enough information to be confident about the taxonomic position of GAV for the first time. It is a member of the nidoviruses, a group of viruses found in animals and typified by a large RNA genome and complicated ways of expressing their proteins. This scourge of modern prawn farming may have an ancient origin. It is the first nidovirus found in an invertebrate, and since marine invertebrates were abundant prior to the evolution of land animals, it may be an example of an ancestral form of the virus.

Cowley, J. A., Dimmock, C. M., Spann, K. M. & Walker, P. J. (2000). Gill-associated virus of *Penaeus monodon* prawns: an invertebrate virus with ORF1a and ORF1b genes related to arteri- and coronaviruses. *J Gen Virol* **81**, 1473–1484.





Passing the acid test

Microbes will grow in the most unlikely places. For example, the liquid of a pilot bioreactor designed to leach gold from arsenopyrite/pyrite ore from Kazakhstan at a temperature of 30 °C and a pH around 1.7 does not sound like a promising location. However, Russian researchers have found the first member of a previously unknown family in exactly this location. Their studies, in collaboration with the GBF National Research Centre for Biotechnology in Germany, have ranged from tempting it to consume 101 different organic compounds, mass spectrometry of its cell constituents and sequencing parts of its DNA.

It has some pretty unusual characteristics. To begin with, it is not actually a bacterium. Instead, it is an archaeon, a member of the third great kingdom of life on this planet. These single-celled organisms are as different from bacteria as we are, with the differences lying in fundamental cellular activities. The organism, *Ferroplasma acidiphilum*, enjoys a very acidic environment at pH 1.7, uses carbon dioxide as its

sole source of carbon for building cell materials and obtains all its energy from the oxidation of iron. This is undoubtedly the physiological key to its presence in the bioreactor because of all the iron seeping from the pyrite ore. The structural key is that the cells, which divide by budding, are entirely wall-less and have exposed membranes, the characteristics of which are probably essential to survival in such a hostile environment.

F. acidiphilum is sufficiently different from other archaea that it belongs to a new family, *Ferroplasmaceae*. It will be interesting to see whether other organisms join it in the future.

Golyshina, O. V., Pivovarova, T. A., Karavaiko, G. I., Kondrat'eva, T. F., Moore, E. R. B., Abraham, W.-R., Lünsdorf, H., Timmis, K. N., Yakimov, M. M. & Golyshin, P. N. (2000). *Ferroplasma acidiphilum* gen. nov., sp. nov., an acidophilic, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of the *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the *Archaea*. *Int J Syst Evol Microbiol* **50**, 997–1006.

Toxic shock

The European Community permits the use of some antibiotics as additives in animal feeds for their growth-promoting properties. Although these antibiotics are not used in human medicine, there are concerns that they may encourage the development of antibiotic-resistant bacteria. The German researchers Bernd Köhler, Helge Karch and Herbert Schmidt have now reported on another possible danger.

*Escherichia coli*s sometimes capable of producing a toxin that causes diarrhoea and haemorrhagic colitis. On rare occasions, the infection can lead to kidney failure

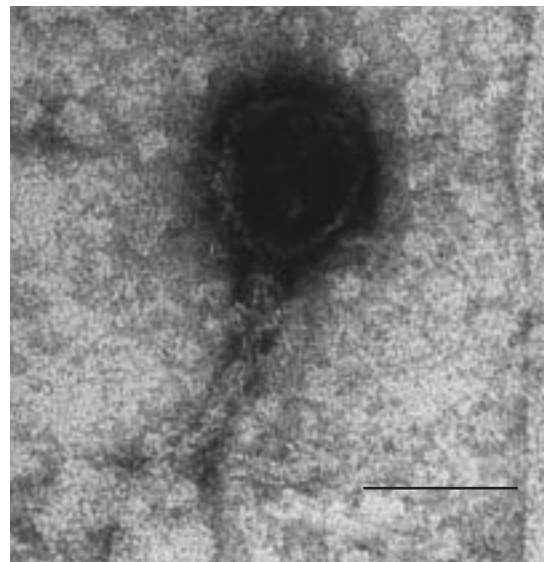
and life-threatening blood or neurological disorders. There are very many versions of these Shiga-toxin-producing *E. coli* (STEC) and they frequently occur in farm animals. The infamous *E. coli* O157:H7 is one of them.

The genes for the toxin are actually not part of the genome of *E. coli*, but of a parasitic virus within it (called a phage). The toxin is probably produced as part of the phage life-cycle, at the point where it has started to multiply within its bacterial host, prior to bursting out and seeking more bacteria to parasitize. This stage is triggered by environmental stress – such as the

presence of antibiotics, perhaps. The German group wanted to know if the feed additives could really do this.

They grew clinical isolates of STEC strains in media containing antibiotics used as animal growth promoters: the concentrations of the antibiotics were selected to allow the bacteria to grow poorly. Then they tested the growth medium for the presence of both Shiga toxin and phage particles. The tests always found the two together. Two of the growth promoters, olaquinox and carbadox, strongly enhanced phage release in all of the strains tested, while the other two (tylosin and monensin) did not.

Of course, bacterial cells growing in laboratory media are in a very different environment from an animal's gut. However, growth promoters are used with clear regulations on the amounts permitted in feed and studies have measured the concentrations in animals' digestive tracts. The sub-lethal amounts used in this study were within the range really experienced within animals. The researchers had to draw the conclusion that



some growth promoters might increase the amount of phage present in intestines and so possibly contribute to the spread of Shiga toxin genes to all the bacteria living there. This is obviously a topic that we will hear about again.

Köhler, B., Karch, H. & Schmidt, H. (2000). Antibacterials that are used as growth promoters in animal husbandry can affect the release of Shiga-toxin-2-converting bacteriophages and Shiga toxin 2 from *Escherichia coli* strains. *Microbiology* **146**, 1085–1090.

ABOVE:
Stx2-phage 933W isolated from *Escherichia coli* O157:H7 strain EDL933. Bar, 50 nm.
PHOTO COURTESY H. SCHMIDT, UNIVERSITÄT WÜRZBURG, GERMANY

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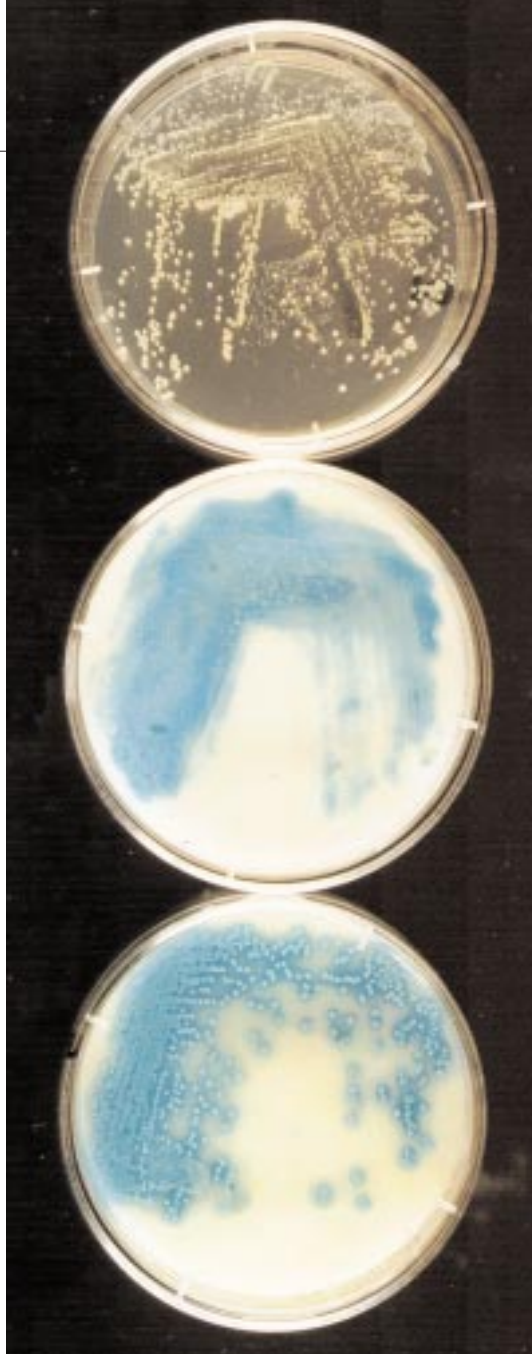
Turned on by milk

Mastitis is an unpleasant infection of cows. Not only is it painful for the animals and means their milk is unfit for consumption, but it often does not respond to treatment with antibiotics. The bacterium *Staphylococcus aureus* is the most common cause. It can colonize the mammary glands within hours, multiplies rapidly and becomes resistant to attack by the immune system. Current data suggests there is a particular set of genes that become active in milk to aid its success in this environment. Knowledge about their identity might allow the design of new prevention measures.

This was the aim of a group of scientists from the Netherlands. Their experiments mutated a pathogenic strain of *S. aureus* with a transposon. This is a short length of DNA that has the ability to insert itself anywhere in a chromosome. Transposons occur naturally and were first detected in the 1940s by Barbara McClintock in maize plants. The Dutch researchers used a transposon containing the gene for a protein that they could easily detect. However, it lacked any 'commands' to turn the gene on. So the protein would only appear when the transposon landed next to suitable 'instructions' from *S. aureus* genes. The researchers were, of course, looking for genes that were switched on by milk and so grew *S. aureus* on media containing it. Once they detected the protein, all they had to do was isolate and sequence the DNA surrounding the transposon and they would have a milk-activated bacterial gene in their hands.

They eventually identified 28 different genes in *S. aureus*. One group of genes was involved in cell wall synthesis. Other genes had roles in synthesis of DNA or of sensors for monitoring the environment. The function of several further genes could not be identified. One encouraging aspect was that several of the genes had already been fingered by other scientists as important for virulence of *S. aureus*. The picture of what happens during mastitis is becoming clearer.

Lammers, A., Kruijt, E., van de Kuijt, C., Nuijten, P. J. M. & Smith, H. E. (2000). Identification of *Staphylococcus aureus* genes expressed during growth in milk: a useful model for selection of genes important in bovine mastitis? *Microbiology* **146**, 981–987.



ABOVE: Colonies of a staphylococcal transposon mutant (transposon insertion in a homologue of the *Bacillus subtilis* *phoR* gene) on three different growth media containing the β -galactosidase substrate X-Gal: top, LB medium; middle, raw bovine milk; bottom, 1 vol. 2x LB medium and 1 vol. milk. PHOTO COURTESY A. LAMMERS, INSTITUTE FOR ANIMAL SCIENCE AND HEALTH, LELYSTAD, THE NETHERLANDS

Hybridization in miniature

Microbiologists have always been interested in accurate and rapid methods for classifying bacteria. A formidable set of tools has been developed over the years as the accepted best methods for dealing with particular groups of bacteria. Unfortunately, there is always a trade-off between speed, cost and precision.

One important way of classifying bacteria at the species level is DNA–DNA hybridization. DNA is extracted from a test strain and mixed with DNA from an authentic strain. This is done in conditions designed to make the DNA of the two strains stick together. The amount that sticks indicates the relationship of the bacteria. There should be 100 % hybridization if the strains are identical.

Reliable methods for these hybridizations are time-consuming and require large amounts of DNA. The procedure described in this paper is as accurate as conventional methods, requires only one-hundredth the amount of DNA and should be easy to automate. It relies on hybridization to authentic DNA covalently attached to tiny plastic wells. This is the type of procedure used in DNA sequencing, so robots already exist that could perform the manipulations. The authors point out that this test is currently under-utilized despite its value because of the difficulties in doing it. Their procedure may be the start of its introduction into routine use.

Christensen, H., Angen, Ø., Mutters, R., Olsen, J. E. & Bisgaard, M. (2000). DNA–DNA hybridization determined in micro-wells using covalent attachment of DNA. *Int. J. Syst. Evol. Microbiol.* **50**, 1095–1102.

Seafood cocktail

White spot syndrome virus (WSSV) causes devastating disease in shrimps, crabs, crayfish and other aquatic invertebrates. Not only is the identity of the virus uncertain, but also the way in which it can attack so many different animals. A collaboration between researchers in the Netherlands and the National Taiwan University has been using DNA sequencing to investigate this puzzle. The very large amount of DNA contained in the virus has made this difficult. In addition, there are regions that look like genes but are unlike ones identified in other organisms. However, the scientists have now succeeded in identifying two WSSV genes, opening the way for comparisons with other viruses.

The genes are for the two parts of ribonucleotide reductase, an enzyme used in making the components of DNA. Its identification in WSSV is particularly valuable because its important role means it has been conserved

during evolution. The researchers could compare it with sequences for these genes from many other organisms to see WSSV's evolutionary relationships. The WSSV genes turn out to be related to ones found in eukaryotes and their viruses, although it is unlikely that they share a recent common ancestor. More interestingly, they are not closely related to those found in baculoviruses. This is the viral group that seems most like WSSV on the basis of morphology and replication characteristics. Although more information is needed, it looks like WSSV is going to be the first representative of the Whispovirus genus, and perhaps an entirely new virus family.

van Hulten, M. C. W., Tsai, M.-F., Schipper, C. A., Lo, C.-F., Kou, G.-H. & Vlak, J. M. (2000). Analysis of a genomic segment of white spot syndrome virus of shrimp containing ribonucleotide reductase genes and repeat regions. *J. Gen. Virol.* **81**, 307–316.