

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.

THIS PAGE (RIGHT):

During the development of *D. discoideum*, amoebae enter an aggregate by chemotaxis toward cAMP. This experiment shows that cells of a cheater mutant (labelled with green fluorescent protein) and those of the wild-type (detected by phase contrast) enter an aggregate with approximately equal efficiency. Each amoeba is 8–10 µm in diameter. See the following website for an animated view of aggregation: <http://cpmcnet.columbia.edu/dept/gsas/anatomy/Faculty/Kessin/index.html>

COURTESY DEE N. DAO (WHO THANKS MARY WU AND THERESA SWAYNE OF THE COLUMBIA UNIVERSITY CONFOCAL FACILITY FOR ASSISTANCE)

OPPOSITE PAGE (LOWER):

Multiple resistance of transgenic tobacco plants. Plants were inoculated with TuMV (row 2) or TSWV (row 3) first, then those plants that did not show systemic symptoms after 34 days were inoculated with TSWV (row 2) or TuMV (row 3). Rows 1 and 4 were non-transgenic controls inoculated with TuMV and TSWV, respectively. The plants were photographed after 49 days.

COURTESY DENNIS GONSALVES, CORNELL UNIVERSITY, NY, USA

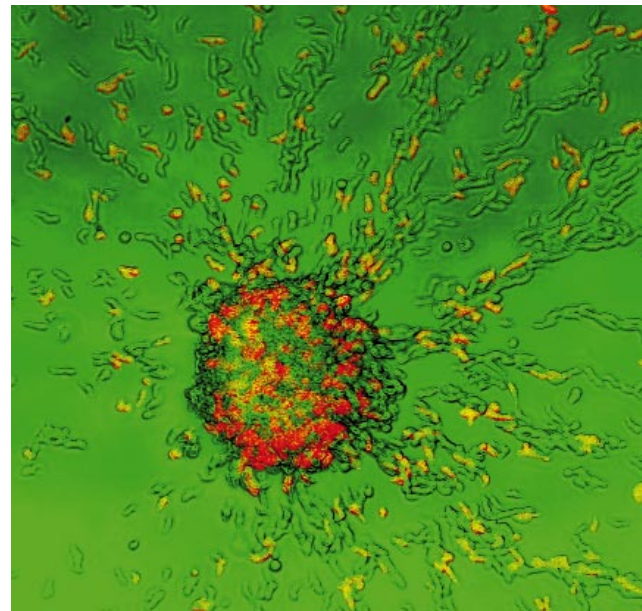
## Recombination network

Molecular biology provides a new way of tracking epidemics, and can shed light on the origin of diseases. There is an epidemic of cotton leaf curl disease, which has spread through the major cotton-growing areas of Pakistan since 1985, caused by a novel series of forms of begomovirus. Begomoviruses cause many important plant diseases and typically carry all their genetic information on two circular single-stranded DNA molecules (DNA-A and DNA-B). The virus spreading through Punjab and Northern Sindh is unusual in apparently only containing DNA-A, along with a second very small circular strand of DNA that resembles part of the genome of a nanovirus and occasionally a third circle of DNA that is a defective piece of DNA-A. There are at least four types of this Pakistan cotton leaf curl virus (CLCuV-PK) DNA-A. All share sequences with a begomovirus that infects the vegetable okra, while the differences between some types are as much as between other begomovirus species.

The implication from this, and other, research was that CLCuV-PK in Pakistan was very prone to recombining its genetic information, with both itself and other begomoviruses. This requires plants to be infected with more than one begomovirus simultaneously. Researchers in Pakistan, Spain and the UK, led by Bryan Harrison and David Robinson from the Scottish Crop Research Institute, have been investigating exactly how many types of begomovirus can be found in leaves from cotton, okra and other plants in Pakistan. They have now reported a series of molecular biological experiments which identify substantial stretches of DNA-A from each type of CLCuV-PK very precisely, and pay particular attention to regions that seem prone to recombination.

Their results indicated that many plants in Pakistan were infected with more than one begomovirus. In one series of tests they found signs of two, or even three, types in 14 out of 43 plants. This happened in cotton, its relatives, and in totally unrelated plant species like tobacco and bottlegourd. Another test detected recombinant begomovirus sequences in 8 out of 18 plants. When the researchers examined begomoviruses extracted from plants growing outside the region of the cotton leaf curl epidemic, they could detect single infections with viruses that matched parts of CLCuV-PK, but not the whole of it. The relationships between the begomoviruses in Pakistan look like a network maintained by frequent recombination, a much more complex situation than reported in other parts of the world. New variants, like those causing the current epidemic of CLCuV-PK, can emerge and undergo further recombination, generating even more variants. The reasons for these constant genetic changes still await discovery.

**Sanz, A. I., Fraile, A., García-Arenal, F., Zhou, X., Robinson, D.J., Khalid, S., Butt, T. & Harrison, B.D. (2000).** Multiple infection, recombination and genome relationships among begomovirus isolates found in cotton and other plants in Pakistan. *J Gen Virol* **81**, 1839–1849.



## Developmental cheating

You always have the idea that microbes lead a lonely, single-celled life. However, individuals of some species can get together for a very definite purpose. The amoeba-like cells of the eukaryotic slime mould *Dictyostelium* normally live alone, engulfing their bacterial prey. When the supply of bacteria run out, they emit a chemical signal and 100,000 *Dictyostelium* individuals will come together and differentiate into a stalk with a mass of spores on top. Eighty percent of the cells make it into spores. The remainder will die, sacrificing themselves to shoot the spores away from the barren area and into the path of passing insects that will disperse the spores.

This type of mass suicide for the benefit of the species also happens in the myxobacteria, a group of prokaryotic microbes. These hunt as packs, secreting enzymes to digest other bacteria and then absorbing the resulting soup. When they run out of prey, they too aggregate and develop into a fruiting structure that unfortunately probably requires the death of most of the cells.

These microbes may provide insight into the evolution of the multicellular lifestyles adopted by most plants and animals. But they also interest scientists studying parasitism because of an unusual problem. Unlike most animals, the cells that get together are not genetically identical. Although differentiation may be good for the species, it is not necessarily good for each individual. Any *Dictyostelium* that finds itself in a stalk has come to a dead end. As Rich Kessin and his colleagues, Dee N. Dao and Herbert L. Ennis, at Columbia University, USA, point out, over evolutionary time, and given the genetic diversity in the wild, strains (called cheaters) should evolve that refuse to contribute to the stalk of *Dictyostelium* or the fruiting structures of myxobacteria. These strains would, in effect, be parasitic on their own species. The researchers predict that cheater strains of *Dictyostelium* should only form spores, and ignore signals

## Gene silencing

When viruses infect plants, they take over the plant to make large amounts of the virus, rather than the normal components of a plant cell. Not surprisingly, many plants have developed ways to detect this abnormal activity and eliminate it. The plant's defences can act at the most fundamental level of gene activity and specifically destroy any instructions for making viral components as soon as they are synthesized. This process is called post-transcriptional gene silencing (PTGS).

Although the mechanism of PTGS is not fully understood, biotechnologists think it can be exploited to develop new varieties of virus-resistant plants and are exploring ways to do this. Tomato spotted wilt virus (TSWV) and turnip mosaic virus (TuMV) are, despite their names, among the 10 most important viruses that cause disease in vegetables. They both infect a much wider variety of plants than their names imply. Researchers at Cornell University, USA, have been investigating whether transgenic plants can be protected from both these diseases simultaneously by PTGS.

Several research groups have protected plants by adding whole viral genes to them. However, there are concerns that this might produce altered forms of virus. Dennis Gonsalves and his colleagues have now reported that only parts of a viral gene are required, provided they are attached to another gene that triggers PTGS. They have produced tobacco plants containing the gene for a TuMV protein joined onto a small part of a gene from TSWV. Some of these plants were resistant to both viral diseases. Interestingly, other plants were only resistant to TuMV and the researchers hope that these may help them discover which are the most effective gene segments for plant protection.

**Jan, F.-J., Fagoaga, C., Pang, S.-Z. & Gonsalves, D. (2000).** A single chimeric transgene derived from two distinct viruses confers multi-virus resistance in transgenic plants through homology-dependent gene silencing. *J Gen Virol* **81**, 2103–2109.

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## Hawaii 2-0

The native trees of Hawaii have their own unique fungi, found nowhere else in the world. Cletus Kurtzman and his colleagues have now described one of them, the single-celled yeast *Pichia hawaiiensis*. It was found on rotting bark from *Charpentiera* trees in Volcanoes National Park on the island of Hawaii over 20 years ago, but its identity has always been uncertain. The traditional way of identifying yeasts is to test their ability to digest a large number of compounds. This can work very well, but runs into problems in some closely related species.

The American researchers have applied a new molecular biological method to sort out these difficulties. This relies on technical improvements so that large pieces of DNA can now be isolated and sequenced routinely. Earlier research by this group had shown that, in most ascomycete yeasts, one region of the gene for a structural component of ribosomes (26S rDNA) was sufficiently variable to distinguish one from another. When they applied this method to their Hawaiian isolates, it was obvious that they were different from all currently recognized species, although closely related to *Pichia populi* and *Williopsis californica populi*.

**Phaff, H.J., Starmer, W.T. & Kurtzman, C.P. (2000).** *Pichia hawaiiensis* sp. nov., occurring in decaying bark of *Charpentiera* trees in the Hawaiian archipelago. *Int J Syst Eval Microbiol* **50**, 1683–1686.

from other cells to participate in a stalk. They might even actively drive wild-type cells into becoming the stalk. One new difficulty for such strains might be an inability to form a fruiting body without wild-type cells.

But is there any evidence for cheater strains? There are strains of myxobacteria that are very bad at making spores on their own, but are efficient when paired with a normal partner. Myxobacterial spores are sticky and this may help cheaters to attach themselves to an exploitable strain. There is also suggestive evidence from some wild slime moulds, but the Columbia group now have direct evidence in the form of a cheater mutant (*chtA*). They isolated it by selecting cells of *Dictyostelium discoideum* that over many generations always ended up in spores. The *chtA* mutant cannot produce spores unless it is mixed with a few wild-type cells, which always form the stalk. The final signal for spore production comes from the stalk, so the *chtA* mutant relies on exploiting its law-abiding fellows. The mutation in the cheaters is in a protein that probably marks other proteins out for destruction. The targets are not known yet, but might include ones that regulate the final steps in spore production. The group is in hot pursuit of the target proteins.

This ability to make an unfair contribution to the next generation is not confined to microbes. In most animals, all cells apart from the gametes are genetically identical, and the cells that give rise to the gametes are themselves set apart early on in the embryo. Only they have any possibility to acquire an unfair share of the future. Despite this limited potential for cheating, a system called meiotic drive has developed that allows some gametes, particularly in *Drosophila* and in mice, to make unequal contributions to offspring. Cheating must be defeated somehow or else evolution would not occur.

**Dao, D.N., Kessin, R.H. & Ennis, H.L. (2000).** Developmental cheating and the evolutionary biology of *Dictyostelium* and *Myxococcus*. *Microbiology* **146**, 1505–1512.

OPPOSITE PAGE (FAR RIGHT): EPEC interacting with microvillus-like processes on HEp-2 cells. COURTESY ROBERT FITZHENRY, GADI FRANKEL, FABIENNE LAMOUREUX AND ALAN PHILLIPS

THIS PAGE (RIGHT): A scuba diver (Susanne Menger, Max Planck Institute for Marine Microbiology, Bremen, Germany) carrying out fine-scaled *in situ* measurements of temperature and pH at the hot water vent in Palaeochori Bay (Milos, Greece) in 8 m of water. COURTESY WIEBKE ZIEBIS, MAX PLANCK INSTITUTE FOR MARINE MICROBIOLOGY, BREMEN, GERMANY

THIS PAGE (BELOW): Ultrathin section of cells of *Thiocapsa litoralis* BM5<sup>1</sup> from late-exponential growth phase showing a typical four-cell aggregate. The formation of thick cell septa and a mucous capsule keeps the cells together. Cells are approximately 1 µm in diameter. PHOTO L.L. MITYUSHIMA COURTESY JOHANNES F. IMHOFF, INSTITUT FÜR MEERESKUNDE, KIEL, GERMANY

## Microbiology *Pseudomonas* special issue

*Microbiology* is publishing a special issue of the journal in October 2000, part of which will be devoted to pseudomonads. This issue will include peer-reviewed research papers from leading groups.

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## Purple haze

The beaches of the White Sea have shallow lagoons containing mats of microbial communities. Many of the microbes are photosynthetic, even ones that live on the surface of the sulphur-rich mud. They have special pigments to exploit the dim light effectively and use the sulphur compounds in place of water in reactions that convert light into a form of energy that is useful to a living cell. *Thiocapsa litoralis* is a newly discovered example of these bacteria. Its non-motile spherical cells stay together in small flat clusters, called platelets, surrounded by a thick capsule. The cells are pink to rose-red in colour and contain globules of elemental sulphur, formed as intermediates in metabolism.

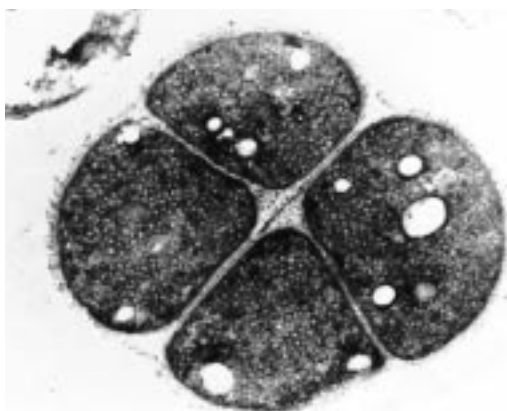
Its identity, like that of all the *Thiocapsa*, is not determined by appearance alone. Another important feature is the sequence of part of a structural component of its ribosomes. These complex cell organelles synthesize proteins and can only tolerate limited alterations without suffering impaired function. By carefully choosing which areas to examine, taxonomists can see evolutionary changes to this molecule that translate



into the differences between species, genera or even whole kingdoms. As part of a collaborative project, researchers at the Institute of Microbiology in Moscow and Institut für Meereskunde at Kiel in Germany studied this feature in a newly isolated strain from the White Sea that looked like a *Thiocapsa*. The study confirmed that it certainly was a *Thiocapsa*, but sufficiently different from all known species of this genus to be a brand new species, *Thiocapsa litoralis*.

**Puchkova, N.N., Imhoff, J.F. & Gorlenko, V.M. (2000).**

*Thiocapsa litoralis* sp. nov., a new purple sulphur bacterium from microbial mats from the White Sea. *Int J Syst Evol Microbiol* **50**, 1441–1447.



## If you can't stand the heat...

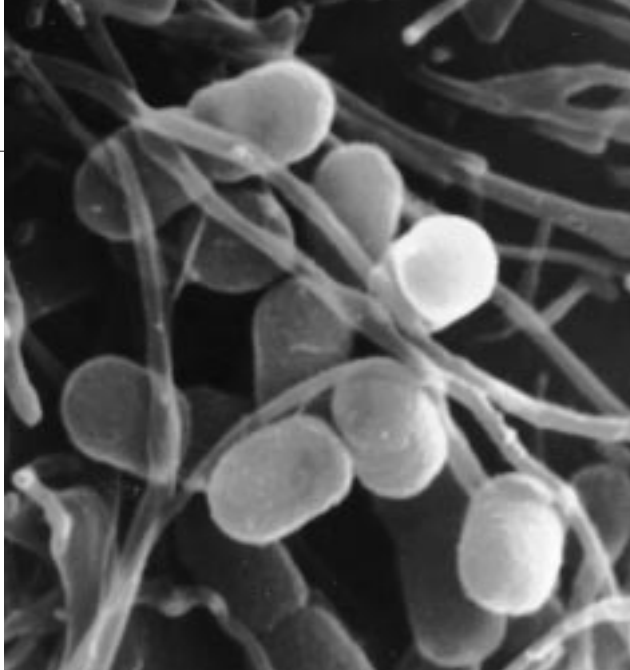
The prokaryotes now known as *Archaea* were originally detected as the only living cells in extreme habitats such as super-heated water or concentrated brine. Taxonomists have now classified prokaryotes into two domains, the *Archaea* and the *Bacteria*. It made a nice story that *Archaea*, which perhaps resembled the first life on Earth, only survived in undesirable places that more advanced organisms had abandoned. However, it is becoming apparent that this is far from the truth. It all depends on how you go looking for signs of life.

Microbiologists have known for a long time that if you search for bacteria by their ability to grow in the laboratory, you will miss ones that do not like the growth conditions on offer. So, they have a number of other ways to detect bacteria. One of these uses rRNA, which is part of the protein synthetic machinery of all cells. Cells need to make many proteins, so each has lots of rRNA molecules. Another useful feature of rRNA is that it contains conserved parts that evolve very slowly over geological time, as well as parts that evolve at a faster rate. Microbiologists can therefore use this information to identify bacteria at species, family and even kingdom level.

A group of researchers at the Max Planck Institute for Marine Microbiology in Bremen, Germany, have now reported their work on the bacteria living around a hydrothermal vent in a sandy bay off the island of Milos in Greece. This vent is another symptom of the geological processes that produce earthquakes and volcanoes in the region. Working in 8 m of water, scuba divers could collect sediment cores and simultaneously take their temperature. Back in Germany, the researchers sliced up the cores and extracted the rRNA. They then measured how much there was from either *Archaea* or *Bacteria* at different temperatures and depths.

The rRNA sampling showed that almost all the prokaryotic cells were in the top 2 cm of sediment, with much more near to the vent than in the cooler regions only 2 m away. The really surprising result was the proportion of *Archaea*. These were always a minor part of the microbial community, reaching only 11.9% in sediments at 82 °C. This matches some earlier reports from deep-sea vents and terrestrial hot springs, but is the first from a systematic study of the microbes living along a thermal gradient. However, the factors that allow *Bacteria* to dominate in a high temperature environment that was once believed to be the realm of *Archaea* remain elusive.

**Sievert, S.M., Ziebis, W., Kuever, J. & Sahn, K. (2000).** Relative abundance of *Archaea* and *Bacteria* along a thermal gradient of a shallow-water hydrothermal vent quantified by rRNA slot-blot hybridization. *Microbiology* **146**, 1287–1293.



## Bridging the gap

Diarrhoea is unpleasant at any age but strains of *Escherichia coli* called EPEC are a common cause of life-threatening diarrhoea in infants in the developing world. EPEC change the surface of intestinal cells. The microvilli, which normally absorb nutrients from food, are destroyed and bacteria become cradled in cup-shaped protrusions of the cell membrane. The EPEC strains force these changes onto the intestinal cells via the proteins they secrete. One of these, Tir, ends up in the intestinal cell membrane and another, intimin, can attach to Tir. EspA forms a structure on the surface of the EPEC cells that is needed to secrete EspB and Tir, as well as bridging the gap between the bacterial and intestinal cells. The importance of each of these, or other, proteins in the disease symptoms and exactly what they do are still unclear.

However, a collaborative project between Alan Phillips at the Royal Free Hospital in London and Jorgé Girón at Benemérita Universidad Autónoma de Puebla in Mexico, along with Gadi Frankel at Imperial College in London, have now pinned down a second role for intimin. They compared classic EPEC strains with others that lacked the ability to produce either intimin, Tir, EspA or EspB. The researchers grew layers of cells in culture and then put the bacterial cells onto them and took photographs over the next few hours with an electron microscope. Within an hour of adding EPEC bacteria, microvillus-like processes (MLP) had appeared as tubes on the surface of infected cells that grew to enmesh and anchor the bacteria. After another hour the MLP had degenerated to leave typical localized adhering clusters surrounded by cell debris. These had completely covered the cell surface after 6 hours. The scientists obtained similar results when they used their bacterial strains with samples of real intestine that had been removed from children, after the consent of parents and an ethical committee.

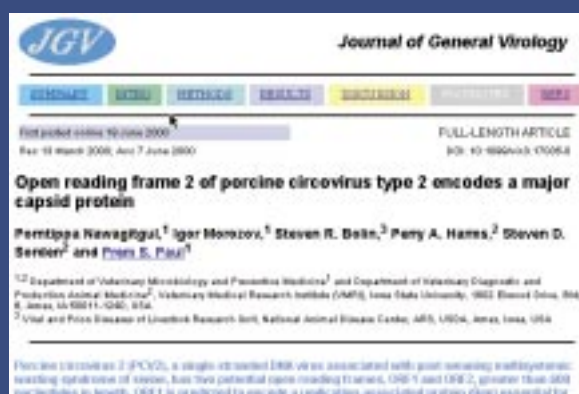
When they repeated their experiments with the strains that lacked individual proteins, one role of intimin became obvious. It was essential to trigger MLP production and elongation. Without intimin, the MLP were few and short and very few bacteria became attached to the cell layer. With it, MLP would even grow to envelop intimin-coated latex beads. This suggests that although Tir is one attachment point, there must be at least one other that is a normal part of intestinal cells. Additionally, proteins like Tir and EspB may be required for MLP degeneration, since much larger, cage-like structures appeared on cultured cells that were incubated with bacterial strains lacking them.

**Phillips, A.D., Girón, J., Hicks, S., Dougan, G. & Frankel, G. (2000).** Intimin from enteropathogenic *Escherichia coli* mediates remodelling of the eukaryotic cell surface. *Microbiology* **146**, 1333–1344.

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## Antifungal toxin from fishery waste bacteria

The newest member of the genus *Paenibacillus* can produce an antifungal compound, the first member of its genus found to do so. Researchers in Korea isolated it among the bacteria that emerged from rice husk and fishery waste. They spread the diluted waste over laboratory media, with chitin as the sole source of nutrition. The reason for this restricted diet was that chitin is a major part of the cell wall of many fungi. Anything that could live on it alone might also be able to destroy fungi, and the bacteria were tested for this ability. One isolate was particularly impressive at inhibiting fungal growth, and it has turned out to be a *Paenibacillus*. Members of

this genus were already known for their production of enzymes that degrade natural polymers as well as antibacterial compounds, but none has shown antagonism to fungi before.

*Paenibacillus* have large rod-shaped cells that can sometimes switch to growth without any need for oxygen. Like all members of the family *Bacillaceae*, the cells can turn into survival structures called endospores in adverse environmental conditions. The distinguishing feature of *Paenibacillus* is the size and sequence of one region of its 16S rRNA molecule. When the researchers checked out their new strain, although it was definitely a member of *Paenibacillus*, it

was significantly different from the 24 other species. As well as differences in rRNA sequence, it had rather different nutritional requirements. The most interesting feature, however, was its ability to synthesize an antifungal toxin. This chemical was a small cyclic peptide from the iturin class. This means that *Paenibacillus koreensis*, as the new isolate has now been called, poisons fungi as well as taking their cell walls apart.

**Chung, Y.R., Kim, C.H., Hwang, I. & Chun, J. (2000).** *Paenibacillus koreensis* sp. nov., a new species that produces an iturin-like antifungal compound. *Int J Syst Evol Microbiol* **50**, 1495–1500.