

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.

Rapid identification of TB

Tuberculosis is an increasing public health problem in many countries. The World Health Organization estimates that there are 20 million cases of TB worldwide, with 8 million new cases and 3 million deaths each year. The speedy identification of patients is essential for TB control. Kits can identify the presence of DNA from the *Mycobacterium tuberculosis* complex (MTBC), but growth of cells in culture is still needed both to confirm the presence of a live infection and to test the antibiotic susceptibility of the strain. Considerable effort is therefore going into developing systems with improved sensitivity for dividing *M. tuberculosis* cells. The BACTEC 460 TB system from Becton Dickinson Ltd takes 4–8 days to give a result, requiring the BACTEC NAP enzyme assay and a DNA test for confirmation. Researchers at the Central Tuberculosis Laboratory of Singapore General Hospital have been comparing it with the BD ProbeTec ET system from the same company. This system can give a result in a day, and the researchers wanted to know if it matched the BACTEC NAP system for sensitivity and specificity.

A total of 145 clinical specimens were used, and these were obtained from fluids such as blood, pus and urine, as well as from patients' lungs and other tissues. Conventional procedures were used to select for MTBC organisms, ending up with a BACTEC 12B culture vial. The researchers then used the manufacturer's recommended methods to test for MTBC using both the BACTEC NAP and BD ProbeTec ET systems. The test systems were capable of detecting the presence of MTBC and determining whether the organism was actually a closely related species incapable of causing TB.

The researchers worked out that 89 of the specimens contained MTBC, while the other 56 contained other *Mycobacterium* species. The BD ProbeTec ET system correctly identified 87 out of the 89 MTBC isolates, and all of the others. Three of the non-tuberculous mycobacteria were initially mis-identified by BACTEC NAP, but came up correctly when the researchers altered the growth conditions slightly. It was concluded that the BD ProbeTec ET system is reliable for identification of MTBC isolates, and that its speed, and the fact that the test reagents can be stored at room temperature, offers distinct advantages.

Wang, S. X., Sng, L. H. & Tay, L. (2004). Preliminary study on rapid identification of *Mycobacterium tuberculosis* complex isolates by the BD ProbeTec ET system. *J. Med. Microbiol.* 53, 57–59.

The importance of biofilms in plague

It would be good to know exactly what makes some bacteria into pathogens, and which features of their host they exploit. That would help with the design of new medical treatments as well as strategies to prevent infections. However, many experiments infecting mammalian cell cultures or animals with bacteria would be needed to find out this information. Apart from ethical and financial reasons for wanting to minimize the number of these experiments, it would be easier to understand the outcome of them in genetically simpler systems. Scientists are therefore investigating alternatives, and researchers at the London School of Hygiene and Tropical Medicine have been seeing what can be learnt from infecting nematode worms with plague bacteria.

Researchers have studied the 1 mm long nematode worm *Caenorhabditis elegans* in incredible detail. They know the fate of each of its 1,030 cells and have an extraordinary collection of mutants. It was the first animal to have its entire genome sequenced. As a consequence, the researchers can test strains of worms with traits that might be important in allowing or preventing infections.

The bacterial genus *Yersinia* contains a number of species. The most notorious is *Y. pestis*, which causes bubonic and pneumonic plague. Other species, such as *Y. pseudotuberculosis*, cause very unpleasant food-borne diseases of the digestive tract. Some scientists think that *Y. pestis* evolved from *Y. pseudotuberculosis* between 1,500 and 20,000 years ago. All the tools of molecular biology can be applied to these bacteria, as well as collections of isolates from many parts of the world.

The researchers already knew that some strains of *Yersinia* could infect the nematode by forming a layer of bacteria over its head, preventing it from feeding. This layer is called a biofilm, and occurs in several bacterial diseases. The researchers therefore focused on trying to understand what genes in the bacteria were essential for forming a biofilm on nematodes. They tested 41 strains of *Y. pseudotuberculosis*, and discovered that most strains could not infect the nematodes. There was also no obvious similarity among the six bacterial strains that caused a severe infection by growing into a biofilm over the front half of the worm and preventing it moving. The researchers tested whether strains that could form a biofilm on an inanimate polystyrene surface were any better as pathogens, and there was again no obvious relationship. In contrast, all three of the *Y. pestis* strains included in the tests caused severe infections of the nematodes. However, the researchers were able to identify several genes within the nematodes that help them resist the formation of bacterial biofilms and are now investigating the exact role of these genes.

Joshua, G. W. P., Karlyshev, A. V., Smith, M. P., Isherwood, K. E., Titball, R. W. & Wren, B. W. (2003). A *Caenorhabditis elegans* model of *Yersinia* infection: biofilm formation on a biotic surface. *Microbiology* 149, 3221–3229.

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Further evidence for simian origin of HIV

The origin of the AIDS epidemic in humans is likely to have started in the first half of the twentieth century by transmission of an immunodeficiency virus from African non-human primates to humans. Researchers have identified many simian immunodeficiency virus (SIV) viruses in monkeys and apes, confirming that these infections are more common than was thought a few years ago. There is a lot of variation among the viruses, adding weight to the idea that they have an ancient relationship with African non-human primates. It is commonly accepted that a SIV called SIVcpz, which has a low prevalence in wild chimpanzees, is the ancestor of HIV-1 while a second virus from sooty mangabeys has evolved into HIV-2. These viruses often cause no detectable illness in their animal hosts but may pose a real risk of providing further immunodeficiency viruses to infect members of the human population, who encounter the animals as pets or bushmeat. Researchers are therefore interested to know how many other immunodeficiency viruses occur naturally in African monkeys and great apes.

While researchers in the Netherlands were screening serum samples from various non-human primates for antibodies against SIV, they spotted an unusual result in a sample from a local zoo taken from a Schmidt's guenon, a subspecies of the red-tailed guenon. The results of further tests indicated that that this was a distinct variant of SIV, which the researchers called SIVschm. The most closely related virus to it, SIVgsn, had been isolated from greater spot-nosed monkeys, but the two were not particularly similar. However, the most exciting feature was that the genome of SIVgsn contains a gene that was thought to be unique to HIV and the SIV viruses from chimpanzees. The identification of another virus with this gene provides more information about the origin of the human immunodeficiency virus HIV-1.

Verschoor, E. J., Fagrouch, Z., Bontjer, I., Niphuis, H. & Heeney, J. L. (2004). A novel simian immunodeficiency virus isolated from a Schmidt's guenon (*Cercopithecus ascanius schmidti*). *J. Gen. Virol.* 85, 21–24.

The 'crypton' factor

Transposable elements (TEs) are an important part of most genomes. For example, almost half the human genome consists of various types of TEs. They are regions of DNA that once had, and sometimes still have, the ability to move around the genome. Researchers are still learning about this type of mobile DNA, particularly how and why it moves. Tim Goodwin and his colleagues Margaret Butler and Russell Poulter at the University of Otago in New Zealand have recently discovered a new type of TE that they have named crypton. They had detected an enzyme in higher organisms that carries out the essential step of re-integrating mobile DNA into the genome, and was already known in bacteria. The Ngaro 1 and DIRS1 groups of retrotransposons both contain the gene, and other researchers have identified it in the ciliate *Euplotes crassus*. The researchers' recent studies have now revealed more about TEs with this type of tyrosine recombinase enzyme.

The researchers have been studying pathogenic fungi such as *Coccidioides posadasii*, *Cryptococcus neoformans* and *Histoplasma capsulatum* that can infect the respiratory tract and also act as life-threatening opportunistic pathogens, especially to immunocompromised individuals. The genomes of these fungi have been sequenced, and the researchers searched for matches to the characteristics of cryptons. The fungi contained several copies, with the number differing between strains of

the same species. The usual explanation for this is that the TE was active comparatively recently. As the researchers discovered more about cryptons and their tyrosine recombinase genes, they became convinced that these were part of a new and very different sort of transposable element.

One unusual feature in *C. neoformans* was that the gene for the tyrosine recombinase contained introns. These regions of the DNA sequence are removed as the cell gets the gene transcript ready for translation into a protein. Introns are present in eukaryotic genes, but are rarely found in bacterial ones. The fungi in which the researchers detected cryptons belong to different major divisions that have evolved separately for over 400 million years. All the evidence indicates that cryptons existed prior to this separation.

The genome of two strains of *H. capsulatum* has been sequenced. The researchers detected 35–40 cryptons in one, and about 10 in the other, all in different locations. This suggests that they have moved around the genome since these two strains diverged from their common ancestor. Movement of a TE can be bad news, since if it lands inside a gene it will affect its normal function. Some fungi have a system that puts mutations into any DNA sequence that appears multiple times in a genome, since such sequences would not be normal genes and mutations should inactivate them. The researchers spotted

evidence that some fungi have been trying to stop the cryptons moving. There were many mutations within the *H. capsulatum* and *C. posadasii* cryptons, typical of this defence process.

The researchers also found a tantalizing suggestion of what else an organism might do to a crypton when they looked at the genome of *Candida albicans*, the fungus that causes thrush. There was the sequence for a protein that looked as if it started as a tyrosine recombinase, but had now developed into something else. *C. albicans* might have managed to exploit the crypton for its own ends. This new class of TE, as well as giving an insight into the way that DNA can recombine, may also provide ideas about evolution.

Goodwin, T. J. D., Butler, M. I. & Poulter, R. T. M. (2003). Cryptons: a group of tyrosine-recombinase-encoding DNA transposons from pathogenic fungi. *Microbiology* 149, 3099–3109.

RIGHT:
Field-emission environmental scanning electron micrograph of *Bacillus odyseeyi* spores.
COURTESY JAMES KULLECK,
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Is there life on Mars?

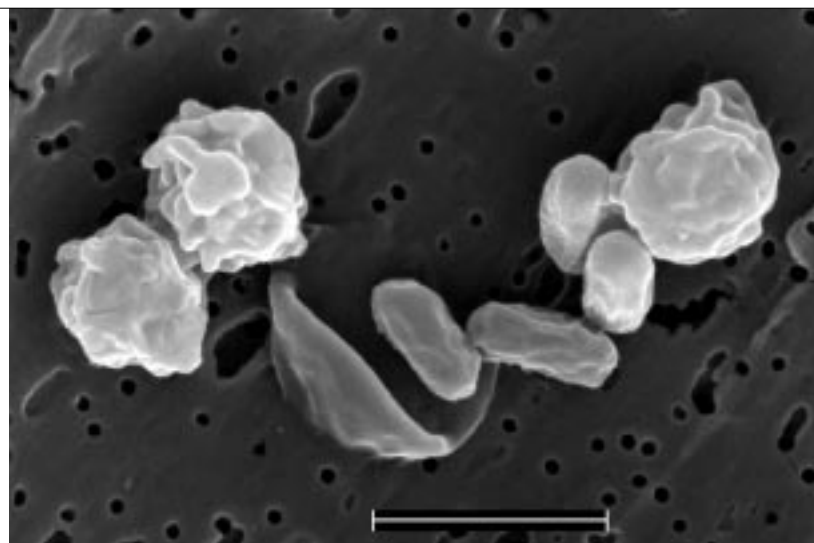
Scientists, and science fiction writers, have speculated about this for centuries. It would be unfortunate if any of the unmanned space probes

sent to the red planet were accidentally accompanied by life from Earth. Spacecraft are therefore cleaned of microbial contamination while they are being prepared for launch. Myron La Duc and his colleagues, Masataka Satomi and Kasthuri Venkateswaran, working at the Spacecraft Assembly and Encapsulation Facility II in the Kennedy Space Center in the USA, have recently reported that a very small number of organisms were left on the surface of the Mars Odyssey spacecraft as it was readied for launch in April 2001. In February they counted around 30 organisms per 25 cm², far below the number on most terrestrial surfaces. Standard tests showed that most were bacteria belonging to many different genera, including *Acinetobacter*, *Curtobacterium*, *Ralstonia* and *Bacillus*, but there was also one species of fungus, *Aureobasidium pullulans*.

The researchers focused on the *Bacillus* isolates because this genus is well known for producing spores that are very resistant to destruction. One strain had unusual and distinctive spherical spores composed of a series of layers around a core. The outermost rather loose layer might have been responsible for adhering efficiently to the spacecraft surfaces. A series of biochemical tests, and examination of a region of a gene that is characteristic in many bacterial species, indicated a close relationship with several *Bacillus* species, but no exact match. Therefore, the researchers became convinced they had a new species, which they named *Bacillus odyseeyi*, after the spacecraft.

Mars Odyssey has been orbiting Mars since October 2001, with no intention of landing. The big question is whether *B. odyseeyi*, or other microbes that have resisted all human attempts to remove them, could survive the highly oxidative UV and gamma radiation-rich environments they would encounter in space and the surface of Mars. If any did, this could be a problem for assuring that any apparently extraterrestrial life is truly alien. The researchers therefore tested how well the spores resisted the lethal effects of hydrogen peroxide, UV light, desiccation and gamma radiation from a radioactive cobalt source. Although all, except desiccation, killed many of the spores, a surprisingly large number survived. Compared with a standard reference *Bacillus* strain, the spores of *B. odyseeyi* survived between 3 and 10 times better. Whether this would be sufficient to survive a trip to Mars, only more experiments will tell.

La Duc, M.T., Satomi, M. & Venkateswaran, K. (2004). *Bacillus odyseeyi* sp. nov., a round-spore-forming bacillus isolated from the Mars Odyssey spacecraft. *Int J Syst Evol Microbiol* 54, 195–201.



Animal origins of human T cell leukaemia virus

Primate T lymphotropic virus type I virus causes a very aggressive form of leukaemia or lymphoma. It includes different strains that affect either humans and/or non-human primates (monkeys and apes) of the old-world. The strains of this virus that infect people, human T cell leukaemia virus type I (HTLV-I), show remarkable genetic stability. There are four major geographic subtypes and researchers have strongly suggested that some of them originated when the virus was transmitted from monkeys or apes to humans. The evidence comes especially from identifying African strains of simian T cell leukaemia virus type I (STLV-I) in wild-caught chimpanzees and mandrills that are similar to some types of HTLV-I that infect humans. However, most strains of STLV-I have been isolated from captive animals in Europe, North America and Asia, making both the origin of their viral infections, and the relationship with HTLV-I, less easy to ascertain.

A collaboration between researchers at the Centre Pasteur in Yaoundé in Cameroon, and colleagues at the Institut Pasteur in Paris has now surveyed over 61 wild-caught gorillas and chimpanzees in Cameroon for the virus. Most of the animals had been kept as pets after hunters had killed their mothers, and any infections were probably transmitted from the animals' mothers. The animals had either been confiscated by the Ministry of Environment and Forestry, or taken directly to a zoo or animal sanctuary. The researchers tested for antibodies characteristic of virus infection and found signs in two animals, a young female gorilla and chimpanzee. To find out how similar these viruses were to other strains of HTLV-I and STLV-I, the researchers sequenced two fragments of the genome; the complete long terminal repeat, which is quite variable, and the gp21 *env* gene. The virus infecting the chimpanzee turned out to be more similar to HTLV-I than any isolate STLV-I from other chimpanzees. Both viral isolates matched the B subgroup of HTLV-I, most isolates of which come from humans in central Africa.

The researchers point out that only a proper survey of primates in the wild, examining, for example, the viral content of faeces, will reveal the true prevalence, geographic and subspecies distribution of the STLV-I viruses. However, the close relationship between the STLV-1 isolates identified in this study, and the HTLV-I strains characteristic of infections of the human inhabitants of the same region, reinforces the idea that STLV-I has been transmitted from animals to humans.

Nerrienet, E., Meertens, L., Kfutwah, A., Foupouapouognigni, Y., Ayoub, A. & Gessain, A. (2004). Simian T cell leukaemia virus type I subtype B in a wild-caught gorilla (*Gorilla gorilla gorilla*) and chimpanzee (*Pan troglodytes vellerosus*) from Cameroon. *J Gen Virol* 85, 25–29.

Benefits of retrovirus infection?

Retroviruses have the unique ability to integrate their genome into the DNA of the host cells. As a consequence, any host cells that survive a retroviral attack can contain viral genes. It is therefore not entirely surprising that projects to sequence genomes, like the Human Genome Project, have found retroviruses, although the amount has been surprising. There are estimates that 8% of each human's DNA consists of retroviral genes. As a consequence, scientists wonder if these so-called endogenous retroviruses (ERVs) actually confer a benefit on their hosts.

Ideas that ERVs could protect their host from infection by exogenous (e.g. horizontally transmitted) retroviruses have developed, and also a hypothesis that ERVs are essential in the development and function of the placenta. Researchers in the 1990s realized that two ERVs were always switched on in the human placenta during pregnancy. Proteins produced by one bore a remarkable similarity to proteins that could suppress the immune response, while the other affected cell shape. Could it really be possible that remains of an ancient retrovirus help the foetus invade its mother's tissues and fend off her immune response?

Massimo Palmarini and his colleagues in the USA have been studying a retroviral disease transmitted from sheep to sheep, and come up with more facts to add to this debate. Jaagsiekte sheep retrovirus (JSRV) causes a major infectious disease resulting in lung cancer. However, every sheep already has about 20 copies of a very similar retrovirus (enJSRV) nestled among their genes. The researchers discovered they are switched on in several tissues and that at least one had all the instructions to make virus particles, but with two very small changes. These differences made laboratory cultures of cells expressing a particular enJSRV less susceptible to release JSRV viral particles. This could be a good example of an ERV providing protection from a viral disease, but more experiments convinced the researchers that enJSRV may also be important in sheep reproduction.

During the second week after fertilization, the outermost layers of an embryonic sheep attach to the endometrium lining the uterus of its mother. The next step starts the development of the placenta, which will nourish the embryo, remove its waste products, defend it from its mother's immune system and provide it with oxygen until it is time to be born. The complexity of these roles, and its necessity for successful reproduction, is another of the puzzles of evolution. How can adaptation allow the evolution of an organ with so many complicated and essential functions? A closer look at placental development and enJSRV is giving researchers hints towards an answer.

During embryo implantation, unusual multinucleated cells are formed in the placenta. Once formed, these multinucleated trophoblast cells expand and invade the mother's endometrium to get closer to blood vessels. In the endometrium of the uterus, enJSRVs respond to levels of the pregnancy hormone progesterone and are particularly abundant in the endometrium during the time when an embryo begins to implant. Further, the enJSRVs are specifically expressed in the multinucleated trophoblast cells of the placenta. The researchers felt that the fact that they could see enJSRV proteins on the surface of the placenta at this key moment in pregnancy could not be a coincidence. They wondered if an interaction between the enJSRV proteins on the mother's endometrium and other proteins on the embryo helped implantation and formation of the placenta. After all, the invasive behaviour at the start of placental development was reminiscent of some cancerous cells, and wild JSRV causes cancer.

An organ to act as a placenta has evolved repeatedly, in fish, reptiles and amphibians, as well as mammals. Even the structure of placentas within the mammals varies widely, indicating that it has evolved several times. enJSRVs are present in the genomes of sheep and goats, and two of them look as if they were present before these groups diverged approximately 4–10 million years ago. There are even vague similarities in the genomes of cattle that diverged 18 or 19 million years ago. The complexity of the placenta has also changed, supporting the idea that enJSRVs assist in creating the elaborate invasive tissues of the embryo. Further research could not only indicate whether a virus really has an essential role in mammalian reproduction, but would also provide greater understanding of how retroviruses and immune tolerance works.

Palmarini, M., Mura, M. & Spencer, T. E. (2004). Endogenous betaretroviruses of sheep: teaching new lessons in retroviral interference and adaptation. *J Gen Virol* 85, 1–13.

JMM Special Issue

Spotlight on colonic spirochaetes of medical and veterinary significance

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Anaerobic spirochaetes of the genus *Brachyspira* are of considerable veterinary significance, causing dysentery and diarrhoea in a number of animal species. In recent years, the potential role of some *Brachyspira* species as intestinal pathogens in humans has been brought under the spotlight. Selected scientific papers presented at the *Second International Conference on Colonic Spirochaetal Infections in Animals and Humans*, held in Edinburgh, UK, on 2–4 April 2003, will be brought together in a special focus issue of the journal. The issue is intended to give a broad understanding of current research and thinking about these bacteria and their potential to cause disease in animals and man.