Hot off the Press

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.

AIDS – does HIV-2 hold the key to understanding HIV-1?

The AIDS pandemic continues to spread unchecked in most of the world, with more than 34 million people currently infected with the human immunodeficiency virus (HIV). Most have HIV type 1, but a second closely related virus, HIV type 2 (HIV-2), is responsible for a significant minority of infections. Jacqueline Reeves and Robert Doms from the University of Pennsylvania, USA, have been reviewing the state of knowledge about this second virus, because it is similar but less pathogenic and may give important ideas about how to combat HIV-1. People infected with HIV-2 can live for many years without symptoms, frequently more than a decade, and with few viruses in their blood. It is transmitted much less effectively than HIV-1, and the mortality from HIV-2 is two-thirds lower. However, if the infection does finally progress to AIDS, both the symptoms and outcome are very similar. The reviewers have tried to pick out the differences between the two viruses that make HIV-2 endemic with a stable prevalence rate in most countries, while HIV-1 is pandemic, with increasing prevalence in developing countries.

HIV-2 originated in sooty mangabey monkeys, but on seven independent occasions it was transmitted to humans in West Africa. Guinea-Bissau, The Gambia, Senegal and Guinea are its strongholds, but it is also found in countries with links to the colonial power Portugal, including southwest India, Angola, Mozambique and Brazil. Even so, it normally only accounts for a few percent of HIV infections.

The reasons for HIV-2's lower pathogenicity are probably related to two factors. First, people infected with this virus maintain an immune response that both limits the amount of new virus that is produced by their cells, and continues to protect against other pathogens. Laboratory tests of cells and fluid taken from people with HIV-2 shows some ability to destroy the HIV-1 virus, although whether this can happen *in vivo* to people who are unfortunate enough to contract a second infection is controversial. The body may be able to detect and counteract some HIV-2 strains more easily because many have errors in a gene that provides a facility to evade the immune system.

The second feature of HIV-2 that is very different from HIV-1 is the way it enters cells. Both viruses have sugar-coated envelope proteins that protrude from their surface and latch onto human cells. The protein then changes shape and attaches to a second co-receptor to trigger a series of events that end up with the virus and human cell fusing, so that the virus can deliver its lethal genetic package. HIV-1 picks out a particular feature on the cell surface, called the CD4 receptor, and invariably uses one of two co-receptors. HIV-2 also uses the CD4 receptor, but can also enter cells that lack it, and exploits a very large number of co-receptors. The source of these differences are a few subtle changes in the structure of the viral envelope protein, with the consequence of making the virus more ready to fuse with human cells. CD4-independent strains are more sensitive to the immune system, perhaps due to these slight changes. There may be something in this that can be used to develop a vaccine to HIV-2, and perhaps point out ideas towards a vaccine against HIV-1.

Reeves, J.D. & Doms, R.W. (2002). Human immunodeficiency virus type 2. *J Gen Virology* 83, 1253–1265.

Novel *Rhodococcus* species from medieval Czech grave

The Margrave Jošt Lucemburský was an important Czech ruler who reigned over Moravia at the turn of the 14th century. Among other things, in March 1393 he gave the Mayor of Brno and the town councillors and officials the privilege of serving high quality imported wine and mature Schweinditz beer. He was buried in St Thomas' church in Brno, and his grave was opened in 1999 by the Brno City Museum for archaeological and anthropological research. Researchers from the Czech Republic and other countries have been studying the micro-organisms that they found in the grave of this early appreciator of fermentation technology.

These included three colonies of pink-pigmented bacteria from a femur. The bacteria had the typical appearance of members of the genus *Rhodococcus*. When the researchers compared the sequence of the 16S rRNA gene, which is frequently used to characterize bacterial species, with that of representative members of the genus, it turned out to be distinctly different. Together with some physiological characteristics, these were sufficient to indicate that the bacteria belonged to a new species, which was named *R. jostii* in honour of the Margrave on whom it was found.

Takeuchi, M., Hatano, K., Sedlacek, I. & Pacova, Z. (2002). *Rhodococcus jostii* sp. nov., isolated from a medieval grave. *Int J Syst Evol Microbiol* 52, 409–413.

OPPOSITE PAGE:
St Thomas' Church, Brno, Czech Republic, in which Jošt Lucemberský, a 14th century Margrave (see inset), is buried. A new species of *Rhodococcus*, *R. jostii*, has been isolated from his recently excavated grave.
COURTESY LYDIE ZUKALOVA, FOREIGN RELATIONS DEPARTMENT, BRNO (WWW.BRNO.CZ)

RIGHT:
A sooty mangabey monkey, the species in which HIV-2 originated.
PHOTO MARK MURCHISON, KATHERINE PHILLIPPI-FALKENSTEIN AND RON VEAZEY, TULANE NATIONAL PRIMATE RESEARCH CENTER, LOS ANGELES, USA

Charcoal keeps coils in culture

Helicobacter pylori is the cause of chronic gastritis and peptic ulcers and is associated with the development of gastric cancer in humans. Members of this genus have also been found causing gastrointestinal disorders in animals as diverse as dogs, mice, poultry and cheetahs. The bacteria have very idiosyncratic requirements for growth outside their host and are probably often missed. In particular, the bacteria sometimes travel to infect the liver and bile tree and are then especially difficult to culture. Yet another problem is that the bacterial cells change shape from spiral to spherical when they are grown in the laboratory. Regardless of the reason for this change, media that maintain the bacteria in their normal spiral shape are preferable.

Researchers at the University of Lund in Sweden, working with colleagues at the National University of Ireland, Galway, have been investigating exactly which medium is best for five *Helicobacter* species, both to permit them to grow at all, and also to keep their spiral morphology. They tested both liquid and solid media, and also the effect of adding compounds like activated charcoal, porcine gastric mucin and β-cyclodextrin. Activated charcoal stimulated growth best, even though it has no nutritional value. Toxic compounds, such as hydrogen peroxide, become adsorbed on the charcoal, so this points firmly at a sensitivity to toxic compounds in *Helicobacter* species.

Activated charcoal and β-cyclodextrin also slowed down the change from a spiral to a spherical shape. One hypothesis for this change is that the bacteria are responding to stress, which matches with the fact that both these compounds can hold toxins away from the bacteria. These experiments indicate that adding charcoal to growth media could help diagnostic laboratories grow *Helicobacter* species, and also maintain them in their normal morphology for longer to aid identification.

Taneera, J., Moran, A.P., Hynes, S.O., Nilsson, H.-O., Al-Soud, W. & Wadström, T. (2002). Influence of activated charcoal, porcine gastric mucin and β-cyclodextrin on the morphology and growth of intestinal and gastric *Helicobacter* spp. *Microbiology* 148, 677 – 684.

Prion-like proteins in *Saccharomyces* and *Candida*

Prions have changed from one of the most obscure topics in biology to being well known to the public as well as scientists because of BSE. This disease of cattle is the consequence of a single protein changing its shape and then forming clumps within the brain that are ultimately fatal. The way that the disease is transmitted, through contact with the mis-shaped protein, is one of its most surprising aspects, and has made researchers look again at several other conditions that are inherited in strange ways. Some of these have also turned out to be caused by proteins that can act as prions.

One of these is [*PSI*$^+$], characteristic of the yeast *Saccharomyces cerevisiae*. Sup35p is the name of the protein that causes the problem. After changing shape, it becomes deposited within the cell, rather than carrying out its normal function. It is required as part of the essential cellular machinery for synthesizing proteins, and a similar protein is needed by all living cells, including mammalian ones. Researchers at the Universities of Kent in the UK and Lisbon in Portugal have been investigating why the *S. cerevisiae* protein can transform into a prion, while others do not. For example, the Sup35p of another yeast, *Candida albicans*, has never been detected in aggregates like a prion, even when it is mixed with the prion form of Sup35p from *S. cerevisiae*.

The Sup35p protein contains two distinct regions. One of them, the C domain, is essential for its normal function and is similar in all known Sup35p proteins. The other region, called the N domain, is much more variable, and the conversion into prion aggregates in *S. cerevisiae* cannot happen without it. There are five stretches of repeated amino acids within the N domain, and removing even one of them prevents the prion-like behaviour. When the researchers investigated Sup35p from *C. albicans*, they were surprised that its N domain had many of the same features, including the repeated regions of amino acids. There are a large number of proteins that have runs of one amino acid, glutamine, and several dozen of these polyglutamine tracts have been found in *C. albicans* alone. What is unusual is that the lengths of the tracts, i.e. the numbers of glutamines, vary between different *Candida* strains. It is also know that in human diseases, like Huntington's disease, for which proteins containing polyglutamine tracts have been implicated, the severity of the disease increases as the length of these tracts increases in a particular protein. The question is, therefore, what is the significance of these strain-specific variations in polyglutamine tract lengths to the function of Sup35p and / or to *Candida* itself.

The researchers checked that the *C. albicans* protein really had the same function as the one from *S. cerevisiae* by deliberately disrupting the gene for Sup35p in some *S. cerevisiae* cells, and then providing them with the one from *C. albicans*. The *S. cerevisiae* cells could use this gene to make the essential protein, although they needed more of it than of their own protein, implying that it did not work as efficiently. It also could not be induced to change into a prion-like form by Sup35p aggregates within the *S. cerevisiae* cell.

The question still remains of why these similar proteins have very different abilities to undergo the shape-change and aggregation that are characteristic of prions. From their experiments, combined with information from other research groups, the researchers think that the subtle control that cells exert over the ways that proteins fold may be important.

Resende, C., Parham, S.N., Tinsley, C., Ferreira, P., Duarte, J.A.B. & Tuite, M.F. (2002). The *Candida albicans* Sup35p protein (CaSup35p): function, prion-like behaviour and an associated polyglutamine length polymorphism. *Microbiology* 148, 1049–1060.

TOP RIGHT
The badger (*Meles meles*), the animal at the centre of the bovine TB controversy in the UK.
PHOTO ALEX TILL, CORNWALL COLLEGE, CAMBORNE

LOWER RIGHT:
Electron micrograph of badger herpesvirus showing full and empty capsids.
PHOTO EM UNIT, VETERINARY LABORATORIES AGENCY, NEW HAW

OPPOSITE PAGE:
The Grand Canal, Venice, from where *Ilyobacter inseutus* was isolated.
PHOTO JANET HURST, SGM

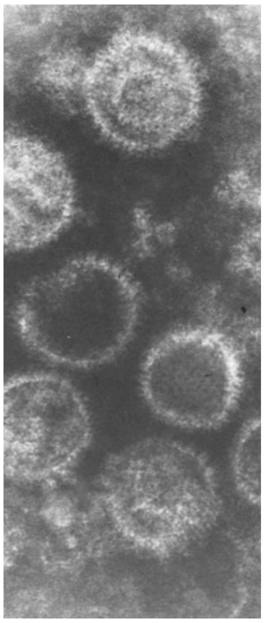
Something in the air

Amazing things can turn up in the air. Researchers in Germany have found a new species of bacteria floating around a composting facility in Kassel, Niederzwehren. The bacteria were trapped on a filter and then cultivated on conventional microbiological growth media. Some were readily identified, but one was more elusive. Peter Kämpfer and his colleagues pinned it down to the genus *Nocardiopsis*, bacteria where the cells are arranged as easily fragmented filaments. However, working out which of the 10 species of *Nocardiopsis* they had caught was more difficult.

These species are distinguished by the fats found in their cells, the composition of their cell walls and by the sequence of one of their genes, that for 16S rRNA. Compared with the others, this isolate had some distinct differences. It contained different proportions of some fats, was able to make use of a different range of growth substrates and also had differences in the sequence of the 16S rRNA gene. As a consequence, the researchers are confident that they have uncovered a new species. Given its source, *N. compostus* seems an entirely appropriate name.

Kämpfer, P., Busse, H.-J. & Rainey, F.A. (2002). *Nocardiopsis compostus* sp.nov., from the atmosphere of a composting facility. *Int J Syst Evol Microbiol* 52, 621–627.

Novel virus may hamper use of a TB vaccine for badgers



The role of badgers in transmitting tuberculosis to cattle in the UK is controversial. One solution that researchers at the Veterinary Laboratories Agency have been working towards is to develop a TB vaccine for badgers, so preventing them from passing on the disease. As part of these experiments, they have recently discovered a new virus which may affect the health of badgers. They were examining the emaciated corpse of a young adult female badger. The researchers managed to grow some of the badger's lung-cells in artificial culture, and were surprised by the presence of dead cells containing small symmetrical particles that looked like herpesviruses. Their analysis of several genes from the virus indicated that it was a new member of the gammaherpesviruses. This type of virus had never been found in a badger before. Closely related viruses can sometimes cause cancer or suppress the immune system, so the researchers wanted to know more about it.

The researchers attempts to get the virus to infect cultures of other animal cells were unsuccessful, except for mink cells. Since mink are closely related to badgers, this indicated that the virus is very specific for its host. One thing that they do not know yet, and are anxious to discover, is how commonly badgers are infected with this virus. It may well not cause any obvious symptoms, but if it reduced the effectiveness of their immune systems, the badger could become more susceptible to other diseases, such as TB. In addition, it can be more difficult to obtain a protective response from a vaccine in animals suffering from problems with their immune responses. Either of these considerations will add new factors to the debate about badgers and TB.

Banks, M., King, D.P., Daniells, C., Stagg, D.A. & Gavier-Widen, D. (2002). Partial characterization of a novel gammaherpesvirus isolated from a European badger (*Meles meles*). *J Gen Virol* 83, 1325–1330.

An extraordinary new species from the canals of Venice

Researchers at the Universities of Munich and Konstanz in Germany have scooped up a truly unusual spherical bacterium from the muddy sediment of the canals of Venice. They have recently named it *Ilyobacter insuetus* (*insuetus* is a Latin adjective meaning extraordinary or unusual) in recognition of the extraordinary fact that the only two compounds that it will grow on are quinic and shikimic acids. Despite their strange names, there are large amounts of these materials in plants because they are used to synthesize wood. The researchers tested over 30 other substrates, including sugars such as glucose that can be used by very many bacteria, but *I. insuetus* left them untouched. In addition, this species is killed by oxygen from the atmosphere and uses a novel strategy for disassembling its sole food source to obtain carbon and energy.

Its taxonomic position was not obvious because when the researchers did the appropriate tests, its nearest relatives were members of both the genera *Ilyobacter* and *Propionigenium*. Although they were all similar, based on the sequence of several genes, there were such significant differences in their cell physiology that the researchers think that once more information is available it will be clear that these bacteria are actually members of several currently unrecognized genera.

Brune, A., Evers, S., Kaim, G., Ludwig, W. & Schink, B. (2002). *Ilyobacter insuetus* sp.nov., a fermentative bacterium specialized in the degradation of hydroaromatic compounds. *Int J Syst Evol Microbiol* 52, 429–432.

Friendly gut bug produces killer protein

Probiotics have been defined as living micro-organisms which when eaten in adequate numbers exert positive health benefits beyond inherent basic nutrition. A probiotic microflora is believed to act through its metabolic activities and its physical presence, with benefits such as the competitive exclusion of medically significant pathogens, stimulation of the immune system and treatment or neutralization of the side effects of antibiotic therapy. Many probiotics have the desirable property of producing antimicrobial substances called bacteriocins. These small proteins are secreted by the bacteria to kill or inhibit other bacteria. Bacteriocin-producers contain genes that give them immunity to their own bacteriocins. A very large number of bacteriocins have been identified, and researchers know about their synthesis and secretion, at least in general terms.

Now, researchers from University College Cork in Ireland, in collaboration with Norwegian scientists, have for the first time isolated the genes responsible for bacteriocin production by a probiotic isolated from the gut of a human, along with the bacteriocin itself. The probiotic bacterium was *Lactobacillus salivarius* UCC118, a member of the lactic acid bacteria that are generally considered to be a beneficial component of the intestinal flora. This strain can synthesize a bacteriocin called ABP-118 that inhibits a number of food-borne and medically significant pathogens, including species of *Bacillus*, *Listeria*, *Enterococcus* and *Staphylococcus*, without apparently affecting other lactic acid bacteria. This is fortuitous, since bacteriocins often inhibit bacteria related to the producer.

After isolating the bacteriocin from the growth medium of an *L. salivarius* UCC118 culture, the researchers could sequence the protein and from that deduce the type of gene that should encode it. With this information, they analysed a region of the genome of the bacterium which turned out to have 16 genes with all the instructions for synthesizing and secreting the bacteriocin ABP-118, along with protection from its effects. The bacteriocin turns out to be made of two proteins, only one of which is active against other bacteria. The other protein enhances this toxicity, but is harmless on its own. Both of the proteins are a little longer when they are first synthesized within the cell, than after they are secreted into the environment. The extra region instructs the cell to secrete the proteins and it is then chopped off. The two genes for the secretion system were among the ones found by the researchers, as was the gene that confers immunity on the cells to their own bacteriocin, and a further three that provide the system to detect and transmit signals to initiate synthesis of the toxic proteins. The researchers initially identified the genes only by their similarity to ones in other bacteria, but were later able to carry out a series of experiments to confirm their identification. In one of these, they added the gene they thought conferred immunity to ABP-118 to a strain of bacteria that was usually killed by it. These bacteria became immune to the bacteriocin, indicating that this was indeed the function of the gene product.

Among the other eight genes, the researchers think that some may be instructions for more types of bacteriocins, but none of their experiments indicated that these genes worked. This is something that they are continuing to investigate, along with the regulation of the production of ABP-118. Although the health-contributing effects of this bacterium may be difficult to assess within the intestine, its ability to inhibit or kill pathogenic bacteria can only benefit its host.

Flynn, S., van Sinderen, D., Thornton, G.M., Holo, H., Nes, I.F. & Collins, J.K. (2002). Characterization of the genetic locus responsible for the production of ABP-118, a novel bacteriocin produced by the probiotic bacterium *Lactobacillus salivarius* subsp. *salivarius* UCC118. *Microbiology* 148, 973–984.