



Science writer **Meriel Jones** takes a look at some recent papers in SGM journals which highlight new and exciting developments in microbiological research.

A soapy solution to HIV?

Roner, M.R., Sprayberry, J., Spinks, M. & Dhanji, S. (2007). Antiviral activity obtained from aqueous extracts of the Chilean soapbark tree (*Quillaja saponaria* Molina). *J Gen Virol* **88**, 275–285.

The Chilean soapbark tree (*Quillaja saponaria*) is a small evergreen tree found in Chile and Peru. Extracts from the bark are used as soap because the saponin chemicals within it act as detergents. They are used in the food and beverages industry in the USA to make frothy foams, and even to create the foam in some fire extinguishers. *Quillaja* extracts are also a traditional medicine in South America for some chest problems, while in modern medicine, purified *Quillaja* saponins are used in vaccines to enhance their protective activity.

Researchers in Texas have been investigating a new role for these triterpenoid saponins as antiviral agents that appear to work through novel interactions with both the virus and

host cells. Viruses can only replicate within living cells, so antiviral agents can be effective by preventing viruses attaching to cells or interfering with steps within the viral replication cycle. The researchers tested whether *Quillaja* saponins reduced infection of human or animal cell cultures by six viruses, including HIV and herpes simplex virus. Concentrations of *Quillaja* saponins below 1.0 mg ml⁻¹ did not affect the growth of the cells, but had direct antiviral activity at a 10-fold lower concentration for five of the six viruses.

Much more interestingly, treatment of the cell cultures with *Quillaja* saponins for an hour made the cells very resistant to viral infection for up to 16 hours after the saponins were washed away.

Incubation in *Quillaja* extract at levels as low as 0.0001 mg ml⁻¹ completely blocked the binding of HIV to the cells, despite the virus remaining fully infectious. Less than 0.25 % of the virus attached to the cells and then caused only 4 % of the expected level of active viral infection.

The most likely mechanism for this protection against such a wide range of viruses is through modification of surface features that the viruses use to recognize and enter cells. The fact that the *Quillaja* extract provided such effective protection in laboratory tests on cell cultures prompted the researchers to suggest that it is a good candidate for use within spermicidal agents to protect against sexually transmitted viruses. The fact that *Quillaja* extracts are approved for use in food and beverages in the USA, indicating their harmless nature to people, is an important first step towards this use.

Star Wars fantasy comes true!

Sassera, D., Beninati, T., Bandi, C., Bouman, E.A.P., Sacchi, L., Fabbri, M. & Lo, N. (2006). 'Candidatus Midichloria mitochondrii', an endosymbiont of the tick *Ixodes ricinus* with a unique intramitochondrial lifestyle. *Int J Syst Evol Microbiol* **56**, 2535–2540.

A character from the *Star Wars* films has been discovered inside ticks on planet Earth. Or, rather, researchers have named a remarkable new genus of bacteria after the midichlorians, imagined by George Lucas as microscopic creatures residing within cells and able to 'communicate with the Force'. The new bacterium, 'Candidatus Midichloria mitochondrii', is the only bacterium known to be able to invade and live inside mitochondria, the intracellular organelles that generate energy to keep cells alive. The bacteria were identified in a collaboration between researchers from Australia, Italy and the Czech Republic who decided that naming them after the midichlorians was particularly appropriate.

The researchers examined cells from the blood-sucking tick *Ixodes ricinus* by electron microscopy and they were struck by

the sight of up to 20 bacteria packed into the mitochondria of ovaries in all the females. The bacteria were only found in the ovaries of the female ticks. Even though large numbers of mitochondria were invaded and destroyed by the bacteria, the eggs of the infected ticks appeared to develop normally. 'Candidatus M. mitochondrii' proved impossible to grow in the laboratory, so the researchers used molecular biology methods to work out the sequences of two of the bacterial genes. These turned out to be a good match to sequences from several other unculturable bacteria, but were sufficiently different to show that the researchers had found a new candidate genus and maybe even a new family of bacteria. When male ticks were tested, 44 % were found to contain these bacteria, even though nothing was visible on electron microscope images.

I. ricinus ticks are notorious for transmitting Lyme disease and other human and animal pathogens. The researchers suggest that 'Candidatus M. mitochondrii' may interact with pathogens transmitted by the ticks. Its presence in the ovaries of all female *I. ricinus* ticks suggests it may have a role in their biology and the discovery of the symbiont adds to the information about the bacterial community associated with this arthropod.

Mobile genes and meningitis

Dunning Hotopp, J.C., Grifantini, R., Kumar, N. & 9 other authors (2006). Comparative genomics of *Neisseria meningitidis*: core genome, islands of horizontal transfer and pathogen-specific genes. *Microbiology* **152**, 3733–3749.

Most *Neisseria* species live harmlessly on warm-blooded animals, including people. *N. meningitidis* is the best known species because it can travel from inside the nose to the bloodstream and cross the blood–brain barrier. Some strains of *N. meningitidis* cause epidemic bacterial meningitis that can rapidly affect large numbers of people who live in close contact. However, up to 20 % of people carry *N. meningitidis* in their upper respiratory tract and remain perfectly healthy. Understanding the nature of potentially pathogenic strains is therefore important. It is also a challenge because of the way that this species can change its complement of genes. It has a natural ability to take up DNA from the environment and incorporate it into its own genetic structure.

The complete genome sequences of three strains of *N. meningitidis* are available. Comparative genome hybridization was used to examine 53 *Neisseria* isolates, representing the whole range of types within *N. meningitidis*, as well as two strains of *N. gonorrhoeae*, which causes gonorrhoea, and three non-pathogenic species. The technique uses microarrays containing DNA designed to match each of the 2,158 genes within one sequenced *N. meningitidis* strain along with additional DNA representing unique regions from other *Neisseria* genome sequences. DNA extracted from each of the *Neisseria* isolates was then tested with this microarray to see which genes were in each isolate.

The researchers identified the core *N. meningitidis* genome from the genes present in all the strains. The data also showed that *N. meningitidis* strains had acquired groups of genes, called genetic islands, from *N. gonorrhoeae*, non-pathogenic *Neisseria* species and other bacterial species that colonize the respiratory tract, as well as exchanging them between themselves. The results suggested that some of these transfers might have involved bacterial viruses or other mechanisms that make DNA more mobile. It was also very clear that there is an efficient system to restrict the types of DNA that could be taken up by *N. meningitidis*. The results also helped to characterize genes that determine cell-surface structure and to identify some aspects of metabolism that may be essential for survival within human cells. However, one of the most important outcomes of this study is the large amount of data on which genes are present or absent from which strains – this will be valuable to all researchers trying to understand the nature of pathogenicity in *N. meningitidis*.

► Acridine orange/DAPI-stained human macrophage containing *Citrobacter koseri*. S. Townsend, Nottingham Trent University

◀ 'Candidatus Midichloria mitochondrii' inside the mitochondria of the oocytes of the tick *Ixodes ricinus*. Luciano Sacchi, University of Pavia, Italy

A 'tail' about brain abscess formation

Townsend, S.M., Gonzales-Gomez I. & Badger, J.L. (2006). *fliP* influences *Citrobacter koseri* macrophage uptake, cytokine expression and brain abscess formation in the neonatal rat. *J Med Microbiol* **55**, 1631–1640.

The bacterium *Citrobacter koseri* can cause very serious infections, particularly in the brain of new-born and young children. Most of these meningitis cases occur out of the blue with no obvious source of infection. Around a third of infected infants die and many of the survivors have severe neurological damage. This is because the infection results in intense inflammation of the brain, frequently causing abscesses that do not respond to antibiotic therapy. In fact, *C. koseri* infections are more likely to cause brain abscesses than any other bacterial species. The bacterial cells survive and grow within macrophages, cells of the immune system that are supposed to engulf and digest pathogens at an early stage in the immune response. A better understanding of the disease process would certainly help to develop improved treatments.

Researchers have now identified one bacterial gene with a role in the uptake and survival of *C. koseri* inside macrophages. They tested the ability of mutant strains of *C. koseri* to survive with macrophages, and found seven mutants that did not survive as well as the wild-type, an indication that the mutation had occurred within an important character. They evaluated each mutant in cell cultures and found differences in their abilities to enter and survive within macrophages. Despite these differences five had the same effects on neonate rats as the wild-type, but one was no longer lethal since the bacteria were very efficiently removed by the immune system without any signs of brain damage.

The seventh mutant (SMT350) was much more virulent, killing all the rats within only 2–3 days, before brain abscesses could develop, and leaving most of the bacteria outside the macrophages. The researchers worked out that this mutant lacked a protein essential for flagella, the surface structures that bacteria use to propel themselves through liquids, and tests showed that SMT350 were indeed unable to move. This fitted with information from other research groups that flagella seem to be important for bacteria to invade animal cells. It also hinted that the flagella might conceal a toxic factor on the bacterial surface that was exposed in its absence.

Macrophages produce interleukin-12 and -10 as signals to the immune system to induce resistance to intracellular infections. Each interleukin induces a different type of response and when the researchers measured their levels, it appeared that the *C. koseri* flagellum, rather than any other bacterial cell component, had a dramatic immunosuppressive effect. This may be why the infection persists so effectively and causes brain abscesses. The possibility that the flagellum plays a key role in determining the course of this infection is a significant step forward in understanding this disease.

