

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

## New approach to TB control using granulysin

**Liu, B., Liu, S., Qu, X. & Liu, J. (2006).** Construction of a eukaryotic expression system for granulysin and its protective effect in mice infected with *Mycobacterium tuberculosis*. *J Med Microbiol* **55**, 1389–1393.

Tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, kills around 3 million people each year. Even more worryingly, resistance to existing anti-TB drugs is increasing, so new therapies are needed. Researchers at the School of Medicine in Wuhan University in China have been exploring whether a peptide antibiotic made by some human cells can be developed as an effective treatment. Granulysin is produced by human cytotoxic T lymphocytes and natural killer cells, and acts in combination with a second pore-forming protein called perforin and proteinases called granzymes to destroy cancerous and virus-infected cells within the body. However, in laboratory tests, granulysin can kill or inhibit many bacteria, including *M. tuberculosis*, fungi and parasites on its own.

The researchers have demonstrated that human granulysin provides protection to mice infected with *M. tuberculosis* when it is made by their own cells. To do this the researchers isolated the sequence for the granulysin gene and inserted it into an expression plasmid, a circular piece of DNA containing all the instructions to allow any animal cell to synthesize granulysin. They checked that this worked using a cell culture, and then injected mice with the granulysin expression plasmid. Some of the mice were then infected with *M. tuberculosis*. After a month the mice had the sort of inflammation and lesions that occur in tuberculosis.

However, these symptoms were much milder in the mice immunized with the granulysin expression plasmid. There were significantly fewer bacteria in these mice as well, with many bacteria showing signs of damage. This is the first evidence that a granulysin expression vector can protect against *M. tuberculosis* infection and suggests a new approach to TB control.

## SARS in horseshoe bats

**Ren, W., Li, W., Yu, M. & others (2006).** Full-length genome sequences of two SARS-like coronaviruses in horseshoe bats and genetic variation analysis. *J Gen Virol* **87**, 3355–3359.

Severe acute respiratory syndrome (SARS) took the medical world by surprise when it was first reported as a new disease in Asia in February 2003. There was concern because of its rapid worldwide spread and high mortality rate, with an estimated 774 deaths among the 8,098 people who contracted SARS in the 2003 outbreak. Rapid work identified the causal agent as a coronavirus. Although no new cases have been recorded since late 2004 among the public, researchers still want to know more about where the virus came from.

Researchers from China have already detected virus from the SARS cluster coronaviruses in at least five horseshoe bat species from the genus *Rhinolophus*, suggesting that bats might be the natural home of the virus. The sequence of all of the genes of some of these group 2b coronaviruses (G2b-CoV) is now known, and is similar to that of viruses isolated from humans and civets in 2003. The researchers have now characterized two additional G2b-CoV genome sequences from bats and have compared all of the genome sequences to see whether this gives clues about why the virus was so virulent or how it was transmitted between different animal hosts.

The bat Gb2-CoV isolates have an identical genome organization and share an overall identity of 88–92 % among themselves, and between them and isolates from civets or humans. However, there is considerable diversity in their detailed DNA sequences, even though researchers think that they all evolved from a common ancestor of the SARS virus. The researchers came up with different relationships among the strains depending on which gene sequences they used. This often indicates that there has been recombination between viruses in the past, although more sequences would be needed to prove this. One isolate from bats, Rf1, had a unique feature that might make it an evolutionary intermediate between the virus that infects bats and strains found in people. Further tests suggested that the viruses in bats had evolved independently for a long time, whereas the ones from humans and civets had recently undergone strong positive selection, reinforcing the idea that something has recently allowed the virus to cross between species.

▶ **Left.** Alternatives to fossil fuels are a challenge for the 21st century as demand steadily increases, but reserves become depleted. *Jeremy Walker / Science Photo Library*

▶ **Right.** Hibiscus flower from Rarotonga, with nitidulid beetles (*Aethina* sp.). *André Lachance*

◀ **Coloured X-ray** of a patient's chest showing disseminated tuberculosis (TB) in the lungs. The lungs contain lesions (tubercles, pink) consisting of infected dead tissue. *Du Cane Medical Imaging Ltd / Science Photo Library*



## Microdiesel from *E. coli* – an alternative to fossil fuels?

**Kalscheuer, R., Stölting, T. & Steinbüchel, A. (2006).** Microdiesel: *Escherichia coli* engineered for fuel production. *Microbiology* **152**, 2529–2536.

Practical alternatives to fossil fuels are a challenge for the 21st century. Not only are the number of exploitable oil reserves around the world decreasing as demand increases, but using this non-renewable resource also contributes to global warming. Among the solutions is biodiesel, an alternative to petroleum-based diesel made from plant oils. Its major drawback is that the acreage of oil crops like oilseed rape, soybeans and oil palm needed to meet the world's current demands would leave little space for food crops. However, most of the carbon in plants is within structural materials like cellulose and starch rather than the seed oils. Biodiesel would be much more practical if it could be made from these chemicals. A second problem is that biodiesel is currently produced by converting the plant oils to fatty acid methyl esters (FAMEs) using methanol, which is both toxic and derived from non-renewable natural gas. Fatty acid ethyl esters (FAEEs), synthesized using ethanol that can be produced biologically, have similar fuel properties to FAMEs, but are more expensive to make by chemical synthesis.

Researchers at the Westfälische Wilhelms-University in Germany have taken a lateral approach to these problems to see whether bacteria can help. Their solution has demonstrated that bacteria can be designed to make 'Microdiesel', resembling biodiesel. The researchers brought together genes from three different bacteria. They had been working with the species *Acinetobacter baylyi* that makes fats to store within its cells. The key enzyme (WS/DGAT) in this biosynthesis turns out to work well with a remarkably broad range of substrates, including many never encountered in nature. This enzyme therefore might make Microdiesel if it was supplied with suitable materials. Unfortunately, *A. baylyi* cannot synthesize suitable amounts of ethanol. The solution was to add two genes from another bacterium, *Zymomonas mobilis*, to the microbiological workhorse *Escherichia coli*, giving it the capacity to synthesize enough ethanol. Adding the gene for WS/DGAT from *A. baylyi* as well created genetically modified bacteria that could churn out up to 26 % of their dry weight as FAEEs, once supplied with sugars and fatty acids.

Higher efficiency and yields would be needed in a practical industrial process, as well as the ability to use crude plant materials as substrates. However, the versatility within bacterial metabolism means that this may well be possible using the right combination of genes and bacteria. The German researchers have shown that the concept works and have opened up the way for further developments.

## Novel yeast distribution in insects

**Lachance, M.-A., Bowles, J.M., Wiens, F., Dobson, J. & Ewing, C.P. (2006).** *Metschnikowia orientalis* sp. nov., an Australasian yeast from nitidulid beetles. *Int J Syst Evol Microbiol* **56**, 2489–2493.

Nitidulid beetles live on fruit, flowers, leaves and other discarded parts of plants, and can be a pest in dried fruit. However, three of these small beetles have posed an interesting question about species distribution, not of beetles, but of yeasts. The beetles were collected from roadsides separated by 11,000 km of land and ocean in cool regions of Rarotonga in the Cook Islands and the Cameron Highlands in Malaysia. A novel species of yeast, *Metschnikowia orientalis*, was discovered in their droppings. Remarkably, *M. orientalis* has not been detected before in extensive collections from habitats in Australia, New Caledonia and Fiji. Researchers from Canada have been surveying the insect-borne yeast flora of these areas. The only tenuous clue linking *M. orientalis* to these two locations is the low maximum temperature at which the yeast will grow. This must not exceed 30–31 °C, which could limit the number of suitable habitats in these tropical regions.

The researchers suspected that they had a novel species when they recovered several strains of a yeast that would apparently only reproduce asexually from two nitidulid beetles in Rarotonga in 1999. In 2005 they found a single isolate from an insect on roadside flowers in Malaysia that had similar growth and DNA characteristics to the Rarotonga yeasts. The only way to distinguish this novel species from other yeasts is to either examine DNA sequences or test for the production of sexual spores once possible isolates are mixed with authentic strains of each mating type.

The question of why the strains were found so far apart, and where others may turn up, is still open.

