

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

## Family affairs

**Lee, K.-B., Liu, C.-T., Anzai, Y., Kim, H., Aono, T. & Oyaizu, H. (2005).** The hierarchical system of the 'Alphaproteobacteria': description of *Hyphomonadaceae* fam. nov., *Xanthobacteraceae* fam. nov. and *Erythrobacteraceae* fam. nov. *Int J Syst Evol Microbiol* **55**, 1907–1919.

Classifying bacteria has always been tricky. They look very similar, so microbiologists have enthusiastically adopted methods based on the sequence of bacterial DNA. The genes that encode parts of the ribosome have changed very slowly during evolution because ribosomes have a vital role in the cell. Sequences of the 16S rRNA gene from hundreds of bacteria have now accumulated, so researchers in Japan have used them to review one class of bacteria, the 'Alphaproteobacteria'. The bacteria within this class have diverse lifestyles, including photosynthetic and non-photosynthetic species as well as ones that require oxygen and others that can do without it.

The researchers used a computer program to compare the sequences of 16S rRNA genes from 249 species to produce a

robust phylogenetic tree. The tree indicated that the natural relationships among the bacteria made them fall into five major clusters. When the researchers studied which species were in each cluster, it was obvious that more taxonomic work was needed before firm conclusions could be drawn about some of the groupings. For example, the *Rickettsiales* cluster included a few species that spend their entire lives within protozoa.

In some regions, however, the relationships were so clear that the researchers recommended the creation of three new bacterial families to record this fact. One was the new family *Hyphomonadaceae* for a series of marine genera such as *Hyphomonas*, *Maricaulis* and *Oceanicaulis*. The second was *Erythrobacteraceae* for genera like *Erythrobacter*, *Porphyrobacter* and *Erythromicrobium* that all contain distinctive lipids and pigments. A third new family, *Xanthobacteraceae*, for genera such as *Xanthobacter*, *Azorhizobium*, *Labrys* and *Starkeya* within the cluster of the 'Rhizobiales', contained the most diverse collection of species.

## Yeast as a human model in *E. coli* research

**Rodriguez-Escudero, I., Hardwidge, P.R., Nombela, C., Cid, V.J., Finlay, B.B. & Molina, M. (2005).** Enteropathogenic *Escherichia coli* type III effectors alter cytoskeletal function and signalling in *Saccharomyces cerevisiae*. *Microbiology* **151**, 2933–2945.

EPEC strains of the intestinal bacterium *Escherichia coli* cause life-threatening diarrhoea in children. These strains latch onto the cells of the gut and inject specific proteins into the human cells, altering their shape and severely damaging the intestinal surface, resulting in diarrhoea. The genes for these proteins lie together on the *E. coli* chromosome at the locus of enterocyte effacement (LEE). Researchers in Spain and Canada have been collaborating to exploit the similarities between ordinary brewer's yeast, *Saccharomyces cerevisiae*, and human cells to learn exactly what LEE proteins do.

As well as providing information about the function of each bacterial protein, the ease of genetic and mutational testing in *S. cerevisiae* means that researchers can check their ideas much more easily than using human cells. The researchers ensured that each LEE protein was synthesized in a series of yeast cells and looked for any effects on growth or shape. *S. cerevisiae* cells divide by forming a bud at one end in a process that requires well-organized activity of skeletal proteins and division of the cell nucleus. One bacterial protein, Map, interfered with many steps in these processes to alter cell shape, division and intracellular signalling. This suggests that it affects several regulatory steps. The researchers created mutations that showed the toxic effects were attributable to one end of the protein.

Other proteins accumulated in particular spots within the yeast cells. Patches of EspF associated with the few buds that

formed in yeast cells expressing this *E. coli* protein. Another protein, EspD, accumulated within the intracellular membrane system. Proteins EspG and EspH are transported into intestinal cells by EPEC strains. EspH activated a yeast intracellular signalling pathway, although it had no effect on yeast cell growth. EspG was distributed throughout the yeast cell and resulted in a loss of co-ordination of budding with division of the nucleus, strongly inhibiting growth. Overall, the data from these studies will help to clarify the role of these proteins in human disease.

◀ Visualization of the microtubular apparatus by immunofluorescence with anti-tubulin antibodies (green) in yeast cells defective in budding as a consequence of the expression of the *Salmonella* virulence factor Map (red). The nuclei were stained with DAPI (blue). *Maria Molina, Madrid*

## Gut flora and autism

**Parracho, H.M.R.T., Bingham, M.O., Gibson, G.R. & McCartney, A.L. (2005).** Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *J Med Microbiol* **54**, 987–991.

As well as their social and communication problems, many children with autistic spectrum disorder (ASD) are reputed to suffer from digestive problems. Parents often say that relieving these problems improves the child's behaviour, but there is little clinical evidence to support this. The bacteria that live in the gut have a major role in its activity and health, but knowledge of what constitutes a 'normal' intestinal microflora is still in its infancy because of the difficulty in measuring the large number and diversity of bacteria in the gut.

Nevertheless, researchers at the University of Reading have made a start on understanding the gut microflora associated with autism. They recorded the numbers of selected bacterial groups in faeces from children with ASD and from two healthy control groups, namely the non-autistic siblings of children diagnosed with ASD and a group of unrelated, healthy children. The researchers used a questionnaire to assess the nature of any digestive problems experienced by each child, what they ate and their history of antibiotic intake.

It turned out that almost all the children with ASD had digestive disorders, along with a quarter of the non-autistic sibling group, but none of the unrelated healthy

children recorded any symptoms. Many of the autistic children were on a gluten- and/or casein-free diet as this has been associated with reduced digestive problems. A further contrast was that none of the unrelated healthy children took probiotics, while these were taken by over half of the children with ASD and 40 % of healthy siblings. Several children had been prescribed antibiotics, but one-third of those with ASD had received more than six courses.

Considering all these factors, it was interesting that only two differences were found in the number of bacteria from the three groups. All the children harboured the groups of bacteria that were monitored, but there was a significant difference in the number of *Bacteroides* between the two healthy groups of children.

The other, and perhaps more significant, difference was that the children with ASD contained the highest number of the *Clostridium histolyticum* subgroup that the researchers had ever seen. However, a further analysis of the figures indicated that there was no significant difference between the numbers in the sibling subgroups (ASD and healthy) as both these subject groups harboured intermediary levels of the *C. histolyticum* subgroup. This suggests that genetics, as well as diet and living conditions, may determine the composition of the gut microflora. In addition, since certain strains of *Clostridium* can sometimes produce powerful neurotoxins, it suggests a further line of research that is worth investigating.

## A tale of two clades

**Likos, A.M., Sammons, S.A., Olson, V.A. & 14 other authors (2005).** A tale of two clades: monkeypox viruses. *J Gen Virol* **86**, 2661–2672.

The orthopox viruses cause diseases like smallpox and monkeypox. Naturally occurring smallpox was eradicated over 25 years ago following a campaign of vaccination and public-health surveillance. Monkeypox was first described as an infection of non-human primates, but between 1970 and 1986, smallpox surveillance programmes turned up 10 cases in humans in West African countries such as Sierra Leone and Liberia, and 394 cases in the Congo Basin. The fatality rate was ~10 %, but previous smallpox vaccination appeared to be protective. There was concern that this disease might replace smallpox, but it became apparent that monkeypox transmission could not be sustained indefinitely between humans without its zoonotic host(s).

In May 2003, monkeypox leapt to the attention of the American Centers for Disease Control and Prevention (CDC) because of reports of a new illness characterized by a rash and fever. The origin turned out to be a consignment of about 800 small mammals imported into Texas from Ghana. Some species carried the monkeypox virus, which then infected pet prairie dogs from which people had contracted the disease. The disease symptoms appeared to be less severe than in the earlier African cases and the outbreak was contained. At the same time, more cases were reported in young people in the Republic of Congo, with the difference that people became very seriously ill and infection was transmitted from person to person.

Inger Damon and collaborators at the CDC, together with colleagues working in public health in Africa and USA, as well as from the WHO, have been comparing details of the patients and viruses. They focused on cases from the Republic of Congo between 1981 and 1986 and the USA in 2003, where they had comparable information about patients. The researchers thought that the youth of patients, and not having been vaccinated against smallpox, all contributed to more severe disease and mortality. However, the statistical analysis showed that the illness was more transmissible, serious and lasted longer among African than USA case patients, regardless of their age or vaccination status.

After analysing the virus genomes, they realized that the viruses, rather than the patients' circumstances, were likely to be the reason for these differences. The researchers examined the sequence of all the genes in three strains of virus isolated from African patients and virus isolated from US human and prairie dog cases, and found some differences that could affect the efficiency of the virus in causing severe, human-to-human transmissible disease. These features have been conserved in the African strains for over 30 years. The Congo Basin type had a feature that may diminish the body's efforts to remove the virus from the bloodstream, while the West African type that infected the Americans contained a new component that could facilitate recognition and clearance of the virus by the immune system.