EVOLUTION

All in a day's work…

A paper just published in *Nature Genetics* shows that a microarray-based method of whole genome re-sequencing can probe the mutations that arise after just a few dozen generations of growth of the laboratory stalwart *Escherichia coli*.

Researchers side-step the problems that might arise from rapid bacterial evolution by relying on laboratory-adapted bacterial strains. But even these laboratory workhorses can be prone to strong selection in certain growth media. Herring *et al.* exploited this problem to test the feasibility of using comparative genome sequencing (CGS) to monitor evolution on a day-to-day basis.

When the sequenced *E. coli* strain MG1655 is grown in minimal medium supplemented with glycerol as the sole carbon and energy source, the growth rate is below the predicted optimum, despite the presence of a complete pathway for glycerol catabolism in the genome. Over time, the growth rate increases to the predicted optimum. The authors monitored the mutations that arose after ~660 generations of growth of MG1655 in glycerol minimal media. They identified the locations of single-nucleotide polymorphisms, insertions and deletions in evolved strains using CGS. The key mutations that adapt MG1655 to grow better in glycerol minimal medium included mutations in genes involved in global transcriptional regulation, and mutations that led to improvements in the kinetics of glycerol catabolism by the rate-limiting enzyme glycerol kinase (GlpK). The evolved phenotypes were faithfully reconstructed by introducing single or combined mutations into the progenitor strain by site-directed mutagenesis, which validated the approach.

METAGENOMICS

A global marine viral metagenome

The first global survey of marine viral genomes has shown that the oceans are awash with viruses. Calculations of viral diversity indicate there might be as many as several hundred thousand distinct marine viral species. Most viral species are widely dispersed, but local environmental conditions dictate which species are most common in a particular oceanic region.

The group of Forest Rohwer and their collaborators collected 184 samples from 68 different marine sites located in four main regions: the Sargasso Sea, the Arctic Ocean, the Gulf of Mexico and the coastal waters of British Columbia. They extracted and amplified DNA from uncultured viral particles and used pyrophosphate sequencing to determine the DNA sequences that were present. Fewer than 10% of the sequences in this viral assemblage were significantly related to sequences in current genomic and metagenomic databanks. The sequences were also phylogenetically distinct from those of known phage genomes, and indicated that marine phage share distinctive characteristics.

The richness of viral species varied along a latitudinal gradient. Diversity was highest in regions nearest the equator and lower towards the poles, in common with other marine biota. The marine virome from British Columbia, a region that is affected by seasonal upwelling and the outflows of many rivers, was exceptionally genotype-rich. The authors speculate that the richness of such regions could be boosted to levels approaching global diversity by the inward migration of viral species from other regions.

> “Most viral species are widely dispersed, but local environmental conditions dictate which species are most common in a particular oceanic region.”

The prevalence of viruses differed among the four oceanic regions. The Arctic Ocean metagenome had the most prophage-like sequences. Cyanophages and a new single-stranded DNA microphage dominated the Sargasso Sea sample. As most viral species are widespread and shared between oceanic regions, the observed geographical distribution is largely the result of differences in the abundance of viral species rather than the exclusion of particular species.

In order to understand the mechanisms of evolution we need to monitor all the mutations that occur and then determine which changes are selected. By analysing the genomes of cells that were frozen periodically throughout the experiment the authors identified when mutations became fixed (between 4—20 days into the experiment) and notably found alternative mutations in GlpK that were lost from evolving populations, as the cells carrying those mutations were out-competed.

This study shows that we now have affordable and reliable techniques to study evolution in action in bacteria.

Susan Jones

ORIGINAL RESEARCH PAPER Herring, C. D. *et al.* Comparative genome sequencing of *Escherichia coli* allows observation of bacterial evolution on a laboratory timescale. *Nature Gen.* 05 Nov 2006 (doi:10.1038/ng1906)

Metagenomic analysis has provided important information on the abundance, distribution and dynamics of marine viruses. A more comprehensive characterization of marine viral diversity and, as a result, a better understanding of marine microbial ecosystems, can be anticipated with further improvements in the power of DNA sequencing technology.

Edward Wawrzynczak

ORIGINAL RESEARCH PAPER Angly, F. E. *et al.* The marine viromes of four oceanic regions. *PLoS Biol.* **4**, e368 (2006)
FURTHER READING Edwards, R. A. & Rohwer, F. Viral metagenomics. *Nature Rev. Microbiol.* **3**, 504–510 (2005) | Tringe, S. G. & Rubin, E. M. Metagenomics: DNA sequencing of environmental samples. *Nature Rev. Genet.* **6**, 805–814 (2005)
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BACTERIAL PATHOGENESIS

Clearing a path

The human intestinal pathogen *Shigella flexneri* negotiates its way through the crowded interior of host cells by destroying the surrounding microtubule network, according to a recent *Science* paper.

Some bacterial pathogens invade the cytoplasm of infected host cells to establish a protective niche and allow the infection to spread to neighbouring cells. Following invasion, *S. flexneri*, *Listeria monocytogenes*, *Burkholderia pseudomallei*, *Mycobacterium marinum* and the spotted-fever Rickettsiae all manipulate the host-cell cytoskeleton, recruiting actin and inducing its polymerization at one end of the bacterial cell to generate the propulsion necessary for motility.

Chihiro Sasakawa and colleagues were interested in the intracellular movements of *S. flexneri*, and particularly what is responsible for the idiosyncratic movement patterns observed when *S. flexneri* is within host cells — the rapid, smooth movements at the periphery of the cytoplasm contrast with the disjointed movements observed in the rest of the cytoplasm, and suggest that an intracellular structure could be affecting *S. flexneri* motility.

Yoshida *et al.* decided to focus their investigations on the role of the type III-secreted effector VirA. VirA is essential for *S. flexneri* entry into epithelial cells and for both intra- and intercellular spreading. Time-lapse photography was used to look at the effect of VirA on *S. flexneri* motility, and it was found that the motility of a *virA* deletion mutant was drastically reduced. The deletion mutant still formed a long actin tail, however, indicating that the absence of *virA* did not affect actin nucleation and polymerization.

To assess whether the cytoskeleton posed an obstacle to *S. flexneri* motility in host cells, Yoshida *et al.* pretreated a fibroblast cell line with the microtubule poison nocodazole before infection with the *virA* mutant. The proportion of motile *virA* deletion mutants was roughly three times greater in the nocodazole-treated cells than in non-treated cells. Immunofluorescence confocal microscopy was then used to take a closer look at *S. flexneri* in the host-cell cytoplasm, and it was noted that some areas of bacterial movement appeared to be devoid of microtubules, and that the actin tail of a motile bacterium seemed to create a tunnel-like area through which other bacteria can follow. Further characterization of this phenomenon using freeze-fracture electron microscopy showed that *S. flexneri* destroys the microtubule network in its local vicinity, and that VirA is required for this destruction.

How does VirA achieve this drastic effect? Analysis of the biochemical activity of VirA revealed that this type III-secreted effector is an α-tubulin-specific cysteine protease and it was found that cysteine 34 was the crucial residue for this activity. Finally, analysis in a mouse infection model proved that the cysteine protease activity of VirA contributes to the pathogenesis of *S. flexneri in vivo*.

So, it seems that *S. flexneri*, the causative agent of bacillary dysentery, forces its way through the host-cell cytoplasm by destroying the microtubules blocking its path, thereby adding a new dimension to bacterial manipulation of the host-cell cytoskeleton.

Sheilagh Molloy

ORIGINAL RESEARCH PAPER Yoshida, S. *et al.* Microtubule-severing activity of *Shigella* is pivotal for intercellular spreading. *Science* **314**, 985–989 (2006)
FURTHER READING Stevens, J. M., Galyov, E. E. & Stevens, M. P. Actin-dependent movement of bacterial pathogens. *Nature Rev. Microbiol.* **4**, 91–101 (2006)