Nature Reviews Microbiology | AOP, published online 3 November 2008; doi:10.1038/nrmicro2035

Nick Waterfield and Maria Sanchez-Contreras holding the tobacco hornworm moth, *Manduca sexta*, one of the model animals used to test the genome libraries. Image courtesy of Nic Delves-Broughton, University of Bath, UK.

TECHNIQUES

Hunting WMDs in pathogen genomes

> "…rapid virulence annotation …uses convenient experimental organisms as surrogates for the mammalian immune system."

Bioinformatics algorithms are becoming increasingly successful for the functional annotation of genomes, but they cannot replace experimental approaches when searching for the highly specialized genes responsible for virulence in pathogens. Such approaches usually involve screening pathogens containing loss-of-function mutations, which can be both time-consuming and fraught with ethical complications. Writing in the *Proceedings of the National Academy of Sciences USA*, Waterfield *et al.* report an alternative approach, which they name rapid virulence annotation, that uses convenient experimental organisms as surrogates for the mammalian immune system.

First the genome of the pathogen is split into segments of around 40,000 bp and cloned into *Escherichia coli*. These modified strains can then be screened for a gain of toxicity. Waterfield and colleagues applied this technique to the genome of *Photorhabdus asymbiotica*, a member of the Enterobacteriaceae that infects insects and humans. The complex life cycle of *P. asymbiotica* includes a phase in which the bacterium lives in the gut of *Heterorhabditis* nematodes, for which the pathogen must produce immune inhibitors, toxins and antimicrobials. The authors therefore screened *E. coli* transformed with the *P. asymbiotica* library against not only a mouse macrophage cell line but also the nematode *Caenorhabditis elegans*, the protozoan *Acanthamoeba polyphaga* and two caterpillars, the tobacco hornworm (*Manduca sexta*) and greater wax moth (*Galleria mellonella*).

Waterfield *et al.* identified 21 discrete regions of the *P. asymbiotica* genome that harbour potential toxicity factors against 1 or more of the test organisms. These included toxin-like genes, polyketide synthases, non-ribosomal peptide-synthesis complexes and putative specialized secretion systems. Of these, the loci involved in human pathogenesis were located by their absence from the genome of the related *Photorhabdus luminescens*, which only infects insects.

Rapid virulence annotation could help efficiently identify virulence factors in the agents of emerging diseases. By relying on a gain-of-function assay this technique should complement traditional mutagenesis approaches by unmasking genes that would otherwise be overlooked owing to redundancy in the pathogen's genome.

Christopher Surridge

ORIGINAL RESEARCH PAPER Waterfield, N. R. *et al.* Rapid Virulence Annotation (RVA): identification of virulence factors using a bacterial genome library and multiple invertebrate hosts. *Proc. Natl Acad. Sci. USA* **105**, 15967–15972 (2008)