TraDIS technique tackles typhoid

October 16 2009

Researchers have used next-generation sequencing to look at the need for every *S. Typhi* gene in a single experiment. Credit: David Goulding, Wellcome Trust Sanger Institute

(PhysOrg.com) -- For the first time, researchers are able to look at the need for every gene in a bacterial cell in a single experiment. The new method will transform the study of gene activity and the search for weaknesses in bacterial armouries.

Using a newly developed, next-gen sequencing method, a team established which genes Salmonella Typhi needs to survive and which are more of a luxury. The results and the method will be a boon to scientists tackling bacterial disease, allowing them to capitalize on the

abundance of genomic sequence data from next-generation sequencing technologies.

Every year 22 million people are infected and 220,000 die from infection with S. Typhi. It is a special threat in the developing world, in areas with poor sanitation or a lack of clean drinking water.

The team were able to look at almost all the genes in *S. Typhi* and showed that it needs only 356 genes for survival: 4162 genes were not essential. Knowing which genes are essential to the survival of pathogens, researchers can seek treatments to target those genes.

"We developed a new method that is ten times more powerful than any previous technique," says Sanger Institute graduate student Gemma Langridge, one of the first authors on the paper. "By combining transposon-induced mutagenesis - a method whereby small chunks of cut-and-paste DNA sequence are inserted into the genome effectively disabling individual genes - and high-throughput sequencing, we have been able to determine which genes are essential for the survival of *S. Typhi* and which are non-essential."

"Crucially, our new method allows us to achieve all this in just a single experiment."

Using the novel method, which the team have named TraDIS (Transposon Directed Insertion site Sequencing), they inserted transposons into the *S. Typhi* genome to generate more than one million mutants. They then grew the bacteria and used next-generation sequencing to directly identify 370,000 insertion sites in the *S. Typhi* genome - an average of more than 80 insertion sites per gene. Previous methods produce only a few mutations per gene.

If a transposon inserts into an essential gene, the gene is silenced and that

mutant cell will not grow and it - and the transposon insert - will be absent from the mutant pool. By sequencing DNA from the entire pool - approximately 1 million mutants in total - the team were able to identify genes in which no transposon insertions had been detected.

In a single experiment using the TraDIS method, the team were able to determine whether or not 99.6% of the *S. Typhi* genes are essential to its survival.

"Sequencing centres such as ours can produce vast amounts of genomic data at a pace unimaginable just a few years ago," explains Professor Julian Parkhill, Director of Sequencing and head of Pathogen Genomics at the Sanger Institute. "One of our aims is to develop high-throughput research methods that can exploit this explosion of genetic data, to ensure these resources can be used effectively. We can now discover which of all the genes in an organism are essential to its survival or required for growth under special conditions, such as infection. Our new TraDIS method will make a dramatic difference to the ability to carry out such genome-wide research."

Importantly, the team applied the method to a clinical problem by looking at how *S. Typhi* might survive in humans. Typhoid can be spread by carriers who, without showing symptoms, act as reservoirs, storing the bacterium in the gallbladder and passing it to others. The most famous such carrier was Typhoid Mary, who worked in the food industry in the US and spread typhoid fever without exhibiting any symptoms herself.

But, bacteria cannot survive in the fairly hostile environment of the gall bladder unless they are tolerant to bile - the fatty fluid secreted by the gall bladder. Looking at genes involved in bile resistance, allows us to see which genes are essential for helping *S. Typhi* persist in a carrier.

"We grew the bacteria in ox bile to pick out genes required for bile

tolerance," says Keith Turner, Sanger Institute investigator and a senior author on the paper. "We found 169 genes involved in bile tolerance - many of these had not been suspected before and more than 30 are genes not characterized at all.

"Using TraDIS, we have highlighted several possible new targets for treatment that would pick on *S. Typhi*'s need to survive in the gall bladder."

For the first time, it is possible to paint a comprehensive picture of essential, advantageous or burdensome genes in many phases of the bacterial life cycle, to determine functions necessary to support them throughout their entire disease cycle. Such a picture is important for discovery of new targets for treatment.

This elegant new method exemplifies how high-throughput research allows scientists to determine systematically the function of or requirement for individual genes in a single experiment, opening the door for similar analyses of other pathogenic genomes in the future.

More information: Langridge G C, Phan M-D, Turner D J et al. (2009) Simultaneous assay of every Salmonella Typhi gene using one million transposon mutants. *Genome Research*, Published online before print at doi:10.1101/gr.097097.109

Source: Wellcome Trust Sanger Institute (news : web)