

Botulism bug has few genome wrinkles

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The genome of the organism that produces the world's most lethal toxin is revealed today. This toxin is the one real weapon in the genome of *Clostridium botulinum* and less than 2 kg - the weight of two bags of sugar - is enough to kill every person on the planet. Very small amounts of the same toxin are used in medical treatments, one of which is known as Botox.

The genome sequence, reported in *Genome Research*, shows that *C. botulinum* doesn't have subtle tools to evade our human defences or tricky methods of acquiring resistance to antibiotics. It lives either as a dormant spore or as a scavenger of decaying animal materials in the soil, and doesn't interact with human or other large animal hosts for prolonged periods of time.

Occasionally it gets into a living animal, via contaminated food or open wounds, leading to infant botulism or wound botulism, both of which are serious human infections. The host can be quickly overpowered and, in some cases, killed by the toxin, and *C. botulinum* has a new food source.

"Although in the same group as *Clostridium difficile* - the Cdiff superbug - *C. botulinum* has a genome that is remarkable because it is so stable," commented Dr Mohammed Sebahia, lead author on the paper from the Wellcome Trust Sanger Institute. "Unlike Cdiff, in which more than 10% of genes have been acquired from other bacteria, there is almost no footprint of these in *C. botulinum*."

There are several types of *C. botulinum*: although described as variants

of a single species, they are really very different organisms linked simply because they have the deadly toxin. For each type, there is also a near-identical but harmless relative that lacks the toxin. *C. sporogenes* is the non-malignant, near twin of the organism sequenced.

Professor Mike Peck, from the Institute of Food Research, commented that "It is astonishing that 43% of the predicted genes in the *C. botulinum* genome are absent from the other five sequenced clostridia, and only 16% of the *C. botulinum* genes are common to all five. Our findings emphasise just how different clostridia are from each other."

C. botulinum toxin stops nerves from working - the basis of its use in medicine to control tremors and in cosmetic treatments. For the prey of its opportunistic attacks, death is swift. Perhaps the most important tool it has to act out its stealth attacks is its ability to hibernate when times are hard by forming dormant spores.

More than 110 of its set of almost 3700 genes are used to control spore formation and germination when opportunity arises.

"*C. botulinum* shows us one extreme of the ways that bacteria can make the most of animal hosts," explained Dr Julian Parkhill of the Wellcome Trust Sanger Institute. "Some organisms use subtle approaches, elegantly choreographing their interaction with us and our defences.

"*C. botulinum* takes the opposite approach. It lies in wait and, if it gets the opportunity, it hits its host with a microbial sledgehammer. It then eats the remains and lays low until the next host comes along."

The genome sequence is peppered with genes that produce enzymes to digest proteins and other animal material in the soil. Also found, uniquely in this species, is a range of genes that allow it to attack the many insect and other small creatures that live in the soil. The

'chitinases' produced by these genes can degrade the casing of insects and small crustaceans.

It is not only animals that can feel the wrath of *C. botulinum*, explains Dr Sebaihia: "The soil can be a harsh environment and food can be scarce. To see off the competition, *C. botulinum* comes with its own 'antibiotic' - a chemical called boticin that kills competing bacteria."

Genome sequences can tell us a lot about the biology of the organism, but research into clostridia has been hampered by the lack of a good genetic system. Professor Nigel Minton, Professor of Applied Molecular Microbiology at the University of Nottingham, has developed new methods to knock out genes in clostridia.

"Even after decades of research, only a handful of mutants had been made in clostridia, and none in *C. botulinum*," Professor Minton explains. "We have developed a highly efficient system, the ClosTron, with which we have, in a few months, knocked out over 30 genes in four different clostridial species, including eight in *C. botulinum*. The availability of this tool should revolutionise functional genomic studies in clostridia."

This remarkable, stable genome demonstrates the wide range of strategies used by bacteria to enhance their chances of survival. For the Clostridia, these range from the approach used by *C. difficile* - long-term interaction with hosts, which involves evading the immune system and countering antibiotics - to the single-minded opportunistic approach of *C. botulinum*.

Source: Wellcome Trust Sanger Institute

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