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A. tumefaciens – friend or foe?

In keeping with our garden theme, **Dariel Burdass** takes a look at crown gall, a harmful tumour on trees and shrubs caused by bacteria. She explains how DNA from the bacteria get into the plant genome and stimulate the gall formation, a process that can be exploited by plant biotechnologists.

Agrobacterium tumefaciens

The soil bacterium *Agrobacterium tumefaciens* is mainly found living in the rhizosphere, the area of soil that surrounds the roots of plants. Some strains contain a large plasmid known as the Ti (tumour-inducing) plasmid. A plasmid is a small circular piece of DNA that is separate from the bacterial chromosome. It is these strains that are pathogenic and can infect a wide variety of broad-leaved plants, including fruit trees and shrubs, e.g. pears, apples and roses, causing a tumour-like growth known as crown gall.

Crown gall

The crown of the plant is the area at the base of the stem just before it joins the roots; the majority of crown galls occur here. They vary in size, from 1 to 30 cm, with the majority being around 5 cm across. In the early stage the gall appears as a small round white swelling that is rough and spongy. As it matures it becomes dark brown in colour, hard and woody. The galls can obstruct the movement of nutrients and water through the plant tissue causing the infected plant to become stunted and unhealthy. These plants are also less resilient to changes in temperature.

There is no cure for the infected plant. This has huge financial implications for commercial growers as diseased plants have to be destroyed.

Entering a plant cell

A. tumefaciens can only enter plant cells through a wound. Phenolic compounds such as flavonoids are secreted from damaged tissue. The bacteria are attracted to these signalling molecules by chemotaxis and move towards the wound. *A. tumefaciens* then binds to the plant cell and is ready to transfer its Ti plasmid.

Plants can become damaged during cultivation processes such as grafting and pruning, by soil insects and by the effects of weather such as freezing temperatures and wind abrasion.

The role of the Ti plasmid

The bacterium causes tumours by transferring a small section of the Ti plasmid called T-DNA into the plant cell where it becomes integrated into the plant genome. This process resembles bacterial conjugation. The Ti plasmid carries genes which are responsible for the transfer of the T-DNA. These genes are switched on by the phenolic compounds secreted from the wound. The T-DNA carries genes which encode enzymes that cause the overproduction of the plant hormones auxin and cytokinin. These hormones stimulate the uncontrollable and rapid growth of plant cells, leading to the formation of a tumour. T-DNA also induces the plant cells to produce opines (amino acids) which are used as a food source by the bacterium.

Biotechnology applications

Although *A. tumefaciens* is a plant pathogen, its abilities can be exploited by scientists for beneficial purposes. It is widely used as a vector for transferring genetically engineered DNA into the genome of plant cells. For example, the genes that encode tumour production on the T-DNA section of the Ti plasmid are replaced in the laboratory by genes that encode a desirable trait such as herbicide tolerance. These engineered genes then become incorporated into the plant genome.

◄ Crown gall on *Euonymus* sp. *Geoff Kidd / Science Photo Library*

Weed management

If weeds are poorly controlled then this affects both the quantity and quality of the crop that is harvested. This is because weeds compete with the plant for food, light and water. The herbicide glyphosate is used to control weeds in fields where soya beans are grown. Glyphosate rapidly breaks down on contact with the soil, reducing its effect on the environment and making it more desirable to use than other more persistent chemicals. Unfortunately, glyphosate is non-selective and does not discriminate between weeds and crops. It can only be applied to the weeds before the soya plants emerge from the soil.

Genetic engineers have developed transgenic soya plants that are tolerant to the herbicide glyphosate. This means that the weeds can be sprayed even after the crop has started to grow.

How is this done? Restriction enzymes are used to cut out the T-DNA. The sequences at either end of the T-DNA are necessary for the transfer of the genetic material and remain. The foreign gene encoding the herbicide-resistant trait is inserted between these two end sections. A marker gene is also incorporated into the plasmid to identify plants that have accepted and expressed the foreign gene. This recombinant plasmid is then transferred back into *A. tumefaciens*.

The next stage in the process is the infection of the soya plant with the engineered plasmid and the integration of the foreign gene into the plant genome. Transgenic soya plants are generated containing the foreign gene, making them resistant to the herbicide glyphosate.

Useful resources

Madigan, M.T., Martinko, J.M. & Parker, J. (2003). *Brock Biology of Micro-organisms*, 10th edn. New Jersey: Pearson Education.

http://helios.bto.ed.ac.uk/bto/microbes/crown.htm

SGM supports training boost for student biology teachers

This event was supported by a grant from the SGM Public Understanding of Science Fund. See **www.sgm.ac.uk/grants**

Last session, every trainee teacher of secondary biology across Scotland attended a 3-day *Biotechnology for Scotland* residential school, hosted by the University of Aberdeen. The aim was to bring them up-to-date with recent developments, in recognition of the need for a teaching force that is well informed about the rapidly changing field of biotechnology. Delegates took part in lectures, laboratory sessions, industry visits and other activities. The programme built on the experiences from a pilot event run in Glasgow in 2003. Parts of it were intended to help the students raise, with their own pupils, some of the societal issues related to the new technologies.

The SGM grant helped to run the practical microbiology sessions where the students gained hands-on experience of activities suitable for use with pupils in the school laboratory. The procedures included examples of bioremediation with the fascinating use of *Photobacterium phosphoreum* as a biosensor and of soil micro-organisms to degrade hydrocarbons.

'Microbiology made easy' consisted of experimental work designed to engage early secondary pupils. It is hoped that this will help pupils to more readily understand the science behind everyday issues of hygiene and other directly relevant applications of microbiological principles. All of the participants were provided with a full set of practical support materials and resources such as SGM's *Microbes and Food* posters.

This event resulted from collaboration between the five Initial Teacher Training Institutes in Scotland and was facilitated by the Scottish Institute of Biotechnology Education and the Science and Plants for Schools Biotechnology Scotland Project (SAPS Scotland).

Dr Peter Shand, a lecturer in the School of Education at Aberdeen said, '*The event has allowed teacher training institutes to develop closer links with each other and with the various organizations involved in biotechnology education*'. Professor Ian Booth of Aberdeen was closely involved with the development of the programme. He also gave a talk about harmful and beneficial micro-organisms which was of direct relevance to the Scottish school curriculum. The university provided first-class laboratory facilities and identified speakers. Other microbiology-based activities included a visit to the Glendronach distillery and the development of resources on the role of micro-organisms in waste management.

Feedback from the attendees was very positive. '*I really enjoyed the week. I thought the resources and facilities were excellent. I feel more confident now about delivering biotechnology in the future. It is good to know that there are people and resources available to help.*'

Plans are well underway for a third residential event for PGCE Biology students in Scotland. For the first time other science subject specialists will join the biologists. At least one of the sessions will provide microbiology activities.

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