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Antibiotic resistance and Syngenta's Bt 10 maize
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On 22nd March 2005, the journal Nature revealed that Syngenta had inadvertently produced and distributed a variety of GM maize, Bt10, which did not have regulatory approval.[1] Between 2001 and 2004, several hundred tonnes of the Bt10 maize had been distributed and grown in the US and probably exported elsewhere. The breach was reported by the company to the US authorities in December 2004, but was not made public until 3 months later. The Bt10 maize is one of Syngenta's experimental lines of insect resistant maize incorporating a toxin gene from the bacterium, Bacillus thuringiensis, and was not intended to be commercialised. Originally, in making reassurances about safety, the company emphasised the similarity between the insecticidal Cry1a toxins produced by Bt10 and another GM maize variety Bt11, which has approval in the USA.[2] However, later it emerged that Bt10 also contains a gene that gives resistance to the antibiotic ampicillin.[3] Syngenta will not disclose the full details of how Bt10 has been genetically modified, but have said that it also contains the pat gene, which gives tolerance to the herbicide glufosinate (Liberty).[4]

Syngenta state that "there are no human, health or environmental concerns with Bt10 maize".[5] However, in 1996, the UK Government voted against the approval of another Syngenta GM maize variety, Bt176, because of the risk to human health associated with the ampicillin resistance gene it contained. Syngenta have confirmed that Bt10 contains the same ampicillin resistance gene together with the same promoter and terminator sequences as Bt176.[4] This briefing examines the issues involved and why the presence of the ampicillin gene in Bt10 cannot be dismissed as unimportant. In this respect, it is important to realise that the ampicillin resistance gene in Bt10 maize, amp^r, does not give resistance to ampicillin alone, but also to some other members a group of antibiotics known as the beta-lactams or broad spectrum penicllins, including:[6] benzyl penicillin, ampicillin, amoxycillin, phenethicillin, carbenicillin, methicilin, flucloxicillin and cloxacillin

What are antibiotic resistance genes and why are they used in GM plants?

Antibiotic resistance genes are used in genetic modification as marker genes – to identify when genes have been successfully transferred from one organism to another. This is necessary because the transfer process is very inefficient with only a small proportion of attempts being successful. The bacteria or plant cells that have been successfully modified and acquired an antibiotic resistance gene along with other gene(s) which give desired characteristics such as insect resistance, will survive in the presence of the antibiotic which would normally kill the cell if it had not been transformed.

In the case of Syngenta's GM maize varieties, the ampicillin resistance gene is used at a very early stage in the construction of the plasmid vectors used to transform the maize itself. The gene has no purpose in the final plant and mechanisms exist to remove marker genes.[7]

How could the ampicillin resistance gene cause problems?

The concerns surrounding ampicillin resistance genes in GM plants centre on the possibility that the resistance gene could be transferred into bacteria in the environment or in the intestines of an animal or human that eats the GM crop. If this did happen, the gene may eventually be transferred into a bacterium that causes disease and which will then be resistant to treatment with ampicillin or other beta-lactam antibiotics. Resistance to antibiotics is becoming an increasing problem in the treatment of infectious diseases.

The emergence of antibiotic resistance and its spread across many organisms has been widely associated with misuse of antibiotics in human and veterinary medicine and with connections between the two. Research in the 1970s detected the spread of antibiotic resistance between animals on farms and from farm animals to man[8] and led to restrictions on the routine use of antibiotics in animals. The same researchers called for the use of antibiotic markers to commonly used antibiotics not to be used in genetic engineering research, considering that the incidence of undesirable antibiotic resistance could be increased if bacteria acquired the genes.[9]

What evidence is there that the gene could be transferred?

It is well known that bacteria can exchange genes quite freely. This movement of genetic material between organisms is known as 'horizontal transfer' to differentiate it from the vertical transfer between one generation and the next. Over the past twenty years there has been a burgeoning literature about gene transfer between micro-organisms leaving the impression, reinforced by the way in which antibiotic resistance has spread between bacterial species, that it is an extremely important and influential process. The extent to which horizontal gene transfer can take place between plants and bacteria is much more controversial, however.

There are three mechanisms by which horizontal gene transfer in bacteria is thought to take place:[10]

- **Transformation:** The uptake of free ('naked') DNA from the environment and its incorporation into the bacterial genome.
- **Conjugation:** Movement of DNA between bacteria following cell-to-cell contact and affected by plasmids or transposons.
- **Transduction:** The transfer of genetic material from one bacterium to another by a bacteriophage (an infective particle of bacteria)

For plant DNA to be transferred to a microorganism, transformation is the most likely mechanism to be involved. In this situation, the DNA in the GM plant would have to be released following plant decomposition in the environment or digestion once it has been cooked and eaten. This 'naked' DNA would then have to be taken up by a bacterium it comes into contact with. There are several obstacles to this taking place. Degradation or digestion may break up DNA into pieces that are smaller than the gene itself, in which case a whole operational gene will not be transferred. Furthermore, not all species of bacteria can take up DNA by transformation, some similarity between the DNA taken in and an organism's own DNA maybe needed for successful integration, and the efficiency of uptake can be affected by environmental conditions. Therefore, the transformation of bacterial DNA by plant DNA is likely to be a very rare event.

However, rare events can arise if the scale of contact is great or prolonged enough and continuous consumption or growing of a GM crop may represent one such situation. Furthermore, because of the large numbers of bacteria in the intestine and environment, difficulties in culturing some of them coupled with other methodological problems means that detecting rare transformation events is extremely difficult. As recent analyses have emphasised,[11,12] absence of evidence of horizontal gene transfer cannot be taken as evidence of absence. The selective pressures placed on an organism will be crucial in whether an organism which acquires a gene through horizontal gene transfer becomes established and multiplies and there will be an inevitable time lag involved, so significant transformations may not be detected for a long time. Therefore, negative results have to be viewed with considerable caution. For example, studies failing to detect transfer of ampicillin genes from GM maize, Bt176, to soil microorganisms,[13] included many samples but overall less than 2g of soil was examined.[11] Studies that concluded that an ampicillin marker gene did not survive passage through the intestinal tract of chickens, used only five chickens given GM feed for five days.[14] It is the nature of the gene and potential impacts if a rare transfer event were to arise that is a more important question than its frequency and one that makes antibiotic resistance so important.

In addition, there is evidence that suggests that horizontal gene transfer from plants to bacteria is not a purely theoretical issue. This includes:

- In the laboratory under optimal conditions, when there is sequence similarity between the plant transgene and the bacteria involved and selection pressure, horizontal gene transfer from plant material to bacteria has been detected.[15]
- Research showing that some genes are likely to survive the passage through the human small intestine and would be able transform bacteria in the large bowel.[16]
- Studies showing DNA can survive in the saliva of sheep for periods of time sufficient to transform bacteria.[17] Transformation of oral bacteria in human saliva by naked DNA has been shown.[18]
- Intact transgenes from GM maize were found intact in the rumen for five hours after feeding maize grains, and thus available to transform bacteria. In contrast, genes did not survive intact in silage.[19] Natural transformation of rumenal bacteria by naked DNA has been demonstrated.[20]
- DNA from GM crops has been shown to persist in soil for two years.[21]
- Gene transfer from GM soya to unidentified microorganisms in the intestines in samples taken of one of seven patients with illeostomies.[22]

As well as the potential for transformation of bacteria by genes from plants being real, the evolution of antibiotic resistant strains of disease causing organisms has already arisen though uptake of naked DNA. Gene transfer by transformation is considered responsible for the evolution (through a different mechanism than that encoded by the amp^r gene) of Beta–lactam resistant strains of *Haemophilus influenzae* and *Neisseria. gonorrhoeae*, as well as penicillin-resistant *Streptococcus pneumoniae*.[23]

The special issues associated with ampicillin resistance in Bt 10

In producing GM crops such as Bt10, Bt11 and Bt176 maize, Syngenta use a plasmid known as pUC18 that carries the ampicillin resistance gene. Plasmids are circular pieces of DNA, constructed in the laboratory to facilitate the transfer of genes into the host organism, but plasmids also occur naturally in bacteria. Bt10, like Bt176 (but not Bt11), contains an intact ampicillin resistance gene with promoter and origin of replication (*ori*) sequences derived from the pUC18 vector – these sequences regulate when the gene is copied by how much. Although the amp^r gene exists in nature, its natural plasmid carrier *ori* sequence gives a low copy number – about 4-18 copies per cell. The pUC18 vector does not occur in nature and has a mutation in the *ori* sequence that mean it can produce over 150 copies.[24] Scientific advisors in the UK consider will this high copy number increases the risks arising from transfer as much higher levels of the enzyme which causes resistance by breaking down ampicillin would be produced. [25] Because of this, the UK voted against the approval of Bt176, although this was overturned in Europe and marketing consent was given.

Is ampicillin an important antibiotic?

Since the potential for horizontal transfer of the amp^r gene cannot be ruled out, the consequences of increased resistance have to be considered in the context of how important the antibiotic is. A survey of antibiotic prescriptions in England and Wales between 1994 to 1998, shows that the 'broad-spectrum penicillins', which includes the beta-lactam antibiotics, were the most commonly prescribed antibiotics, making up 40% of all antibiotic prescriptions.[26] These antibiotics also have similar importance across the rest of Europe[27]. According to Glaxo SmithKline, ampicillin is indicated in the treatment of "ear, nose and throat infections, bronchitis, pneumonia, urinary tract infections, gonorrhoea, gynaecological infections, septicaemia, peritonitis, endocarditis, meningitis, enteric fever, [and] gastro-intestinal infections".[28]

In veterinary medicine, around 50 tonnes of beta-lactams are used in the UK each year .[29] Beta-lactams are used to treat skin, genito-urinary, upper respiratory tract infections, pneumonia and gastroenteritis in a wide range of species.[30] Clearly, beta-lactams are an important front-line antibiotic in both human and veterinary medicine.

Conclusions

Some scientists and regulators argue that there are no risks from the horizontal gene transfer of the ampicillin resistance gene from GM crops because:[25,31]

1) the probability of DNA survival in segments large enough to be taken up and be functional is very low;
2) the probability of bacteria taking up, incorporating and expressing DNA is virtually zero;
3) the clinical significance is virtually zero because ampicillin resistance is widespread and can be overcome by antibiotics other than ampicillin.

It is quite clear that the beta-lactam, broad spectrum penicillins are of considerable clinical importance. Despite claims of widespread resistance, others have emphasised how resistance is far from ubiquitous. The UK's Defra Antimicrobial Resistance Coordination (DARC) Group, said in 2004 that "a significant number of species of veterinary bacteria remain fully susceptible to beta -lactam compounds, such as ampicillin, despite continued therapeutic use of these compounds for decades. Considered against this background of extremely low or no detected resistance in certain bacterial species of veterinary origin, any occasional transfer of resistance genes to these organisms would be a very significant event and we do not feel that the potential hazard to animal health should be characterized as slight in such circumstances."[32] Similarly in human medicine, although beta-lactam resistance in increasing in some organisms, many remain sensitive and any factors which may increase the scope for further antibiotic resistance remain undesirable.

Research has shown that DNA can survive intact in the mouth, intestinal tract and soil for sufficient periods of time for bacterial transformation with intact genes to take place. Whilst transformation events may be rare, the scale and frequency of exposure between a GM food and bacteria in a human or animal body or environment, mean such events will arise. The difficulties in identifying when horizontal gene transfer has taken place has led scientists to conclude that the most important consideration is what the likely selection pressures will be, as these will be a more important factor in driving outcome than frequency of transfer events.[11,12,15] In relation to ampicillin resistance, the widespread use of the beta-lactams antibiotics, suggests that a selection advantage would be gained if resistance was acquired. The ampicillin resistance gene in Bt10, as in Bt176 maize, poses particular problems because of the regulatory *ori* sequence that accompanies it that could give an additional selection advantage.

Therefore, Syngenta and the US regulators are wrong to dismiss the risks of the use of ampicillin resistance genes in plants. The assumptions they make and evidence they rely on to support their claims always take an optimistic view of the likelihood of harm arising which is not supported by the latest scientific thinking. A precautionary approach to antibiotic resistance marker genes, as endorsed by the UK's Advisory Committee on Novel Foods and Processes,[33] would be more rigorous about the uncertainties and, considering the importance of beta lactam antibiotics in the treatment of common infections, rule out the use of antibiotic resistance marker genes in GM crops. Whether the presence of ampicillin marker genes in Bt10 or Bt176 maize has led to the emergence of new strains of resistant bacteria may not be evident for some time. The lessons from the use of routine antibiotics on farms show that even isolated, single farm events, can be significant.

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