

An assessment of the risks associated with the use of antibiotic resistance genes in genetically modified plants: report of the Working Party of the British Society for Antimicrobial Chemotherapy

P. M. Bennett^{1*}, C. T. Livesey², D. Nathwani³, D. S. Reeves⁴, J. R. Saunders⁵ and R. Wise⁶

¹Department of Pathology and Microbiology, University of Bristol, Bristol BS8 1TD; ²Central Veterinary Laboratory, New Haw, Surrey KT15 3NB; ³Ninewells Hospital, Tayside University Hospitals, Dundee DD1 9SY; ⁴Journal of Antimicrobial Chemotherapy, Editorial Office, Birmingham B1 2JS; ⁵School of Biological Sciences, University of Liverpool, Liverpool L69 7ZB; ⁶Department of Medical Microbiology, City Hospital NHS Trust, Dudley Road, Birmingham B18 7QH, UK

Development of genetically modified (GM) plants is contentious, in part because bacterial antibiotic resistance (AR) genes are used in their construction and often become part of the plant genome. This arouses concern that cultivation of GM plants might provide a reservoir of AR genes that could power the evolution of new drug-resistant bacteria. We have considered bacterial DNA transfer systems (conjugation, transduction and transformation) and mechanisms of recombination (homologous recombination, transposition, site-specific recombination and DNA repair) that together might productively transfer AR genes from GM plants to bacterial cells, but are unable to identify a credible scenario whereby new drug-resistant bacteria would be created. However, we cannot entirely rule out the possibility of rare transfer events that involve novel mechanisms. Hence, we also considered if occasional transfers of AR genes (*bla*_{TEM}, *aph*(3'), *aadA*) from GM plants into bacteria would pose a threat to public health. These AR genes are common in many bacteria and each is found on mobile genetic elements that have moved extensively between DNA molecules and bacterial cells. This gene mobility has already severely compromised clinical use of antibiotics to which resistance is conferred. Accordingly, the argument that occasional transfer of these particular resistance genes from GM plants to bacteria would pose an unacceptable risk to human or animal health has little substance. We conclude that the risk of transfer of AR genes from GM plants to bacteria is remote, and that the hazard arising from any such gene transfer is, at worst, slight.

Keywords: GM plants, transfer of resistance genes, spread of resistance genes, risk-hazard assessment

Introduction

The development and cultivation of genetically modified (GM) plants with traits introduced by 'genetic engineering' is contentious, not least because the procedures use bacterial antibiotic resistance (AR) genes to tag DNA sequences that are manipulated in a bacterial surrogate host before their introduction or reintroduction into the plant. The resistance gene, although of no value in the final cultivar, is nonetheless integrated into the plant genome together with the gene(s) of interest where, in the main, it remains silent. There is concern in some quarters that cultivation of GM plants with bacterial resistance genes integrated into their genomes might provide a source of drug resistance genes that could catalyse the development of previously unencountered drug-resistant bacteria of clinical importance. How seriously should such fears be taken and how much credence should be given to claims that large-scale cultivation of GM plants carrying bacterial AR genes poses an unacceptable threat to future chemo-

therapy of infectious diseases? These were the questions addressed by the Working Party.

GM crops are created in the laboratory by precision genetic engineering, in the sense that specific genes of the plant are altered or removed, or particular additional genes are introduced by *in vitro* techniques. Genetic engineering technology has provided manipulative methods that permit the introduction into crop and ornamental plants of desirable traits, or the removal of unwanted ones, more or less at will and with almost surgical precision.¹ The cultivars so created have directed changes to their genomes. This is in contrast to the traditional method of developing new cultivars. Until the last decade or two, desired traits were necessarily bred into plants by identifying rare variants with new characteristics, either naturally occurring or as a result of forced mutation, and crossing and backcrossing these until a cultivar with the desired characteristics was obtained. Although the process is time consuming, and substantially 'hit-and-miss', it was effective, as attested by the enormous numbers of crop and ornamental

*Corresponding author. Tel: +44-117-9287897; Fax: +44-117-9287896; E-mail: Peter.M.Bennett@bristol.ac.uk

Review

plant cultivars available today. However, unlike GM technology, the genetic changes involved in the creation of a new cultivar by traditional methods are largely unknown. Nonetheless, despite our ignorance of the genetic changes that have occurred, this type of genetic manipulation is viewed as both benign and acceptable, presumably because it is seen as 'natural', and long experience has demonstrated it is not harmful to human well-being.

In contrast, genetic modification technology allows much more precise genetic changes to be made.² Genes may be added, changed, deleted, or have their expression altered, to add, remove, or enhance a particular trait (e.g. improved seed yield, shorter stems that better resist wind damage, herbicide resistance, frost resistance, salt tolerance, etc.). Unlike traditional crop development methods, the use of genetic modification techniques in the creation of new cultivars requires precise and detailed information about the genes involved and the biochemistry of their products, and the genetic changes undertaken are carefully targeted. Nonetheless, this form of genetic manipulation is viewed in some quarters at best with suspicion and at worst with outright hostility, because it is seen to transgress the natural order, by meddling with nature. Is there a rational justification for such hostility? Are GM crops inherently less safe than other cultivars? Do GM crops carrying bacterial AR genes present a threat to human or animal health because they are likely to compromise antibiotic therapy in the future? This document attempts to answer the second of these questions.

Generation of GM crops

Genetic modification techniques involve cloning plant genes into a bacterial cell, usually a suitable non-pathogenic laboratory strain of *Escherichia coli*, where they are manipulated before being put into the target plant. To achieve this, an appropriate small fragment of the plant DNA, carrying the gene(s) to be manipulated, is inserted into a small bacterial DNA molecule called a cloning vector.³ Many cloning vectors are small bacterial plasmids that carry AR genes with which they can be both identified and selected in the laboratory. The plant DNA in the cloning vector is changed as required for the trait of interest. All such DNA manipulations are carried out in the test tube and a bacterial host is used to recover and amplify the new gene arrangement. This is then put into a plant cell of the appropriate species in a process called transformation. From single, transformed cells, new plant cultivars are propagated.^{1,4}

Genetic constructs, assembled *in vitro* and recovered in a bacterial host, can be delivered into plant cells in different ways.^{1,4} For example, one exploits the tumour-inducing (Ti) plasmid of *Agrobacterium tumefaciens* that naturally transfers part of itself, called T-DNA, into certain plant cells where it is integrated into the plant cell DNA.^{5,6} In the natural system, the T-DNA re-programs the plant cell to produce plant growth regulators, which promote plant cell proliferation, and small nitrogenous organic molecules (opines), which are secreted and then used by *A. tumefaciens* as nutrients for growth,^{7,8} (i.e. the bacteria genetically modify plant cells to create nutrient-generating systems). These naturally transformed plant cells become visible on plants as crown gall tumours. When this natural system is used to genetically engineer plants, the genes on the T-DNA responsible for mediating the change in plant cell metabolism are removed and replaced by the manipulated plant DNA and, if necessary, a linked resistance gene to facilitate selection of the desired DNA rearrangement. The natural transfer properties of the Ti plasmid or a surrogate plasmid, are then used to deliver the modified T-DNA sequence into

the plant cell where the natural integration properties of the T-DNA insert the entire gene ensemble into the chromosomal DNA.^{6,9-11}

Engineered plant DNA can also be delivered into plant cells by particle bombardment (biolistic transformation), when the DNA is literally shot into plant cells on DNA-coated metal particles (often gold).¹²⁻¹⁴ In this case, the DNA is permanently established in the plant cell by recombination into the plant cell genome. Insertion may be locus-specific by homologous recombination (see sub-section Homologous recombination in section Bacterial DNA transfer and recombination systems), but can be random, being mediated by a recombination mechanism intrinsic to the plant cell. Once established in a chromosome of a plant cell, bacterial DNA sequences are chemically indistinguishable from the rest of the plant cell DNA. The origin of the sequence, in terms of its subsequent treatment in the cell (i.e. replication and segregation) is irrelevant. To all intents and purposes it becomes plant cell DNA. This is an important point to bear in mind when considering possible retransfer of resistance genes from GM plants to bacteria, because many bacteria have DNA recognition systems that identify foreign DNA and degrade it¹⁵ (see section What if antibiotic resistance gene transfer occasionally occurs from GM plants to bacteria?).

Bacterial AR genes are used in two different contexts to generate GM plants. In one context, a bacterial resistance gene is used to select transformed plant cells, when there is no direct selection for the trait of interest.¹⁶ To this end, the transcription and translation signals that promote expression of the resistance gene in bacteria are removed and are replaced with signals appropriate for plant cells. The resistance gene with its replacement expression signals is joined to the manipulated plant gene to provide a selectable, linked marker. Transformed plant cells, selected for acquisition of the resistance trait, carry not only the AR gene but also the linked plant gene. Once the appropriate plant cell constructs have been identified, the job of the AR gene is complete and the gene is essentially redundant, although it will be an inherited trait.

In the second, and more common, context, AR genes on the cloning vector are used simply to recover and keep track of the cloning vector and its plant DNA insert in the bacterial host,³ before the final genetic construction is returned to the plant system. This is necessary because plant genes are not expressed in bacteria and so do not alter the phenotype of the bacterial host. Accordingly, the plant genes are tracked by genetic linkage. In this situation the normal transcription/translation signals of the resistance genes needed for expression in bacteria are retained. The resistance genes generally also end up in a transformed plant cell, simply because they are linked to the plant genes of interest and cannot easily be uncoupled from them. When put into a plant cell, these bacterial resistance genes are silent because they lack the appropriate and necessary signals for expression in cells of higher organisms. They are not needed to select transformed plant cells and hence serve no useful purpose whatsoever in the transformed plant cell and could, in principle, be dispensed with before the altered plant DNA is reintroduced into plant cells.

If bacterial resistance genes, with bacterial promoters, were to be transferred from a GM plant to bacteria they would, in all probability, be expressed and would confer AR phenotypes on the recipients. In this sense, therefore, the GM constructions that concern us here are not as precise as they might be; it would clearly be desirable to jettison all resistance genes that are not needed for selection of transformed plant cells before transformation. Molecular genetic systems that will do just that are being developed,^{17,18} however, even if one was available, it could not be used to remove the bacterial resistance genes in the present generation of GM crops, whether silent or not. It will be

Review

several years before GM crops developed with the new technology will be available for cultivation. In the meantime, GM crops with bacterial resistance genes are with us. It would also be desirable to have selectable markers other than bacterial resistance genes with which to select desired transformed plant cells. Again, developments are in progress. In the meantime, it is worth emphasizing that a full inventory of the genetic alterations involved in a particular GM construction, including carriage of a bacterial resistance gene, is made for licensing purposes in the European Union, including the UK.¹⁹ In the UK, such manipulations are permitted only in appropriately authorized laboratories that operate to strict Health and Safety Executive (HSE) guidelines;²⁰ all constructions must be judged biologically safe (i.e. of no danger to human health or the environment) first by local and then by national genetic manipulation safety committees before work commences; and constructs generated abroad must have HSE approval before being imported, either for further GM manipulation or for trial work. Although different approaches to regulation of GM crops have been taken in different countries, the same basic objectives of ensuring human, animal and environmental safety are held in common.²¹ In contrast, cultivars created by conventional breeding programmes, using natural or forced variants, or wild relatives that may contain potential toxins or allergens, are not subject to the same strict regulations, but are assumed by consumers to be both safe and wholesome, neither of which is a necessarily safe assumption.¹

As stated, when the construction of a GM plant requires the expression of a resistance gene in the plant cell, the normal bacterial promoter and translation control signals are removed and replaced with signals appropriate for expression of the resistance gene in plant cells. Reintroduction of these resistance determinants into bacterial cells would not immediately confer resistance, because the genes would not be expressed from the plant cell promoter and so would remain silent. To be expressed in the new bacterial host, further genetic changes would be needed, in particular the substitution of bacterial promoters for plant cell promoters to permit transcription of the resistance genes and acquisition of prokaryotic translation signals to allow translation of the gene transcripts (i.e. translation of mRNA). Each substitution would be a not impossible but rare event. Activation of silent bacterial genes,^{22–25} including resistance genes,^{26,27} has been reported but these mutational events are rare and only involved provision of a suitable promoter. The genes already had functional bacterial translation signals.

Risks associated with use of bacterial resistance genes in the generation of GM plants

The risks of using bacterial AR genes in the construction of GM plants, where they are introduced into a commercial cultivar, can be defined as direct or indirect. Direct risks are those concerning plant tissue that becomes toxic to anyone/anything consuming it (cf. the native crop that is non-toxic). Indirect risks are those pertaining to adverse effects on human health, other than toxicity, such as damage to the quality of life or to the environment, that arise from GM crop cultivation.

Direct risks

These can be evaluated as:

1. Introduction into the plant of a toxic DNA sequence (i.e. the particular section of DNA is toxic to man). This is a strictly hypothetical situ-

ation, since it is generally accepted that all DNA sequences behave chemically in the same way, in that there is no history of any piece of DNA being toxic to humans or any other animal and that large quantities of DNA are eaten by everyone on the planet on a daily basis as an integral component of food without harm;

2. Production of toxicity as a consequence of expression of the bacterial genes (i.e. production of toxic RNA or toxic proteins from the bacterial DNA sequence);

3. Production of a toxic compound arising from the activity of the product of the bacterial gene in the plant.

The evidence to date would indicate an absence of toxicity arising from the genetic alterations involved in GM plant creation, including the acquisition of the resistance genes used in their construction. Animal feeding experiments have indicated no adverse effects, other than when known toxic products have been administered,²⁸ whereas GM crop products have been consumed by farm animals and the American public for more than 15 years with no indication of toxicity of any form.¹⁸ There is also no evidence of additional allergy risk,^{29–31} except when a known allergen is transferred from one plant to another when its allergenic character is retained and cotransferred; witness production of the 2S-albumin protein of the Brazil nut by soy bean.³² These data do not, of course, rule out the development of toxicity for an animal or insect species not tested, particularly when plants are engineered to produce specific insecticidal proteins; witness, for example, the report that the pollen of GM maize (Bt corn), which was genetically modified so as to produce a *Bacillus thuringiensis* insecticidal protein to control the European corn borer beetle, shows a degree of toxicity for caterpillars of Monarch butterflies, albeit under somewhat artificial rearing conditions,³³ giving cause for concern that Bt corn pollen might travel beyond corn fields and cause mortality in non-target lepidopterans. In response to this finding, a field study designed to test the general thesis examined survival of larvae of the black swallowtail butterflies when exposed naturally to pollen from Bt corn. The investigators found no evidence for toxicity arising from GM-maize pollen and concluded that the pollen of the maize tested is unlikely to affect wild populations of black swallowtails.^{34,35} In addition, it should be borne in mind that tons of *B. thuringiensis* insecticidal proteins, in the form of *B. thuringiensis* cells containing the toxins, which are not harmful to humans or animals, are sprayed annually on a variety of plants and trees to control a variety of insect pests. The direct risk to human health as a consequence of consumption of GM crops is likely to be no greater than that associated with non-GM crops, and GM cultivars will have been subject to much more rigorous assessments for detrimental health consequences than so-called naturally produced cultivars.^{1,30} Accordingly, a direct risk to human health arising from the resistance genes in GM crops can be discounted.

Indirect risks

These include:

1. Spread of resistance genes from GM crops to other plants;
2. Increased opportunities for AR genes to spread among human/animal bacterial pathogens (actual or potential).

The risk of any gene introduced into one plant escaping to other closely related plants through cross-pollination has to be real once plants are mass cultivated. Does this matter for bacterial AR genes? Escape of a drug resistance gene(s) to another plant will certainly expand the particular gene pool, but does this significantly affect the

Review

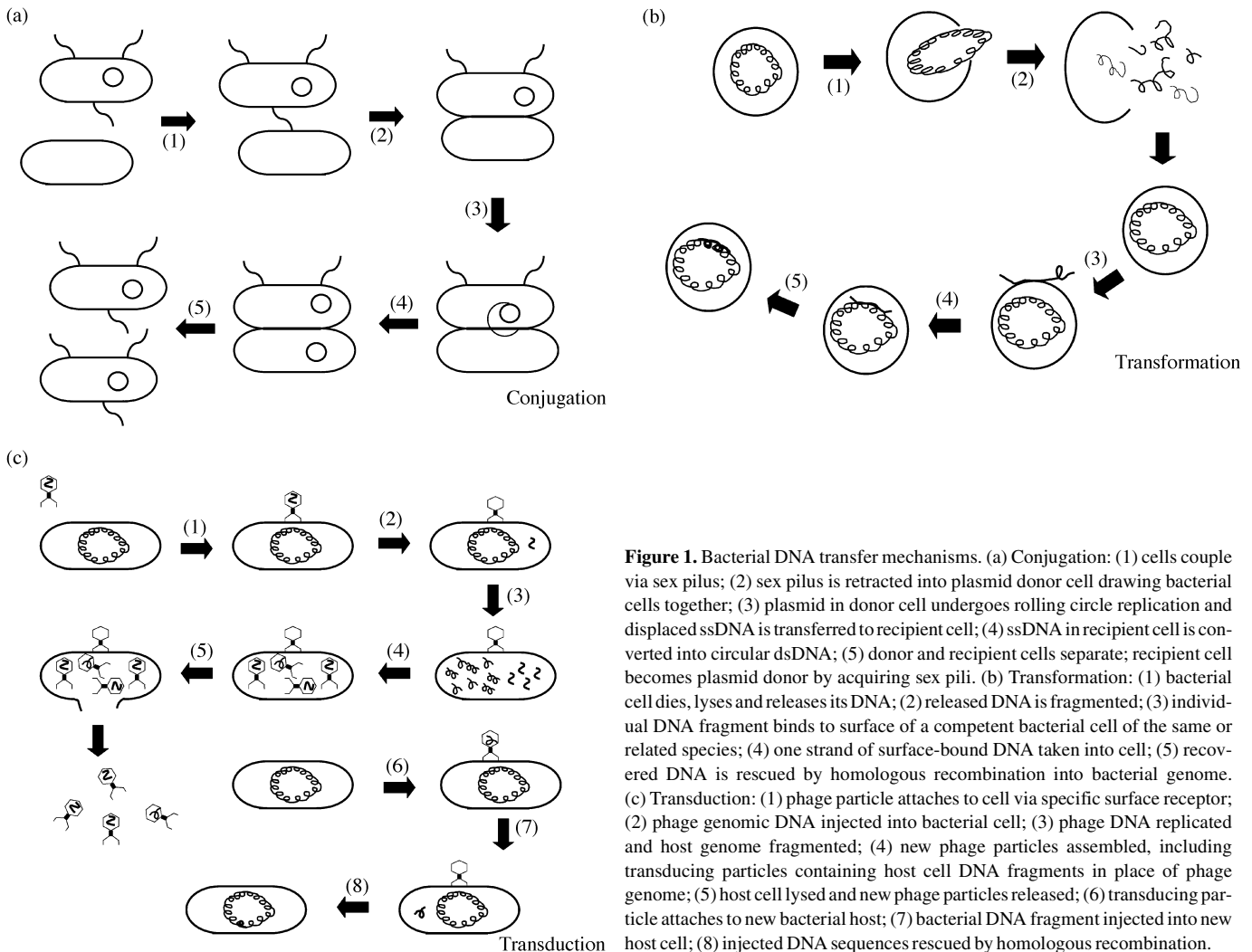


Figure 1. Bacterial DNA transfer mechanisms. (a) Conjugation: (1) cells couple via sex pilus; (2) sex pilus is retracted into plasmid donor cell drawing bacterial cells together; (3) plasmid in donor cell undergoes rolling circle replication and displaced ssDNA is transferred to recipient cell; (4) ssDNA in recipient cell is converted into circular dsDNA; (5) donor and recipient cells separate; recipient cell becomes plasmid donor by acquiring sex pili. (b) Transformation: (1) bacterial cell dies, lyses and releases its DNA; (2) released DNA is fragmented; (3) individual DNA fragment binds to surface of a competent bacterial cell of the same or related species; (4) one strand of surface-bound DNA taken into cell; (5) recovered DNA is rescued by homologous recombination into bacterial genome. (c) Transduction: (1) phage particle attaches to cell via specific surface receptor; (2) phage genomic DNA injected into bacterial cell; (3) phage DNA replicated and host genome fragmented; (4) new phage particles assembled, including transducing particles containing host cell DNA fragments in place of phage genome; (5) host cell lysed and new phage particles released; (6) transducing particle attaches to new bacterial host; (7) bacterial DNA fragment injected into new host cell; (8) injected DNA sequences rescued by homologous recombination.

perceived risk to human/animal health? Does the toxicity argument change? Does the expansion of the resistance gene pool significantly affect the risk of reintroduction of the resistance genes into the bacterial gene pool via a new host? We would judge not. Productive gene transfer from a plant to a bacterial cell (i.e. gene capture and expression) is likely to be a very rare event, if it happens at all (see sub-sections Transformation and Homologous recombination in section Bacterial DNA transfer and recombination systems). There is no reason to believe that gene transfer from one plant species to bacteria is likely to be any more common than from another; however, we are not aware of any attempt to investigate the matter systematically, so categorical assurance cannot be given.

The creation and extensive cultivation of GM crops carrying bacterial drug resistance genes expands the pool of resistance genes in the environment. This need cause no concern in itself, if there is no direct risk to human health or the environment and the risk of transfer of the genes to bacterial pathogens that currently lack them can be discounted. When considering this, it should be emphasized at the outset that there is no documented example of a mechanism that will mediate transfer of genes from higher organisms, such as plants, to bacteria, so as to expand the gene pool of the recipient bacteria (but

see below). However, it should be kept in mind that this may reflect lack of information, rather than lack of transfer.³⁶

Bacterial DNA transfer systems

DNA transfer between bacterial cells has been extensively demonstrated,³⁷ and specialized plasmid-mediated transfer of DNA between certain bacterial species and particular plants has been well characterized.^{7,38} More recently, it has been demonstrated that some broad host range bacterial plasmids can mediate DNA transfer between bacteria and a selection of eukaryotic cells.³⁹ Transfer in the opposite direction has not been demonstrated.

DNA can be transferred from one bacterial cell to another by three mechanisms (Figure 1) discussed next.

Conjugation

This requires the participation of a conjugative plasmid or conjugative transposon (Figure 1a).⁴⁰ Although some of these systems can mediate DNA transfer from bacteria to cells of higher organisms,³⁹ including plant cells, we can find no report of DNA transfer in the reverse direction, from a plant into a bacterial cell, except perhaps in the distant past.⁴¹ Given that this would almost certainly require the

Review

expression of a set of bacterial genes in a eukaryotic background, for which there is no contingency on any of the conjugative plasmids that have been fully sequenced,⁴⁰ the likelihood of gene transfer from GM plants to bacteria by a conjugation mechanism is remote.

Transformation

This requires no connection between the donor and recipient cells, save the DNA itself (Figure 1b). In this case, the bacterial cell takes up 'naked' DNA from its environment and incorporates it into its own genome. Although few bacteria appear to be naturally transformable, exceptions to this generalization include significant human pathogens such as *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis* and *Streptococcus pneumoniae*.⁴² The property is determined genetically and the ability to take up DNA sequences for genetic transformation is termed competence.⁴³ In practice, bacterial transformations involve DNA from the same or a closely related species, because the DNA uptake systems only recognize self-DNA and/or maintenance of rescued fragments requires that they be integrated into the cell's genome by homologous recombination, a process that requires a very close relationship between the DNA sequence being recovered and the DNA sequence at the locus where integration will occur [i.e. the sequence to be rescued and the sequence into which it is inserted essentially have to be one and the same (homologous) (see sub-section Homologous recombination in section Bacterial DNA transfer and recombination systems)]. Hence, we believe that the likelihood of recovery and salvage of a resistance gene from a GM plant by bacteria with no history of carriage of the gene in question is extremely remote.

Transduction

This involves gene transfer by bacterial viruses, called bacteriophages (usually shortened to 'phages') (Figure 1c).⁴⁴ Phages tend to have very narrow host ranges and there is no known example of a phage infecting both bacterial and higher organism cells. Since transduction involves phage reproduction, transfer of genes from a plant to bacteria by this route would require the phage to reproduce in both cell types. There is no precedent for this, so the likelihood of gene transfer from plant to bacteria by this means is also so remote that it can be discounted.

Of course, the arguments briefly outlined above do not consider the possibility of a novel transfer system, yet to be discovered. However, it is unlikely that there is a specific plant-bacteria transfer system that mediates high frequency transfer of DNA from plants to bacteria, because, in general, the transfer would serve no useful purpose, since plant genes cannot be expressed in bacteria without first replacing all the expression signals. There is no sustainable scenario for the argument, either on the basis of selection pressure or spread of selfish DNA (i.e. DNA the sole purpose of which appears to be self-propagation). Further, the known bacterial genome sequences show little or no evidence of gene acquisitions from plants, recently or in the past.^{40,45-47}

For those who wish a more detailed discussion of DNA transfer systems and the arguments for and against DNA transfer from plants to bacteria, this is presented in the section Bacterial DNA transfer and recombination systems, otherwise the reader is directed to section Conclusions regarding recombinational recovery of a bacterial antibiotic resistance gene from a GM plant by bacteria.

Bacterial DNA transfer and recombination systems

Conjugation

This requires the participation of either a conjugative plasmid or a conjugative transposon, each of which encodes a DNA transfer system that has evolved specifically to mediate horizontal transfer of itself⁴⁰ (Figure 1a). Such conjugation systems can also assist in transfer of certain non-conjugative plasmids, providing the non-conjugative plasmid possesses an origin of transfer, *oriT*, and encodes the transfer function(s) specific to process the DNA at *oriT*. Transfer of any other DNA sequence requires it to be attached to the conjugative plasmid or transposon while being transferred. Conjugation is responsible for much of the horizontal gene transfer, particularly of resistance genes, seen among prokaryotic cells, both Gram-negative and Gram-positive.^{44,48} Some bacterial conjugation systems can mediate DNA transfer to eukaryotic cells; indeed, the Ti plasmids of *Agrobacterium* spp. are natural prokaryotic-eukaryotic DNA transfer systems,^{6,7} that can be used to transform plant cells to generate GM crops.^{1,10} However, in these systems DNA transfer has only been reported to occur in one direction, namely, from bacteria to plant cells. Plasmid transfer from bacteria to yeast in laboratory experiments has been documented, but required the use of synthetic chimeric DNA molecules that have two origins of replication, one specific for bacteria, the other for yeast cells.^{39,49} Such chimeric replicons are artificial structures created in the laboratory for the purpose of the experiment and have not been found in nature. Transfer of these chimeric DNA structures in the reverse direction has, to our knowledge, not been reported.

To promote DNA transfer from a plant cell to a bacterial cell, the plant cell would have to carry a plasmid natural to the eukaryotic cell that could promote DNA transfer to bacterial cells (there are no reports of such an element), or a bacterial conjugative plasmid would have to be able to transfer to and be maintained in the plant cell, before retransfer to a bacterial cell. This latter scenario would require expression of a number of plasmid (bacterial) genes in the plant cell. Given the differences in gene expression systems in these two cell types, the probability of this happening for any one transfer gene is remote, let alone for the 20 or so genes needed for conjugal transfer of DNA. Several conjugation plasmids have been fully sequenced and in none of these systems is there any evidence that the transfer genes would be expressed in other than a bacterial cell. Hence, the likelihood of expression of a functional bacterial conjugation system in a eukaryotic cell is vanishingly small, to the point where any risk is purely hypothetical. However, one slightly less fantastic possibility for DNA transfer from plant to bacterium should be considered, namely, retro-transfer (i.e. transfer of DNA from what is formally the recipient in the cross to the donor).

Retro-transfer of DNA

Conjugation involves the coupling of donor (i.e. the cell carrying the conjugative element) and recipient cells and the formation of a DNA transfer pore between the two (Figure 1a). DNA transfer is normally considered to be a one-way process from donor to recipient, driven by replication of the transferring DNA species, but retro-transfer has been demonstrated in bacterial crosses,⁵⁰ defined as transfer of a DNA sequence from the recipient in a bacterial mating to the donor (i.e. the parent with the information for conjugation in the first instance). Retro-transfer can involve plasmids present in the recipient⁵¹ or chromosomal genes^{52,53} and occurs at low frequency. However, there is evidence that the conjugative plasmid in the donor

Review

must first transfer to the recipient before retro-transfer can occur,^{51,54,55} arguing for *de novo* mating (i.e. construction of a new mating bridge, when the original recipient becomes the donor). Thus, since conjugal transfer involves only DNA linked to an origin of transfer, the likelihood of retro-transfer of a marker embedded in one of the chromosomes of the plant cell is remote, but perhaps cannot be ruled out entirely. Even assuming plant chromosomal fragments carrying the bacterial resistance gene were retro-transferred into a bacterial cell, this in itself poses no immediate problem; the most likely fate of such DNA would be degradation, since the transferred DNA would be single-stranded. To pose a problem, the marker would have to be stabilized by integration into one of the replicons in the bacterial cell. The most likely mechanism by which this would occur is homologous recombination (see Homologous recombination below). Two related but different scenarios can be envisaged: recombination between the bacterial resistance gene from the plant and (1) an identical or (2) a similar resistance gene in the bacterial cell (Figure 2). The former situation would create nothing new; the latter would possibly create a new allele of the resistance gene (Figure 2a), the product of which could have properties similar to but different from the products of the original two genes, such as an enzyme with an altered substrate profile. Precedents for this would be the penicillin binding protein (PBP) gene mosaics seen in *N. gonorrhoeae* and *S. pneumoniae*,⁵⁶ which, it must be emphasized, have arisen from DNA transfer from one bacterial species to another.

Retro-transfer of genes from eukaryotic cells to bacteria has not been investigated and so no figure can be assigned as to the likely retro-transfer frequency of a resistance gene embedded in a plant cell chromosome. Given the low frequency of retro-transfer of plasmids between bacterial cells and the evidence that it requires expression of genes (bacterial) on the conjugation element in the recipient,^{51,54,55} the likelihood of retro-transfer of resistance genes in bacterial cell-plant cell crosses, even if it is possible, is so low as to be undetectable in laboratory experiments. However, it should not be forgotten that the number of transfer events in nature is potentially extremely large, so events that are essentially undetectable in laboratory experiments may still occur in nature on rare occasions.

Transduction

This is the transfer of DNA from one cell to another, mediated by a bacteriophage (Figure 1c) and occurs as a consequence of rare errors in phage reproduction, when a small percentage of the phage particles produced contain DNA sequences from the host cell, in place of or in addition to the normal phage genome.^{44,57,58} Transduction is responsible for some of the transfer of bacterial drug resistance genes among clinical strains of *Staphylococcus aureus*,^{59,60} and has been shown to occur among bacteria in natural water systems.^{61,62} Fragments of bacterial genomes or small bacterial plasmids can be moved from one bacterial cell to another in this way. Survival of the DNA in the new cell requires either 'rescue' by recombination or the transferred DNA must be able to replicate independently in its new host (i.e. act as a plasmid).^{59,60} If the DNA is a linear fragment of a larger replicon, such as a fragment of the chromosome of the cell in which the phage reproduced, then any genes on it will only survive if they are rescued by recombination. Recombination can, in principle, be homologous [requiring sequence homology between the sequence to be rescued and the replicon into which the sequence will be incorporated, a mechanism that essentially replaces like with like (see Homologous recombination below)] or illegitimate (i.e. not requiring homology between the sequence to be rescued and the recovery

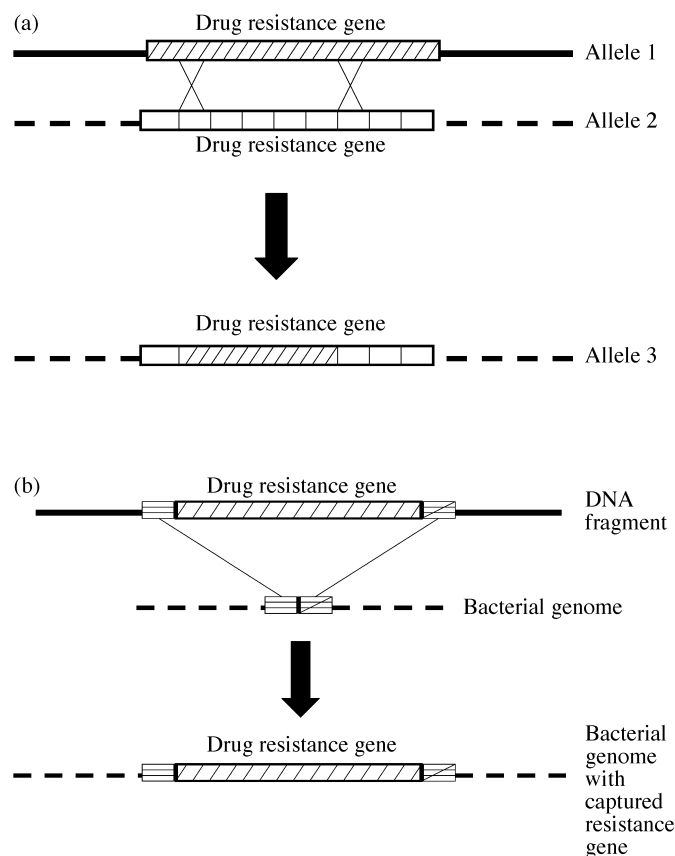


Figure 2. Homologous recombination. (a) Allelic conversion by recombination. Two distinct but related genes may undergo recombination when homologous fragments of the gene sequences are exchanged to create new hybrid sequences. In the case of natural transformation of bacteria, since one gene is present on a DNA fragment only one hybrid structure emerges from the recombination. Distinct resistance gene alleles 1 and 2 are represented by hatched and blocked boxes, whereas hybrid recombinant allele 3 is represented by the hatched and blocked box. Other sequences on the captured DNA fragment are represented by the solid lines, whereas bacterial genome sequences are represented by the dotted lines. (b) Gene capture by homologous recombination. Gene acquisition by this route requires that recombination events at both ends of the sequence to be captured are coordinated. This requires homologies between the sequences flanking that to be captured and the putative rescue replicon (chromosome or plasmid) (homology between flanking sequences is not required). Homologous sequences are represented by the boxes with horizontal bars; points of recombination are represented by the diagonal lines; the drug resistance gene is represented by the hatched box; other sequences on the captured DNA fragment are represented by the solid lines, whereas bacterial genome sequence is represented by the dotted lines.

replicon). Most illegitimate recombination in bacteria can be attributed to the activities of transposable elements or integron/gene cassette systems, but other, as yet undefined, systems may be responsible for a proportion of such events (see Illegitimate recombination below). For plant cell-bacterial cell transduction to occur, the phage would have to be able to recognize not only its normal bacterial host but also cells in the GM plant. Phages generally have narrow host ranges among bacteria, unless the receptor is encoded by an itinerant DNA element such as a plasmid.^{44,63} There are no reports of phages that can infect both bacterial and plant cells, but it is unlikely there has been any great effort made to find such an element, given the above noted reservations. It is not likely that a bacteriophage will infect

Review

plant cells as the cell envelopes are too dissimilar and would not be expected to contain the same, or even functionally similar, phage receptors. The host range of a phage/virus is determined, in the first instance, by the presence on the cell surface of the potential host of the requisite receptor to which the phage/virus binds before introducing its genome into the recipient cell. Thus, for a phage/virus to infect both bacterial and plant cells, both cell types would have to elaborate the SAME receptor, or sufficiently similar receptors so as to be mistaken one for the other at the molecular level. Given the evolutionary separation of prokaryotes and eukaryotes, this is extremely unlikely. However, even if a phage were to interact with and inject its DNA into a plant cell, there would still be the problem of gene expression, given that the production of a transducing phage particle is a reproduction mistake (i.e. the wrong DNA is packaged during the assembly of the next generation of phage particles). Hence, the phage would still have to reproduce in the eukaryotic cell to pick up the resistance gene from the GM plant for transfer to a bacterial cell and this would require expression of phage genes, which have evolved in a bacterial context. This is extremely improbable, given the different gene expression systems in prokaryotic and eukaryotic cells, as already discussed. An alternative would be gene transfer mediated by a plant virus. The virus would have evolved in plant cells, so reproduction in the eukaryotic host would not be a problem, and replication in the bacterial cell would not be necessary. The virus would simply serve as the delivery vehicle for the DNA; but, again, host range limitation, among other difficulties, makes this possibility very unlikely. No plant viruses are known that can cross the plant–bacterial kingdom boundary; but, again, it is doubtful if any serious search for such an entity has been made.

Transformation

This involves the uptake of ‘naked’ DNA and its incorporation into the genome of the recipient cell^{43,64} (Figure 1b). Since the mechanism involves only DNA, this requires release of DNA from the donor cell, by cell lysis, after which the particular gene must be rescued by the receiving cell before it is degraded. There has been considerable debate as to whether DNA survives *ex vivo* for a significant time once released from the cell, given the abundance of nucleases in the environment. That DNA can survive in natural systems (e.g. in association with clay particles in the soil) in a form that can transform bacterial cells, is no longer seriously questioned.^{42,46,65} Survival of DNA in animals, following ingestion of plant tissue, is much more transitory,⁶⁶ and transformation in the ruminant tract is likely to be a very rare event, if possible at all.⁶⁷

Natural transformation is a feature of a small, but growing, group of bacterial species, including human pathogens, such as *Acinetobacter* spp., *Haemophilus* spp. and *Neisseria* spp.⁴² Indeed, transformation has been responsible for the evolution of β -lactam-resistant strains of *H. influenzae* and *N. gonorrhoeae*, as well as penicillin-resistant *S. pneumoniae*.^{56,68} Productive transformation requires the uptake of either a plasmid, which is a very inefficient process with natural transformation systems (in contrast to laboratory transformation³), or rescue of genes or gene fragments on the transforming DNA by recombination.^{43,64} Again, this is most unlikely to involve homologous recombination, which needs sequence homology between the transforming DNA and the DNA with which it will recombine,⁶⁹ although some form of illegitimate recombination, such as transposition or gene cassette/integron involvement, could possibly mediate resistance gene recovery.^{48,70–73}

Although about 50 species have been shown to be naturally transformable at some stage in their growth cycles,⁴² this varies markedly from species to species. Furthermore, not all strains of a particular bacterial species are necessarily naturally transformable. With some species, e.g. *N. gonorrhoeae* and *H. influenzae*, the transforming DNA must carry a nucleotide signature that identifies the DNA as coming from the same or a similar species.⁴³ These sequences are unlikely to occur frequently by chance on other bacterial genomes. Hence, if the DNA presented to the bacterial cell lacks the signature needed for DNA uptake, it transforms poorly, if at all. In other cases, such as *Acinetobacter* spp. and *Bacillus subtilis*, any DNA can, in principle, serve as transforming DNA, in that any DNA can be taken up. Normally DNA from an unrelated species would then be degraded and the components recycled, but an opportunity for transformation before the DNA is degraded would exist, at least hypothetically.

Homologous recombination

Fixing DNA sequences into the bacterial genome by homologous recombination is the most likely way for a bacterial cell to recover a resistance gene from a plant cell⁷⁴ (Figure 2), given that its transfer would most likely involve a small linear fragment of one of the plant cell chromosomes. However, this would require the presence in the bacterial cell of an allele of the drug-resistance gene (Figure 2a) or sequences closely related to those flanking the resistance gene on the chromosome in the plant (Figure 2b). In the former case, when the recipient bacterial cell already has an allele of the resistance gene, transformation and recombination would not markedly alter the genetic composition of the bacterial cell and there would be no significant expansion of the resistance gene pool and, critically, no extension of the bacterial range of the resistance gene. This type of recombinational rescue of a resistance gene embedded in a plant chromosome has been demonstrated in the laboratory⁷⁴ and in simulated natural environments.⁴⁶

To establish a resistance gene from a GM plant in a bacterium *de novo* by homologous recombination would require that, in the plant chromosome, the resistance gene is flanked by DNA sequences that are essentially the same as sequences located close together (or contiguously) on a DNA molecule in the bacterial cell. If there were then simultaneous recombinations in both flanking sequences (Figure 2b), then the resistance gene would be inserted into that replicon in the bacterial cell. If the DNA fragment on which the gene is located was first circularized, then only one region of homology would be needed, and the entire sequence on the fragment, including the resistance gene, would be recovered as an integrated linear array. The likelihood of a linear fragment of plant DNA being circularized is non-quantifiable (the situation is hypothetical and without precedent), but is highly unlikely to be common, if it occurs at all, under natural conditions. Further, in natural transformation systems, DNA is taken into bacterial cells as linear single-stranded (ss) DNA;⁴³ recovery of circular DNA molecules is very inefficient.⁶⁸ Given the difficulties, this hypothetical variation is extremely unlikely.

Transformation with a fragment of plant cell DNA carrying an AR gene is most likely to involve uptake of linear DNA fragments where a double crossover between very similar sequences is needed. To capture a new resistance gene, these must occur on each side of the resistance gene (Figure 2b). Such a mechanism can only insert the resistance gene into a locus that has sequences that are identical, or almost so, to those close to and flanking the resistance gene on the plant cell DNA. It has been reported that in *E. coli*, recombination can

Review

occur at low frequency between homologous sequences as short as 20 bp;⁷⁵ the frequency of recombinations that need simultaneous crossover in two such short sequences is unknown, but will be extremely low. It has been demonstrated, however, that recombinational rescue of relatively large sequences (~20 kb) into a resistance plasmid in *Pseudomonas aeruginosa* by homologous recombination involving relatively small flanking sequences (500–800 bp) can be reasonably efficient.⁷⁶

The likelihood of perfect homology between the sequences flanking the drug resistance gene in the GM plant DNA and a potential, but unrelated locus in a possible recipient bacterium is extremely low, with the probability of finding a particular 20 bp sequence on a DNA molecule being 1 in 4²⁰ or, approximately, 1 in 10¹², meaning it would require a genome of one million million base pairs before there was a significant possibility of finding the 20 bp sequence by chance. This is several orders of magnitude larger than the genome size of any plant yet investigated. Precisely how the efficiency of recombination is affected by less than perfect homology (i.e. various degrees of mismatch) is not known, but any sequence mismatch over short regions of homology is likely to decrease the frequency of recombination significantly.⁷⁵ All the experimental evidence regarding recombination would suggest that recombinational rescue of a bacterial drug resistance gene from GM plant DNA, by bacteria unrelated to that from which the resistance gene was obtained and which do not already carry an allele of the resistance gene, would be an extremely rare event, if it were to occur at all.

Recombinational rescue of a drug resistance gene inactivated by a small deletion, by transformation with DNA from a transgenic plant carrying the resistance gene, has been demonstrated in the laboratory with *Acinetobacter* spp.⁷⁴ The efficiency of transformation was low when relatively crude preparations of DNA were used. It has also been shown that such transformation can occur in soil.⁴⁶ The findings establish that DNA from a GM plant can be transferred to a bacterial cell by a transformation mechanism, given the appropriate conditions. However, given the low frequency of transformation achieved in these experiments under partly optimized conditions, where the bacteria already had a damaged form of the resistance gene in the plant to facilitate gene capture, it seems safe to conclude that the evolution of new forms of bacterial pathogens, with resistance genes from GM plants that reduce antibiotic treatment options, is likely to occur extremely rarely, if at all, outside the laboratory. This conclusion is supported by the experiments of Schlüter *et al.*⁷⁷ who failed to find transfer of a β -lactamase gene from a transgenic potato to the tightly associated bacterial pathogen *Erwinia chrysanthemi* under conditions mimicking natural infection.

Illegitimate recombination

Transposition. This type of mechanism requires a transposable element, or sequences related thereto.⁴⁸ To date, transposable elements have not been used in GM plant construction, so no such sequence has been incorporated into a GM plant. Accordingly, if a transposable element were to be involved in transfer of a resistance gene from a GM plant to bacteria, it would have to be one in the bacteria. However, the likelihood that a transposable element, not linked to the resistance gene, could rescue the resistance gene from a fragment of plant DNA and integrate it into a bacterial replicon is remote. There is no precedent on record, and all experience with many different transposable elements suggests that such an event is extremely unlikely, if not impossible. As a mechanism of mass transfer of resistance genes from GM plants to bacteria, this possibility can be dismissed out-of-

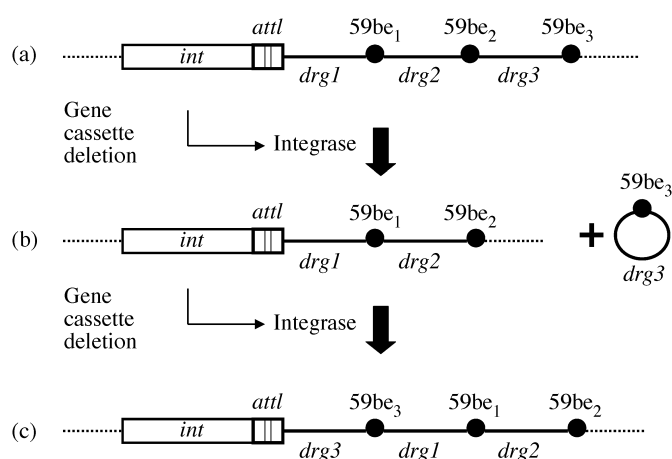


Figure 3. Integron-mediated site-specific recombination. (a) Bacterial integron comprising integrase gene (*int*), attachment site (*attI*) and three drug resistance genes (*drg1*, 2 & 3) punctuated by 59 base elements 1–3 (filled circles). Flanking DNA is represented by the dotted lines. (b) An integrase-mediated site-specific recombination between sequences in elements 59be₂ and 59be₃ releases *drg3* gene cassette with 59be₃ as circular DNA species generating new integron lacking *drg3* and 59be₃. (c) Integrase-mediated site-specific recombination between 59be₃ on the free *drg3* gene cassette and *attI* leads to integration of *drg3* at *attI* and generation of a new integron (with the same gene complement as the first integron but a different resistance gene arrangement).

hand. Rare transfer events, promoted by particular transposable elements, perhaps ones as yet uncharacterized, are a theoretical possibility, but given our present understanding of transposition systems,^{48,73} the molecular problems associated with this scenario would appear to be formidable, if not completely insurmountable, and the risk involved negligible.

Gene cassette/integron. This is the bacterial cell's own gene capture system.⁷¹ It requires a double-stranded (ds), circular DNA gene cassette,⁷¹ with a specific integration sequence known as a 59 base element (59 be), which is needed for site-specific recombination^{78–80} (Figure 3). When integrated in a replicon, DNA arrays are linear and resistance genes are flanked by 59 bes (or equivalents) (Figure 3a). These elements allow cassettes to be excised from one site as small circles of DNA (Figure 3b) and inserted into another (Figure 3c). Recombination into a new location requires a specific sequence (insertion site, *attI*) on the target DNA, usually provided by a genetic element termed an integron,⁷⁰ and the participation of an integrase, an enzyme that specifically mediates gene cassette insertion into integrons.⁷⁹ Gene cassette insertion at secondary sites that fortuitously resemble a normal integron insertion site but which are not part of an integron, has been reported,^{81–84} but expression of the captured gene may be silenced as most genes on gene cassettes lack their own promoters and rely on promoters provided by the receiving integron for expression. Insertions at secondary sites are events that are essentially mistakes. When inserted into an integron, expression of the cassette gene is from a strong promoter on the integron, located in the integrase gene, beside the integration site.⁸⁵ Expression of a cassette gene that lacks its own promoter, when integrated into a secondary site, would be a hit and miss affair; some will be expressed because there is a promoter close to the insertion site, others will fall silent.

DNA captured from a plant cell by a natural transformation system is likely, on initial uptake, to be linear and single-stranded as

Review

natural transformation systems generally do not promote uptake of dsDNA.⁴³ Linear, ssDNA is not a substrate for integrases and would first have to be converted into dsDNA and then circularized to generate a potential cassette. For the latter step to be mediated by an integrase the drug-resistance gene would need to be flanked by 59 bes. Without this arrangement, circularization of the DNA from the plant by an integrase is impossible and, consequently, so is subsequent insertion of the resistance gene from the plant into an integron in the bacterial cell. If the AR gene is not flanked by 59 bes when it is put into the plant, the likelihood of such sequences being acquired subsequently (in the plant or on transfer to the bacterial cell) is remote, in which case, conversion of plant DNA fragments into gene cassettes by an integrase, and insertion into an integron is well-nigh impossible.

Some form of illegitimate recombination, different from the mechanisms discussed and as yet undescribed, could, in principle, circularize linear fragments of dsDNA. For example, T4 phage DNA ligase is used *in vitro* to circularize linear DNA fragments produced in the process of genetic engineering.³ Even so, integration into the bacterial genome via an integron system would only occur if the circular DNA molecule generated also happened to have a 59 be and the bacterium had an integron. The coincidence of the necessary elements needed to rescue a resistance gene embedded in plant cell DNA, by an integrase-dependent mechanism, is likely to occur only on very rare occasions, if at all. Mass transfer of bacterial resistance genes from GM plants to bacteria by this method is, therefore, an exceedingly unlikely scenario, and one with no scientific credibility.

DNA non-homologous end-joining activity in bacteria

Before leaving this topic, one further recombination system should be considered, namely, DNA non-homologous end-joining.⁸⁶ This recombination activity, which was first described in eukaryotic cells, has been shown to be present in some bacteria, particularly those that sporulate, where it functions to limit damage to DNA caused by ionizing radiation. The repair system comprises DNA binding proteins and a specific DNA ligase activity that act to seal double-strand gaps in DNA. Given the ligase function, such an activity could, conceivably, mediate integration of non-homologous DNA at a double strand break in host cell DNA, although such an activity has, to our knowledge, not been demonstrated. Again, the mechanism could not conceivably support mass transfer of AR genes from plants to bacteria. The occasional capture of an AR gene by this route cannot be ruled out.

Conclusions regarding recombinational recovery of a bacterial antibiotic resistance gene from a GM plant by bacteria

Although bacteria have three distinct mechanisms, conjugation, transduction and transformation, whereby DNA can be transferred from one cell to another, realistically it is only the last that is likely to be involved in productive acquisition of DNA from GM plant cells by bacteria. The limitations of the other two systems are judged to be too great to overcome, whereas transformation of bacteria by DNA from a GM plant, albeit under very particular circumstances, has been demonstrated in the laboratory.^{46,74} However, the frequency of gene recovery was very low. Given the partially optimized nature of the experimental system (i.e. the recipient bacteria were provided with a mutant copy of the resistance gene to be rescued to facilitate transformation), the likelihood of transforming bacteria to AR with a plant DNA containing a resistance gene that is unrelated to any gene in the

bacteria is remote, and indeed was undetected in the control experiments of Gerhard & Smalla,⁷⁴ a negative finding supported by the work of Schlüter *et al.*⁷⁷ If, by chance, the potential recipient did carry an allele of the resistance gene that had been inactivated by mutation then, in this particular circumstance, transformation by GM plant DNA could restore gene function, but the result would be neither novel nor significant, because the resistance gene clearly would already have been established in the bacteria by other means (i.e. the transfer would not expand the host range of the resistance gene). However, it should be remembered that the resistance genes used in GM plant generation are already widespread among clinically important bacteria and were chosen precisely for this reason. In the absence of optimization, resistance gene transfer from GM plants to bacteria, even in the laboratory, cannot be demonstrated.⁷⁴ Hence, given the probable rarity of such transfers outside the laboratory, they are unlikely to contribute significantly to the load of bacterial drug resistance and are highly unlikely to create something that has not already evolved or could and probably will evolve much more readily by transfer of the resistance genes between bacteria.

What if antibiotic resistance gene transfer occasionally occurs from GM plants to bacteria?

Although the preceding discussion argues that the risk of productive transfer of resistance genes from GM plants, either directly or indirectly, to bacterial pathogens is essentially hypothetical, nevertheless such gene transfer, although it cannot be demonstrated experimentally, cannot be ruled out entirely. Precisely because such gene transfer events have never been reported, their frequency is likely to be so low as to be unquantifiable in laboratory experiments. Nonetheless, rare transfers may occur, so it is pertinent to ask, if, very occasionally, the resistance genes found in the genomes of GM plants were to be transferred back into bacteria would this have any practical consequences? Would such an event threaten the future of antibiotic therapy?

The three drug resistance genes that have been used in GM plant creation are the TEM β -lactamase gene (*bla*_{TEM}), an aminoglycoside modifying enzyme gene, *aadA*, that confers resistance to streptomycin and spectinomycin, and an *aph* gene [*aph*(3')], also designated *nptII*], encoding resistance to kanamycin/neomycin. These resistance genes are widespread in the bacterial domain and are among the most common, particularly among the Enterobacteriaceae and non-fermentative Gram-negative bacteria such as *P. aeruginosa*, *A. baumannii* and *Stenotrophomonas maltophilia*, to name but three, and particularly among clinical isolates of these bacteria.⁸⁷ The drugs to which these genes confer resistance, with the exception of ampicillin/penicillin, are now used infrequently, and rarely as first choice therapy. Nonetheless, on occasion they are used, so would transfer of these genes from a GM plant to a potential bacterial pathogen compromise therapeutic options? Is it likely that such an event will compromise our present or future capacity to treat certain bacterial infections? The common sense answer, based on all we know, is no. Transfer of the resistance genes that have been used in GM plant development to a wider spectrum of bacteria than is currently the case is most likely to occur between bacteria themselves, using the gene transfer systems that have evolved for the purpose (Figure 1), rather than between GM plants and bacteria, even allowing for the considerable gene amplification that is an inevitable consequence of mass cultivation of the current cultivars of GM crops. The difficulties to be overcome for DNA to transfer from plant cells to bacteria in the field are, in principle, not insurmountable, but do provide a formidable set

Review

of barriers that will be breached rarely, if at all. If it were not so, then we might expect regularly to find examples of plant genes in bacteria. We do not. Examples of possible gene transfer from plants to bacteria in the distant past have been suggested,^{41,45} but, even if true, these are rare examples that essentially prove the point. Genes do not move freely from plants to bacteria and, apparently, never have or, to be more precise, plant genes, now and in the past, are rarely if ever captured and incorporated into bacterial genomes. Many, if not all, bacteria possess efficient 'restriction systems'. These act as natural defence systems that identify and destroy any 'foreign' DNA that does get into the cell, by whatever means, to prevent it being incorporated into the bacterial genome, so maintaining the genetic integrity of the bacteria. Self and non-self DNAs are generally recognized by the patterns of DNA methylation. If certain sites are unmethylated, then the DNA is recognized as non-self and is attacked at these points.¹⁵ These mechanisms serve efficiently to limit genetic acquisition of non-self DNA by bacteria. Bacterial resistance genes embedded in plant DNA would be identified as foreign and degraded and the previous bacterial origin would be irrelevant.

Experience tells us that although there are barriers which limit the horizontal spread of resistance genes among bacteria they are not 100% efficient. Gene transfer routes may appear to be circuitous and involve a number of intermediate hosts, and there may appear to be little or no lateral spread between particular bacterial species, until the resistance genes suddenly appear in the new hosts to confer resistance to antibiotics of choice, selected by the use of those same antibiotics. This is not an encouraging precedent. However, the barriers to plant cell–bacterial cell transfer of genes unrelated to any in the target bacterial cell are likely to be much more formidable than those that limit bacterial cell–bacterial cell DNA transfer, where efficient natural DNA transfer systems have evolved.

One 'disaster' scenario that has been proposed is the transfer of a resistance gene (*bla*_{TEM}) from pollen of GM maize to *N. meningitidis* to create a penicillinase-producing strain, thus compromising the use of penicillin and some other β -lactams to treat meningococcal meningitis. Inhalation of pollen grains could bring plant DNA, and hence the resistance gene, and the pathogen, *N. meningitidis*, together in the nasal tract. Release of DNA from the pollen grains, followed by transformation of the meningococcus to create a β -lactamase-producing, penicillin-resistant meningococcus would clearly be undesirable. A variation on this scenario concerns dry milling of GM plant material, which would produce dust that contains fractured plant cells that makes the release of DNA easier than from intact cells and, therefore, more readily available to transform *N. meningitidis*. However, the numbers of people exposed to this would be considerably fewer than to the plant pollen. Is there a real, as opposed to hypothetical, risk of the *bla* gene transferring successfully from GM maize to the meningococcus? The risk appears remote, for many of the reasons discussed. (The possibility could be tested in the laboratory to determine the upper limits of the risk.) That the TEM β -lactamase gene (*bla*_{TEM}), which is found in both *H. influenzae* and *N. gonorrhoeae* on plasmids, can be conjugally transferred to *N. meningitidis* has been demonstrated in laboratory experiments.^{88,89} Further, there are a few reports of transfer having occurred in nature.^{90–94} Why this particular resistance gene is not found more often in *N. meningitidis* is not known and is somewhat puzzling, since therapeutic practice would seem to provide the necessary selection pressure. Opportunities for transfer from other members of the nasopharyngeal flora, such as *H. influenzae*, clearly exist. These data indicate that capture of the gene from GM pollen is a very remote possibility, since capture from a much closer source appears to be problematic. All the problems of

eukaryotic–prokaryotic DNA transfer discussed above would apply. There is undoubtedly a hypothetical risk, but in the light of our knowledge of gene transfer and gene rescue in bacteria, it has to be deemed slight in the extreme. It is also true that other disaster scenarios can be envisaged, but the likelihood of any one of these hypothetical events occurring is also extremely remote. Can a blanket ban on cultivation of GM plants carrying bacterial drug resistance genes be justified, even in part, because of extremely improbable, unquantifiable concerns?

Ways forward

There are two ways to deal with the situation.

The precautionary principle

Irrespective of how remote the possibility of AR gene transfer from a GM plant to a potential bacterial pathogen in which the resistance gene would compromise therapy, impose a ban on cultivation of all GM plants with AR genes to eliminate the risk entirely. This stance views the possibility of transfer of bacterial AR genes from GM plants to bacteria, however small that possibility and regardless of the resistance gene involved, as unacceptable, even if the result of the transfer would be non-threatening.

The pragmatic approach

Accept that we cannot exist risk-free and assess the cost–benefit balance, weighing the hypothetical risk of transfer of a resistance gene from a GM plant to a bacterial cell becoming a clinical problem against the potential economic and environmental benefits to be gained from GM crop production,^{1,95,96} and if the answer is favourable permit cultivation.

To choose between these options requires risk and possible hazard to be considered, as well as perceived benefits (see Appendix). From the foregoing discussion, it is clear that the risk of a bacterial AR gene embedded in a plant cell genome being transferred productively to a bacterial pathogen with no history of carriage of the determinant is negligible. There is no evidence for transfer of plant genes to bacteria in the recent past and no mechanism is known that will mediate such a transfer event. Hence risk is so tiny as to be unquantifiable. Indeed, the risk that the particular resistance genes used in GM plant development will spread further among human and animal bacterial pathogens than is currently the case, by their natural DNA transfer systems, is very much higher. Whether, in practice, they become established in new bacterial hosts will depend largely on them conferring selective advantage to the recipient bacteria, which, in turn, largely depends on their levels of exposure to the antibiotics. In this respect, it should be noted that cultivation of GM plants with bacterial resistance genes does not involve the use of antibiotics, at all.

Considering hazard, the resistance genes used in GM plant development are 'old' resistance genes that have already severely compromised use of the corresponding antibiotic in most, if not all, cases where their use was appropriate in the past. Would it be disastrous if any of these genes successfully migrated, at very low frequency, from a GM plant into a human or animal pathogen with no history of carriage of the particular or a closely related gene? Although the event would be unfortunate and further avoidable spread of resistance genes is not to be encouraged,^{97–99} were these particular AR genes to spread further among human bacterial pathogens as a consequence of transfer from GM plants, it would be a considerable exaggeration to classify the

Review

result as a disaster, since there are potent alternative antibiotics that can be, and are, used instead of ones that are already compromised. Indeed, if transfer of these resistance genes from GM plants to bacteria that do not have them were to be considered a disaster, then the elements for the catastrophe are already in place among the bacteria themselves, given the proven abilities of bacteria to transfer resistance genes from one to another.

Among the known benefits of GM technology is the development of crops that require less spraying of dangerous chemical insecticides and fungicides, because the GM crops are less susceptible to major pests that decimate the non-GM varieties. Storage properties can be improved, as with the FlavrSavr tomato, with major economic benefits accruing to producers and consumers from reduced waste. In the future, plants may be engineered to produce medicinal compounds. It may be possible to create cultivars that can be raised economically in regions that currently will not support non-GM cultivars, e.g. salt-tolerant cereals, or ones that are more drought-resistant or frost-resistant or grow faster in cold climates.^{1,95} The possibilities are numerous and most, if not all, could greatly improve the human condition on parts of the planet. That these plants might carry a bacterial resistance gene that has already seriously compromised use of the antibiotic presents no real threat to human or animal health or to the environment.

The Working Party finds that there are no objective scientific grounds to believe that bacterial AR genes will migrate from GM plants to bacteria to create new clinical problems. Further, by limiting the use of bacterial resistance genes in GM plant development to a very small number [*aadA*, *bla*_{TEM} and *aph*(3')] that confer resistance to 'old' antibiotics that are now rarely used, because resistance is already common in human bacterial pathogens, also markedly reduces the risk of an adverse clinical situation developing. Hence, use of these bacterial resistance genes in GM plant development cannot be seen as a serious or credible threat to human or animal health or to the environment. Therefore, the Working Party is unable to sustain an evidence-based argument to support a ban on the development or cultivation of GM crops simply because they contain one of the bacterial resistance genes *aadA*, *bla*_{TEM} or *aph*(3'). However, although much of the scientific evidence discussed above could be cited in support of the use of any bacterial AR gene in GM plant construction, on the basis that the risk involved to the efficacy of antibiotic therapy now and in the future is likely to be extremely low, the Working Party consider it extremely undesirable and unnecessary to extend the list of AR genes approved for GM plant development. In particular, the use of any AR gene that if disseminated widely among bacteria would be likely to compromise use of a front-line or currently widely used antibiotic should be strongly discouraged, if not banned. This constraint was largely self-imposed when the three AR genes discussed above were selected for use in GM plant construction. The moratorium should continue, particularly as alternatives to AR genes are being developed.

Conflicts of interest statement

Members of the Working Party wish it to be known that none owns or manages shares in an agrochemical or biotechnology company involved with GM plant development or exploitation, and that no member of the Working Party is a consultant for or is undertaking research supported by one of the aforesaid companies.

Comment on editorial process

The review and decision making process on this article was handled by an Editor who was not connected with the BSAC Working Party. As a co-author of this article, the Editor-in-Chief took no part in the review and decision making process.

References

1. Halford, N. G. & Shewry, P. R. (2000). Genetically modified crops: methodology, benefits, regulation and public concerns. *British Medical Bulletin* **56**, 62–73.
2. Conner, A. J. & Jacobs, J. M. (1999). Genetic engineering of crops as potential source of genetic hazard in the human diet. *Mutation Research* **443**, 223–34.
3. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). Strategies for cloning in plasmid vectors. In *Molecular Cloning, a Laboratory Manual*, vol. 1, 2nd edn, pp. 53–104. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.
4. Newell, C. A. (2000). Plant transformation technology. Developments and applications. *Molecular Biotechnology* **16**, 53–65.
5. Stachel, S. E. & Zambryski, P. (1989). Generic trans-kingdom sex. *Nature* **340**, 190–1.
6. Tzfira, T., Rhee, Y., Chen, M.-H. *et al.* (2000). Nucleic acid transport in plant-microbe interactions: the molecules that walk through the walls. *Annual Review of Microbiology* **54**, 187–219.
7. Kado, C. I. (1998). Evolution of the selfish Ti plasmid of *Agrobacterium tumefaciens* promoting horizontal gene transfer. In *Horizontal Gene Transfer* (Syvanen, M. & Kado, C. I., Eds), pp. 63–74. Chapman & Hall, London, UK.
8. Zambryski, P. (1992). Chronicles from the *Agrobacterium*-plant cell DNA transfer story. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 465–90.
9. Hooykaas, P. J. J. (1989). Tumorigenicity of *Agrobacterium* in plants. In *Genetics of Bacterial Diversity* (Hopwood, D. A. & Chater, K. E., Eds), pp. 373–91. Academic Press, London, UK.
10. Shaw, C. H., Leemans, J., Shaw, C. H. *et al.* (1983). A general method for the transfer of cloned genes to plant cells. *Gene* **23**, 315–30.
11. Zambryski, P., Tempe, J. & Schell, J. (1989). Transfer and function of T-DNA genes from *agrobacterium* Ti and Ri plasmids in plants. *Cell* **56**, 193–201.
12. Casas, A. M., Kononowicz, A. K., Bressan, R. A. *et al.* (1995). Cereal transformation through particle bombardment. *Plant Breeding Reviews* **13**, 235–64.
13. Kemp, A., Parker, J. & Grierson, C. (2001). Biolistic transformation of *Arabidopsis* root hairs: a novel technique to facilitate map-based cloning. *Plant Journal* **27**, 367–71.
14. Rasco-Gaunt, S., Riley, A., Cannell, M. *et al.* (2001). Procedures allowing the transformation of a range of European elite wheat (*Triticum aestivum* L.) varieties via particle bombardment. *Journal of Experimental Botany* **52**, 865–74.
15. Old, R. W. & Primrose, S. B. (1985). *Principles of Gene Manipulation*, pp. 20–9. Blackwell Scientific Publications, Oxford, UK.
16. Flavell, R. B., Dart, E., Fuchs, R. L. *et al.* (1992). Selectable marker genes: safe for plants? *Bio/technology* **10**, 141–4.
17. Daniell, H., Muthukumar, B. & Lee, S. B. (2001). Marker free transgenic plants: engineering the chloroplast genome without the use of antibiotic selection. *Current Genetics* **39**, 109–16.
18. Stewart, C. N., Jr, Richards, H. A., IV & Halfhill, M. D. (2000). Transgenic plants and biosafety: science, misconceptions and public perceptions. *Biotechniques* **29**, 832–43.
19. Martens, M. A. (2000). Safety evaluation of genetically modified foods. *International Archives of Occupational and Environmental Health* **73**, Suppl., S14–S18.
20. Health & Safety Executive. (2000). ACGM Compendium of Guidance: guidance from the health and safety commission's advisory committee on genetic modification. [Online.] www.hse.gov.uk/hthdir/

Review

noframes/ acgmcomp/acgmcomp.htm (5 January 2004, date last accessed).

21. Schilter, B. & Constable, A. (2002). Regulatory control of genetically modified (GM) foods: likely developments. *Toxicology Letters* **127**, 341–9.
22. Hall, B. G. (1999). Transposable elements as activators of cryptic genes in *E. coli*. *Genetika* **107**, 181–7.
23. Hall, B. G. & Xu, L. (1992). Nucleotide sequence, function, activation and evolution of the cryptic *asc* operon of *Escherichia coli* K12. *Molecular Biology and Evolution* **9**, 688–706.
24. Kharat, A. S. & Mahadevan, S. (2000). Analysis of the β -glucosidase utilization (*bgl*) genes of *Shigella sonnei*: evolutionary implications for their maintenance in a cryptic state. *Microbiology* **146**, 2039–49.
25. Parker, L. L. & Hall, B. G. (1990). Mechanisms of activation of the cryptic *cel* operon of *Escherichia coli* K12. *Genetics* **124**, 473–82.
26. Podglajen, I., Breuil, J. & Collatz, E. (1994). Insertion of a novel DNA sequence, IS1186, upstream of the silent carbapenemase gene *cfiA*, promotes expression of carbapenem resistance in clinical isolates of *Bacteroides fragilis*. *Molecular Microbiology* **12**, 105–14.
27. Podglajen, I., Breuil, J., Bordon, F. *et al.* (1992). A silent carbapenemase gene in strains of *Bacteroides fragilis* is expressed after a one-step mutation. *FEMS Microbiology Letters* **91**, 21–9.
28. Ewen, S. W. B. & Pusztai, A. (1999). Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *Lancet* **354**, 1353–4.
29. Chang, H. S., Bae, Y. K., Lim, S. K. *et al.* (2001). Allergenicity test of genetically modified soybean in Sprague Dawley rats. *Archives of Pharmaceutical Research* **24**, 256–61.
30. Lack, G. (2002). Clinical risk assessment of GM foods. *Toxicology Letters* **127**, 337–40.
31. Taylor, S. L. & Hefle, S. L. (2001). Will genetically modified foods be allergenic? *Journal of Allergy and Clinical Immunology* **107**, 765–71.
32. Nordlee, J. A., Taylor, S. L., Townsend, J. A. *et al.* (1996). Identification of a Brazil-nut allergen in transgenic soybeans. *New England Journal of Medicine* **334**, 688–92.
33. Losey, J. F., Rayor, L. S. & Carter, M. E. (1999). Transgenic pollen harms monarch larvae. *Nature* **399**, 214.
34. Gatehouse, A. M. R., Ferry, N. & Raemaekers, R. J. M. (2002). The case of the monarch butterfly: a verdict is returned. *Trends in Genetics* **18**, 249–51.
35. Wraight, C. L., Zangeri, A. R., Carroll, M. J. *et al.* (2000). Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. *Proceedings of the National Academy of Sciences, USA* **97**, 7700–3.
36. Nielsen, K. M., Bones, A. M., Smalla, K. *et al.* (1998). Horizontal gene transfer from transgenic plants to terrestrial bacteria—a rare event? *FEMS Microbiology Reviews* **22**, 79–103.
37. Davison, J. (1999). Genetic exchange between bacteria in the environment. *Plasmid* **42**, 73–91.
38. Fründt, C., Meyer, A. D., Ichikawa, T. *et al.* (1998). Evidence for the ancient transfer of Ri plasmid T-DNA genes between bacteria and plants. In *Horizontal Gene Transfer* (Syvanen, M. & Kado, C. I., Eds), pp. 94–106. Chapman & Hall, London, UK.
39. Courvalin, P., Goussard, S. & Grillot-Courvalin, C. (1998). Gene transfer from bacteria to mammalian cells. In *Horizontal Gene Transfer* (Syvanen, M. & Kado, C. I., Eds), pp. 107–17. Chapman & Hall, London, UK.
40. Thomas, C. M., Ed. (2000). *The Horizontal Gene Pool*. Harwood Academic Publishers, Amsterdam, The Netherlands.
41. Katz, L. A. (1996). Transkingdom transfer of the phosphoglucose isomerase gene. *Journal of Molecular Evolution* **43**, 453–9.
42. Lorenz, M. G. & Wackernagel, W. (1994). Bacterial gene transfer by natural genetic transformation in the environment. *Microbiological Reviews* **58**, 563–602.
43. Dubnau, D. (1999). DNA uptake in bacteria. *Annual Review of Microbiology* **53**, 217–44.
44. Bennett, P. M. & Howe, T. G. B. (1998). Bacterial and bacteriophage genetics. In *Topley & Wilson's Microbiology and Microbial Infections*, 9th edn, vol. 2, Systematic Bacteriology (Collier, L., Balows, A. & Sussman, M., Eds), pp. 231–94. Arnold, London, UK.
45. Doolittle, R. F. (1998). The case for gene transfers between very distantly related organisms. In *Horizontal Gene Transfer* (Syvanen, M. & Kado, C. I., Eds), pp. 311–20. Chapman & Hall, London, UK.
46. Nielsen, K. M., van Elsas, J. D. & Smalla, K. (2000). Transformation of *Acinetobacter* sp. strain BD413(pFG4 Δ nptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. *Applied and Environmental Microbiology* **66**, 1237–42.
47. Bertolla, F., Kay, E. & Simonet, P. (2000). Potential dissemination of antibiotic resistance genes from transgenic plants to microorganisms. *Infection Control and Hospital Epidemiology* **21**, 390–3.
48. Bennett, P. M. (2000). Transposable elements. In *Encyclopaedia of Microbiology*, 2nd edn, vol. 1 (Lederberg, J., Ed.), pp. 704–24. Academic Press, San Diego, CA, USA.
49. Heinemann, J. A. & Sprague, G. F., Jr (1989). Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. *Nature* **340**, 205–9.
50. Wilkins, B. M. (1995). Gene transfer by bacterial conjugation: diversity of systems and functional specializations. In *Society for General Microbiology Symposium 52: Population Genetics of Bacteria* (Baumberg, S., Young, J. P. W., Wellington, E. M. H. *et al.*, Eds), pp. 59–88. Cambridge University Press, Cambridge, UK.
51. Heinemann, J. A., Scott, H. E. & Williams, M. (1996). Doing the conjugative two-step: evidence of recipient autonomy in retrotransfer. *Genetics* **143**, 1425–35.
52. Blanco, G., Ramos, F., Medina, J. R. *et al.* (1991). Conjugational retrotransfer of chromosomal markers in *Azotobacter vinelandii*. *Current Microbiology* **22**, 241–6.
53. Mergeay, M., Lejeune, P., Sadouk, A. *et al.* (1987). Shuttle transfer (or retrotransfer) of chromosomal markers mediated by plasmid pULB113. *Molecular and General Genetics* **209**, 61–70.
54. Ayres Sia, E., Kuehner, D. M. & Figursky, D. H. (1996). Mechanism of retrotransfer in conjugation: prior transfer of the conjugative plasmid is required. *Journal of Bacteriology* **178**, 1457–64.
55. Heinemann, J. A. & Ankenbauer, R. G. (1993). Retrotransfer in *Escherichia coli* conjugation: bidirectional exchange or *de novo* mating? *Journal of Bacteriology* **175**, 583–8.
56. Dowson, C. G., Coffey, T. J. & Spratt, B. G. (1994). Origin and molecular epidemiology of penicillin-binding-protein-mediated resistance to β -lactam antibiotics. *Trends in Microbiology* **2**, 361–6.
57. Masters, M. (1996). Generalised transduction. In *Escherichia coli and Salmonella: Cellular and Molecular Biology*, 2nd edn (Neidhardt, F. C., Ed.), pp. 2421–41. ASM Press, Washington, DC, USA.
58. Weisberg, R. A. (1996). Specialized transduction. In *Escherichia coli and Salmonella: Cellular and Molecular Biology*, 2nd edn (Neidhardt, F. C., Ed.), pp. 2442–8. ASM Press, Washington, DC, USA.
59. Lacey, R. W. (1973). Genetic basis, epidemiology, and future significance of antibiotic resistance in *Staphylococcus aureus*: a review. *Journal of Clinical Pathology* **26**, 899–913.
60. Lacey, R. W. (1984). Antibiotic resistance in *Staphylococcus aureus* and streptococci. *British Medical Bulletin* **40**, 77–83.
61. Miller, R. V. (1998). Bacterial gene swapping in nature. *Scientific American*, January 1998, 67–71.
62. Miller, R. V. & Ripp, S. (1998). The importance of pseudolysogeny to *in situ* bacteriophage-host interactions. In *Horizontal Gene Transfer* (Syvanen, M. & Kado, C. I., Eds), pp. 179–91. Chapman & Hall, London, UK.
63. Stanisich, V. (1974). The properties and host range of male-specific bacteriophages of *Pseudomonas aeruginosa*. *Journal of General Microbiology* **84**, 332–42.
64. Dubnau, D. (1991). The regulation of genetic competence in *Bacillus subtilis*. *Molecular Microbiology* **5**, 11–8.
65. Wackernagel, W., Sikorski, J., Blum, S. *et al.* (1998). Natural genetic transformation of bacteria in soil. In *Horizontal Gene Transfer* (Syvanen, M. & Kado, C. I., Eds), pp. 168–78. Chapman & Hall, London, UK.

Review

66. Duggan, P. S., Chambers, P. A., Heritage, J. *et al.* (2000). Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. *FEMS Microbiology Letters* **191**, 71–7.
67. Mercer, D. K., Melville, C. M., Scott, K. P. *et al.* (1999). Natural genetic transformation in the rumen bacterium *Streptococcus bovis* JB1. *FEMS Microbiology Letters* **179**, 485–90.
68. Saunders, J. R., Hart, C. A. & Saunders, V. A. (1986). Plasmid-mediated resistance to β -lactam antibiotics in Gram-negative bacteria: the role of *in-vivo* recyclization reactions in plasmid evolution. *Journal of Antimicrobial Chemotherapy* **18**, Suppl. C, 57–66.
69. Kowalczykowski, S. C., Dixon, D. A., Eggleston, A. K. *et al.* (1994). Biochemistry of homologous recombination in *Escherichia coli*. *Microbiological Reviews* **58**, 401–65.
70. Hall, R. M. & Collis, C. M. (1995). Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. *Molecular Microbiology* **15**, 593–600.
71. Recchia, G. D. & Hall, R. M. (1995). Mobile gene cassettes: a new class of mobile element. *Microbiology* **141**, 3015–27.
72. Bennett, P. M. (1999). Integrons and gene cassettes: a genetic construction kit for bacteria. *Journal of Antimicrobial Chemotherapy* **43**, 1–4.
73. Merlin, C., Mahillon, J., Nešvera, J. *et al.* (2000). Gene recruiters and transporters: the modular structure of bacterial mobile elements. In *The Horizontal Gene Pool* (Thomas, C. M., Ed.), pp. 363–409. Harwood Academic Publishers, Amsterdam, The Netherlands.
74. Gerhard, F. & Smalla, K. (1998). Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Applied and Environmental Microbiology* **64**, 1550–4.
75. Shen, P. & Huang, H. V. (1986). Homologous recombination in *Escherichia coli*: dependence on substrate length and homology. *Genetics* **112**, 441–57.
76. Stanisich, V. A., Bennett, P. M. & Ortiz, J. M. (1976). A molecular analysis of transductional marker rescue involving P-group plasmids in *Pseudomonas aeruginosa*. *Molecular and General Genetics* **143**, 333–7.
77. Schlüter, K., Fütterer, J. & Potrykus, I. (1995). 'Horizontal' gene transfer from a transgenic potato line to a bacterial pathogen (*Erwinia chrysanthemi*) occurs—if at all—at an extremely low frequency. *Biotechnology* **13**, 1094–8.
78. Stokes, H. W., Gorman, D. B., Recchia, G. D. *et al.* (1997). Structure and function of 59-base element recombination sites associated with mobile gene cassettes. *Molecular Microbiology* **26**, 731–45.
79. Collis, C. M. & Hall, R. M. (1992). Site-specific deletion and rearrangement of integron insert genes catalysed by the integron DNA integrase. *Journal of Bacteriology* **174**, 1574–85.
80. Collis, C. M. & Hall, R. M. (1992). Gene cassettes from the insert region of integrons are excised as covalently closed circles. *Molecular Microbiology* **6**, 2875–85.
81. Francia, M. V., de la Cruz, F. & Garcia Lobo, M. (1993). Secondary sites for integration mediated by the Tn21 integrase. *Molecular Microbiology* **10**, 823–8.
82. Recchia, G. D. & Hall, R. M. (1995). Plasmid evolution by acquisition of mobile gene cassettes: plasmid pIE723 contains the *aadB* gene cassette precisely inserted at a secondary site in the IncQ plasmid RSF1010. *Molecular Microbiology* **15**, 179–87.
83. Recchia, G. D., Stokes, H. W. & Hall, R. M. (1994). Characterisation of specific and secondary recombination sites recognised by the integron DNA integrase. *Nucleic Acids Research* **22**, 2071–8.
84. Segal, H. & Elisha, B. G. (1997). Identification and characterisation of an *aadB* gene cassette at a secondary site in a plasmid from *Acinetobacter*. *FEMS Microbiology Letters* **153**, 321–6.
85. Collis, C. M. & Hall, R. M. (1995). Expression of antibiotic resistance genes in the integrated cassettes of integrons. *Antimicrobial Agents and Chemotherapy* **39**, 155–62.
86. Weller, G. R., Kysela, B., Roy, R. *et al.* (2002). Identification of a DNA nonhomologous end-joining complex in bacteria. *Science* **297**, 1686–9.
87. Towner, K. J. (2000). Resistance to antimicrobial agents. In *Antimicrobial Chemotherapy* (Greenwood, D., Ed.), Oxford University Press, Oxford, UK.
88. Brett, M. S. Y. (1989). Conjugal transfer of gonococcal β -lactamase and conjugative plasmids to *Neisseria meningitidis*. *Journal of Antimicrobial Chemotherapy* **24**, 875–9.
89. Roberts, M. C. & Knapp, J. S. (1988). Transfer of β -lactamase plasmids from *Neisseria gonorrhoeae* to *Neisseria meningitidis* and commensal *Neisseria* species by the 25.2-megadalton conjugative plasmid. *Antimicrobial Agents and Chemotherapy* **32**, 1430–2.
90. Dillon, J. R., Pauze, M. & Yeung, K.-H. (1983). Spread of penicillinase-producing and transfer plasmids from the gonococcus to *Neisseria meningitidis*. *Lancet* **i**, 779–81.
91. Phyllis, B. (1988). Penicillin-resistant *Neisseria meningitidis* in Southern Africa. *Lancet* **i**, 54.
92. Roy, C., Tirado, M., Reig, R. *et al.* (1989). Type TEM β -lactamase activity in a *Neisseria meningitidis* strain. *Enfermedades Infecciosas y Microbiología Clínica* **7**, 206–9.
93. Vazquez, J. A., Enriquez, A. M., De la Fuente, L. *et al.* (1996). Isolation of a strain of β -lactamase-producing *Neisseria meningitidis* in Spain. *European Journal of Clinical Microbiology and Infectious Diseases* **15**, 181–2.
94. Bäckman, A., Orvelid, P., Vazquez, J. A. *et al.* (2000). Complete sequence of a β -lactamase-encoding plasmid in *Neisseria meningitidis*. *Antimicrobial Agents and Chemotherapy* **44**, 210–2.
95. Hammond, J. (1999). Overview: the many uses and applications of transgenic plants. *Current Topics in Microbiology and Immunology* **240**, 1–19.
96. Wolfenbarger, L. L. & Phifer, P. R. (2000). The ecological risks and benefits of genetically engineered plants. *Science* **290**, 2088–93.
97. American Society for Microbiology. (1995). Report of the ASM task force on antibiotic resistance. *Antimicrobial Agents and Chemotherapy* **39**, Suppl., 2–23.
98. Ministry of Health. (1998). *The Copenhagen Recommendations: Report from the Invitational EU Conference on The Microbial Threat, Copenhagen, September, 1998*. Ministry of Health, Denmark. INRA.
99. Ministry of Health, UK. (1998). *Standing Medical Advisory Committee Report The Path of Least Resistance*. The Stationary Office, London, UK.

Appendix: risk pathways

Application in the case of use of a bacterial antibiotic resistance marker gene in GM plants

Risk question

What is the probable consequence of the use of bacterial AR marker genes in GM plants on the incidence and level of antibiotic resistance expressed by bacteria?

Hazard

The hazards are bacterial AR genes.

The potential adverse effect is transfer of bacterial AR genes from GM plants to bacteria and expression of antibiotic resistance by bacteria leading to reduced therapeutic options.

Where bacterial AR genes are already present in a bacterium, incorporation of additional resistance genes may alter the quantitative expression of antibiotic resistance.

Risk pathways

Risk pathways describe chains of events. In the case of bacterial AR genes, this involves the following: release of the hazard (a bacterial AR gene) from the source, exposure of a susceptible target (bacteria),

Review

capture of the hazard (incorporation of the bacterial AR gene into a recipient bacterial genome) and expression of the hazard (expression of the bacterial AR gene by the recipient bacterium and its progeny).

In order for gene expression to occur, the entire chain must be patent. Breaking the chain eliminates risk. Two risk pathways can be conceived for bacterial AR gene transfer in the environment.

Risk pathway 1: gene transfer between bacteria (documented)

1. Presence of a bacterial AR gene in a bacterium
↓
2. Amplification of the bacterial AR gene by bacterial multiplication
↓
3. Exposure of other bacteria to those carrying the bacterial AR gene
↓
4. Transfer of the bacterial AR gene to other bacteria (several routes)
↓
5. Incorporation of the bacterial AR gene into a recipient bacterial genome (variety of methods)
↓
6. Expression of antibiotic resistance by recipient bacterium

Risk pathway 2: gene transfer from plants to bacteria (hypothetical)

1. Presence of a bacterial AR gene in a GM plant
↓
2. Bacterial AR gene amplification by crop cultivation
↓
3. Release of bacterial AR gene from GM plant cells, including via pollen production
↓
4. Exposure of target organisms (bacteria)
↓
5. Uptake of bacterial AR gene (as plant DNA) by target organism
↓
6. Incorporation of plant-derived bacterial AR gene into a recipient bacterial genome
↓
7. Expression of antibiotic resistance arising from plant-derived bacterial AR gene

Risk evaluation

The worst case assumption is that bacterial AR gene transfer occurs via pathway 2, albeit very infrequently.

Scenario 1. Where bacterial AR genes used as marker genes in GM plants are commonly present in and expressed by a range of bacteria (prevailing situation).

Risk pathway 1

There are a number of well-known pathways for gene transfer between bacteria and bacterial AR gene transfer occurs frequently compared with pathway 2.

Risk pathway 2

Bacterial AR gene transfer via pathway 2 is a low frequency event compared with pathway 1, but may involve bacteria that do not normally exchange genes via pathway 1.

Scenario 2. Where an antibiotic resistance gene is not already present in or expressed by bacteria (hypothetical situation).

Risk pathway 1

No gene-transfer because bacteria do not contain the gene.

Risk pathway 2

Gene transfer via this pathway is the only possible method of gene transfer and assumes significance even if the frequency of gene transfer is low.

Given transfer of a bacterial AR gene a further, distinct, risk question arises. What is the consequence for human health of bacteria acquiring the antibiotic resistance gene?

The major risk to human health is if the recipient bacterium is a potential pathogen, or may transmit the resistance gene to one, and where infection is controlled by use of the specific or a related antibiotic.

For bacterial AR genes that are already widespread in nature, the high-probability route for acquiring additional genes is from other antibiotic-resistant bacteria via risk pathway 1. Therefore, risk pathway 2 does not significantly increase risk unless it transfers resistance genes to a previously susceptible bacterium excluded from the mechanisms covered in pathway 1. In the latter case, the risk to human or animal health remains insignificant, unless the recipient is a pathogen or can serve as a gene conduit to a pathogen excluded from mechanisms applying in pathway 1. For the three bacterial AR genes, *aadA*, *aph(3')* and *bla_{TEM}*, reviewed in this report, we consider these hypothetical possibilities to be remote and therefore the risk arising from pathway 2 to be insignificant.

In contrast, for bacterial AR genes that are not yet widespread and that confer resistance to antibiotics to which bacteria are generally susceptible, risk pathway 2 represents a theoretical, but unquantifiable, risk of inducing antibiotic resistance in bacteria of clinical importance. (But it should be reiterated that this option does not apply in the cases specifically examined by the working party, which involve bacterial AR genes widely distributed and freely transmissible among bacteria belonging to many different genera.)

Recommendations

The precautionary assumption is that pathway 2 is patent.

The use, in GM plants, of bacterial AR genes that are already widespread among bacteria does not increase risk, unless bacteria, to which the gene has not previously been transmitted, are recipients via pathway 2 and this opens avenues of spread not previously available to pathogens. Regarding the bacterial AR genes considered in this study, the unanimous view is that their incorporation into GM plants poses no realistic threat to human or animal health.

In contrast, bacterial AR genes that are uncommon in bacterial pathogens and for which any further spread would be undesirable, if not disastrous, should not be used as marker genes in GM plant development.