Opinion

Streptomycetes are special: arcane applications

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The evolution and spread of antibiotic resistance determinants in human and animal pathogens has caused increased morbidity and mortality of infection throughout the world, with correspondingly staggering increases in cost to health-care systems (Davies and Davies, 2010). Although a proportion of this epidemic is a result of mutation, it is considered that most occurs through the acquisition of resistance genes from environmental sources. In particular, the streptomycetes, producers of the majority of known antimicrobials, possess cognate resistance genes associated with the biosynthetic pathway of the compounds made.

A comprehensive review of the antibiotic resistance (AR) genes found in actinomycetes that synthesize antimicrobial agents (mostly streptomycetes) has recently been published (Cundliffe and Demain, 2010). This review classified the resistance genes based on biochemical mechanisms, the majority of which have analogues in clinically isolated pathogens; more than 100 different mechanisms in six different classes were described. Incidentally, there exists little hard evidence that the roles of these many genes actually involve the function of 'self-resistance'.

Another group has compiled a list of AR genes defined by non-functional criteria, primarily nucleotide sequence relationships, and reported some 23 000 resistance genes for 249 different antibiotics (Liu and Pop, 2009). This compilation included mainly gene sequences that have not been definitively identified to have any resistance (protective) functions; putative resistance determinants based on sequence similarities make up the bulk of the list with few characterized at the function and protein level. The authors identified 380 'types' of resistance; this information is available online as the 'Antibiotic Resistance Database' (ARDB).

Another sequenced-based compilation with antibiotic-selected strains identified numerous AR genes present in the natural antibiotic 'resistome' (D'Costa *et al.*, 2007), thus providing ample proof of the wide distribution of potential AR genes in nature. They can be found in all environments, including human and animal microbiomes (Allen *et al.*, 2010). In general, the AR genes are found in the absence of any antibiotic production or exposure; they are 'quasi-'resistance genes. Potential for the evolution of clinically significant resistance in pathogenic bacteria is a question of considerable interest; at the present time a causal relationship between environmental resistance genes and the AR genes in resistant pathogens remains hypothetical. It is worth noting that metagenomic studies of environmental bacteria often indicate the presence of integron structures with gene cassettes closely related to known resistance genes (Gillings *et al.*, 2008); this supports the notion of 'pick-up' and lateral transfer of AR genes in the wild.

In the biological and medical sciences the use of antibiotics and associated AR genes as critical genetic tools is taken for granted, which presents an entirely different scenario! The experimental use of antibiotics and their resistance genes has been of inestimable value in contributing to the major advances made in the life sciences during the past three decades. Their applications in recombinant gene technology not only changed medical practice, but were instrumental in the commercialization of molecular biology as well. Without the use of these antibiotic-related tools there would have been no modern biotechnology!

The use of antibiotics and antibiotic resistance as laboratory tools began in the early 1950s when Esther and Joshua Lederberg employed streptomycin to demonstrate spontaneous mutation by replica plating and also to obtain estimates of the frequency of such events (Lederberg and Lederberg, 1952). Subsequently, antibiotic selection was used to establish the mechanism of conjugation; in particular, the use of streptomycin-resistant strains showed that polarity exists in this process, permitting the discovery of donor and recipient strains and the eventual demonstration of the mechanism of bacterial conjugation (Hayes, 1952). These and other experiments employing AR mutants were landmarks in the discovery

Table 1. Antibiotic/AR gene combinations for cloning.

AR gene	Source[a]	Antibiotic	Application[b]	References
aac	B	Apramycin	A, M	Kuhstoss and Rao (1983); Paget and Davies (1996)
hph	B, A	Hygromycin	A, Y	Gritz and Davies (1983); Kuhstoss and Rao (1983)
aph	B, A	Neomycin, G418	B, E, P	Davies and Jimenez (1980); Southern and Berg (1982); Velten and Schell (1985)
cat	B	Chloramphenicol	B	Desomer *et al.* (1990); Wang (1993)
sat	B	Nourseothricin	P, Y, Pr	Gold *et al.* (1994); Joshi *et al.* (1995); Lussier *et al.* (1997)
pac	A	Puromycin	Ar	Vara *et al.* (1986)
bla	B	Ampicillin	B	Bolivar *et al.* (1977)
tsr	A	Thiostrepton	A	Thompson *et al.* (1982)
erm	B, A	Erythromycin	A, B	Thompson *et al.* (1982)
tet	B	Tetracycline	E, B	Bolivar *et al.* (1977)

a. A: from producing strains; B: mainly Gram-negative pathogens, some Gram-positive.
b. A: actinomycetes; Ar: archaea; B: other bacteria; E: eukaryotes; M: mycobacteria; P: plants; Pr: protozoa; Y: yeast/fungi.

and development of bacterial genetics. The demonstration of the phenomenon of transferable antibiotic resistance came from Japanese workers (Watanabe, 1963), completely transforming the picture with respect to AR development in therapy. There had been no inkling that AR genes are capable of lateral gene transfer between bacterial genera. Applications of the genetic tools discovered were soon adapted to improve a number of industrial processes such as amino acid production.

It was not until the early 1970s that the most significant practical application of antibiotic resistance was revealed by the seminal experiments of Cohen and Boyer, who were successful in achieving laboratory-designed recombinant plasmids by cutting-and-splicing 'foreign' genes into resistance plasmids and then introducing them into different bacterial strains by transformation (Cohen *et al.*, 1973). These elegantly simple gene-splicing experiments employing plasmid-encoded resistance genes and antibiotics completely revolutionized the life sciences. Within a few years the transfer and expression of a variety of heterologous genes on plasmids engineered in *Escherichia coli* and other bacteria could be carried out in essentially any bacterial or eukaryotic cell, and by the 1990s genetic engineering in the *Archaea* completed the phylogenetic tree (Metcalf *et al.*, 1997). Shuttle plasmids capable of replication in different genetic backgrounds have been designed for specific purposes and now provide key elements of genetic toolkits. The essential component of virtually all transfer systems remains an appropriate selectable resistance marker originating from the R-plasmids of pathogens, but increasingly from the antibiotic-producing streptomycetes (Table 1). At present the market for plasmid and AR gene toolkits is considerable! Catalogues have become virtual histories of the development of genetic engineering.

These advances did not take place in the absence of controversy: questions were raised about the use of the bacterial neomycin phosphotransferase gene in the production of insecticide-resistant plants (Velten and Schell, 1985). The possibility of introducing AR genes into the environment was a concern. There is evidence for lateral transfer of AR genes from plants to bacteria (Kay *et al.*, 2002), but, given our current knowledge of the extent and universality of the environmental resistome, this would seem to be a very minor contribution to the AR gene pool. For practical and ethical reasons gene therapy in humans has met with some considerable opposition since the first approved demonstration of the introduction of a bacterial gene into human cells (Kasid *et al.*, 1990); the neomycin resistance gene *aph* was used and has been employed successfully in human and animal studies since that time.

Conclusions

Antibiotic use saves lives but concomitantly selects and maintains AR genes with serious consequences in infectious disease morbidity and mortality worldwide. The expanding roles of antibiotics and AR genes in the practice of the life sciences have been a major spin-off from their functions in infectious disease treatment, and use as selection agents and selective markers in medical research promises major advances in gene replacement therapy. Recent studies have demonstrated that antibiotics may have direct applications other than infectious disease treatment; the ability of aminoglycoside antibiotics to cause translation misreading (Davies *et al.*, 1964) has been applied to induce readthrough of mutant stop codons and restore defective gene function in the mitigation of several human genetic diseases (Hainrichson *et al.*, 2008).

One cannot escape the fact that streptomycetes are an inexhaustible source of bioactive small molecules and that their therapeutic uses are boundless.

Acknowledgements

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