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Various pathways leading to the acquisition of antibiotic resistance by natural transformation

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Natural transformation can lead to exchange of DNA between taxonomically diverse bacteria. In the case of chromosomal DNA, homology-based recombination with the recipient genome is usually necessary for heritable stability. In our recent study, we have shown that natural transformation can promote the transfer of transposons, IS elements, and integrons and gene cassettes, largely independent of the genetic relationship between the donor and recipient bacteria. Additional results from our study suggest that natural transformation with species-foreign DNA might result in the uptake of a wide range of DNA fragments; leading to changes in the antimicrobial susceptibility profile and contributing to the generation of antimicrobial resistance in bacteria.

Horizontal gene transfer (HGT) enables bacteria to utilize genetic variation present in their communities including new genes and traits.[1,2] Natural transformation is characterized by the uptake, integration and expression of extracellular DNA from the environment into a recipient cell that is naturally competent.[3,4] The ability to express natural competence has been described in at least 60 species of bacteria, such as *Streptococcus pneumoniae*, *Helicobacter pylori*, *Neisseria gonorrhoeae* and *Acinetobacter baylyi*. A larger number of bacterial species is expected to have the ability to undergo natural transformation if the relevant conditions can be identified.[5,6] Natural transformation has been demonstrated in a variety of environments, such as in human fluids,[7] in soil,[8-10] in water[11,12] and in foodstuff.[13,14] Natural transformation is suggested to contribute to the genetic variability in human pathogens, allowing adaptation to the host environment.[15,16]

Mobile genetic elements (MGEs), such as transposons and integrons, have a well-established role in bacterial genome dynamics; contributing to prokaryotic evolution and adaptation. Class 1 integrons with the same composition and organization of gene cassette arrays have been found in both Gram-negative and -positive bacteria,[17,18] in different species, in different geographical places and isolated in different periods of time;[19-22] clearly pointing to a role of HGT in the dissemination of integrons. Natural transformation has the potential to promote exchange of DNA among taxonomically diverse bacteria.[23] However, the contribution of natural transformation to the transfer and integration of integrons in the genomes of bacterial populations, and hence to the dissemination of associated antimicrobial resistance determinants, is not established. The heritable stability of DNA taken up by natural transformation will depend on self-replication (plasmids) or recombination with the bacterial genome.[24] Thus, natural transformation has been thought to be of limited importance in the transfer of chromosomal DNA between species due to the lack of sufficient DNA sequence similarity for homologous recombination to occur.

In our recent experimental study,[25] we showed that natural transformation with DNA isolated from various bacterial sources can result in the successful integration of class 1 integrons, gene cassettes and transposons in *A. baylyi* BD413; a well-established model organism for natural transformation studies.[26] We found that several of the gene transfer events investigated, had occurred independent on the genetic relatedness and hence, degree of DNA sequence similarity with the donor bacteria. The integration of synthetic gene cassettes by natural transformation has also been shown in *Pseudomonas stutzeri*.[27] Thus, we now ascertain that natural transformation can facilitate species-foreign DNA acquisitions due to either local DNA sequence similarity provided by broadly dispersed mobile genetic elements, or by transient expression of recombinase genes in DNA fragments present in the bacterial cytoplasm.

Three different mechanisms that contributed to the incorporation of class 1 integrons in the chromosome of the recipient bacterium were detected in our study: transposition promoted by an IS*26*-composite transposon; transposition promoted by Tn*21* transposon; and homologous recombination occurring between similar genes in the flanking regions of the donor class 1 integron and the recipient genome.[25] The acquisition

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Table 1. Minimal inhibitory concentration (MIC; μg/ml) of *A. baylyi* isolates obtained on transformant-selective plates as determined by the E test method

Bacterial isolate[a]	AM	CAZ	CN	CTX	K	SX
A. baylyi BD413 (recipient strain)	1	1.5	0.064	1.5	0.38	16
A. baumannii 064	> 256	n.d.	> 256	n.d.	> 256	> 1024
A. baylyi (AbII)5	12	n.d.	0.5	n.d.	1	32
A. baylyi (AbII)6	1	n.d.	0.38	n.d.	> 256	48
A. baylyi (AbII)L1	32	n.d.	0.38	n.d.	0.5	24
A. baumannii 65FFC	64	> 256	n.d.	> 256	n.d.	> 1024
A. baylyi (AbI)L1	6	8	n.d.	8	n.d.	16
A. baylyi (AbI)L2	4	8	n.d.	8	n.d.	64
A. baylyi (AbI)L3	0.75	1.5	n.d.	1.5	n.d	24
P. aeruginosa SM	> 256	n.d.	16	n.d.	n.d.	> 1024
A. baylyi (Ps)1	6	n.d.	0.19	n.d.	n.d.	24
A. baylyi (Ps)L1	6	n.d.	0.5	n.d.	n.d.	24
A. baylyi (Ps)L2	6	n.d.	0.38	n.d.	n.d.	24
S. enterica Rissen 486	> 256	n.d.	n.d.	n.d.	n.d.	> 1024
A. baylyi (Sr)1	4	n.d.	n.d.	n.d.	n.d.	16
S. enterica Typhimurium 490	> 256	n.d.	n.d.	n.d.	n.d.	> 1024
A. baylyi (St)L1	6	n.d.	n.d.	n.d.	n.d.	64
A. baylyi (St)L2	6	n.d.	n.d.	n.d.	n.d.	32

See reference 25 for details. [a]*A. baylyi* transformants were named based on the donor from which they were acquired: AbII, *A. baumannii* 064; AbI, *A. baumannii* 65FFC; Ps, *P. aeruginosa* SM; Sr, *S. enterica* serovar Rissen 486; St, *S. enterica* serovar Typhimurium 490; antibiotics: AM, ampicillin; CAZ, ceftazidime; CN, gentamicin; CTX, cefotaxime; K, kanamycin; SX, sulphamethoxazole. n.d., not determined.

of class 1 integrons and transposons was shown to occur at low frequencies from related as well as unrelated host species. This work represents the first report that shows that integron-carrying transposons can be transmitted with active transposition during natural transformation between bacterial species. The mechanistic model presented suggests that DNA taken up in a single-stranded form (ssDNA) can be re-annealed to double-stranded DNA (dsDNA) in the bacterial cytoplasm, enabling the MGEs present on such DNA to actively transpose to the recipient's chromosome. We also showed experimentally that the conserved regions of class 1 integrons provide sufficient DNA similarity for recombinational exchange of gene cassettes between class 1 integrons present in unrelated species. In conclusion, MGEs shared among species can provide sufficient DNA similarity for recombination to occur within such elements in the process of natural transformation. Recombination-based replacement of gene cassettes between integrons has also been suggested by others.[28,29]

The majority of the class 1 integrons described so far contributes to the dissemination of antimicrobial resistance genes (in addition to other gene cassettes with unknown function), and our study suggest a new pathway for the dissemination of antimicrobial resistance between genetic divergent bacteria; in addition to plasmid-based conjugation. Such natural transformation events, even if occurring at low frequencies, can be of clinical importance as they result in the initial acquisition of an integron-carrying MGE. The element can subsequently serve as a new genetic platform for the efficient capture or exchange of additional resistance gene cassettes.

In the course of our studies, we also found that the exposure of *A. baylyi* cells to DNA substrates of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Salmonella enterica* serovar Rissen and serovar Typhimurium produced several transformants in which we could not identify the acquisition of a class 1 integron, or other known antimicrobial resistance genes from the donor strain. These transformants showed a reduced susceptibility profile to some antibiotics, mainly to ampicillin and sulphonamides, when compared with the profile of the unexposed recipient bacterium. **Table 1** summarizes the antimicrobial susceptibility profiles of these transformants obtained in our recent study.[25] The profiles obtained suggest that exposure of competent bacteria to heterologous DNA may also lead to the transfer of other smaller DNA fragments, or possibly genetic rearrangements, that affect the antimicrobial susceptibility profile of the recipient cell. According to the level of resistance obtained in some transformants (**Table 1**), we find that the nature and scale of such genetic changes cannot necessarily be predicted or expected from the resistance pattern of the donor strain.

It is known that a broad range of DNA sizes can be acquired after HGT.[30,31] Transfer of smaller DNA fragments (< 1,000 bp) can be associated with the formation of mosaic genes, leading to changes in the antimicrobial susceptibility pattern. Interestingly, the formation of mosaic genes is known to occur in genes related to decreased susceptibility to β-lactams and sulphonamides; and the majority of the mosaic genes known are considered a result of natural transformation events.[32] Mosaic genes are frequently found in the Penicillin-Binding Protein (PBP) genes where blocks of nucleotides found in the susceptible strains are intercalated with blocks from the resistant ones. For example, Lujan and collaborators[33] found evidence that the resistance to penicillin in two isolates of *Neisseria lactamica* was due to the replacement of a region with 175 bp in the *penA* gene from *Neisseria flavescens*, which codes for the PBP2. Resistance to sulphonamides can be due to the mosaic allele formation in the dihydropterate synthase gene, *dhps*. For instance, the comparison between four resistant strains of *Neisseria meningitidis* with four susceptible to sulphonamides demonstrated the presence of exchanged segments of DNA in the *dhps* gene of the resistant strains.[34]

In most studies published so far, the recombination events resulting in mosaic genes occurred between isolates belonging to the same species or same genera. In contrast, our study[25] noted changes in susceptibility profiles resulting from

natural transformation of *A. baylyi* with both related and unrelated donor species and genera. In addition to the two genome sequences published in our recent study,[25] the genome of a third isolate growing on a transformant-selective agar plate, isolate (AbI)L2 was also sequenced. This isolate was obtained from transformation of *A. baylyi* BD413 with DNA from the class 1 integron-carrying *A. baumannii* 65FFC (carrying the carbapenemase IMP-5), and showed decreased susceptibility to ampicillin, ceftazidime, cefotaxime and sulphamethoxazole (**Table 1**). Nevertheless, we were unable to identify transfer of the integron or a β-lactamase gene from the donor. The genome of (AbI)L2 was fully sequenced in an attempt to identify genetic alterations that may explain the reduced antimicrobial susceptibility when compared with the recipient strain *A. baylyi* BD413. However, analysis of the genome sequence did not reveal indications of major DNA acquisitions.

The change in the resistance profile of isolate (AbI)L2 was therefore attempted resolved by examining minor nucleotide changes in genes known to be involved in intrinsic antibiotic resistance in *A. baylyi*, specifically the ACIAD0795, *acrB*, *ampD*, *argH*, *gph*, *gshA*, *hisF*, *mpl*, *oprM*, *pbpG* and *recD* genes.[35] The other PBP-coding genes, including ACIAD1184, *dacC*, *ftsI*, *pbpA* and *ponA* (GenBank accession number CR543861) as well as the *ampC* gene[36] present in the recipient *A. baylyi* were also examined for nucleotide changes. However, non-synonymous nucleotide changes that could explain the reduced antimicrobial susceptibility patterns of the transformants were not identified. These observations suggest that the altered antimicrobial susceptibility profiles in transformants might be due to smaller recombinations events that had occurred in other genes (structural or regulatory) that have not been yet described as involved in resistance, or the outcome of other transferable traits or rearrangements of unknown mechanistic and genetic nature. Recent studies reported co-transfer of numerous chromosomal polymorphisms between *Hemophilus influenza*[37] and *S. pneumoniae* and *Streptococcus mitis* genomes[38] by natural transformation.

In conclusion, our results lead us to suggest that interspecies horizontal gene transfer of chromosomal DNA can lead to multiple genetic changes in the genome of recipient cells with significant impact on the resistance profile. Broader studies are necessary to reveal the exact nature of such changes and the extent of species influenced by natural transformation events influencing their resistance profile. Prediction of the host impact and maintenance of such acquired traits may be evaluated by determination of fitness cost as well as by stability studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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