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The role of mobile genetic elements in bacterial adaptation to xenobiotic organic compounds

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Retrospective studies clearly indicate that mobile genetic elements (MGEs) play a major role in the *in situ* spread and even *de novo* construction of catabolic pathways in bacteria, allowing bacterial communities to rapidly adapt to new xenobiotics. The construction of novel pathways seems to occur by an assembly process that involves horizontal gene transfer: different appropriate genes or gene modules that encode different parts of the novel pathway are recruited from phylogenetically related or distant hosts into one single host. Direct evidence for the importance of catabolic MGEs in bacterial adaptation to xenobiotics stems from observed correlations between catabolic gene transfer and accelerated biodegradation in several habitats and from studies that monitor catabolic MGEs in polluted sites.

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Abbreviations

CB	chlorobenzene
2,4-D	2,4-dichlorophenoxyacetic acid
HGT	horizontal gene transfer
IS	insertion sequence
MGE	mobile genetic element
PAH	polycyclic aromatic hydrocarbon

Introduction

Xenobiotic compounds can be defined in a strict or broader sense. Xenobiotics *sensu strictu* are defined as man-made molecules, foreign to life and, as such, should have never been encountered by bacterial populations before their introduction by man. Examples are polychlorinated biphenyls (PCBs) and various pesticides. However, structurally related compounds (such as some chlorinated hydrocarbons) have been recently detected in nature, (e.g. as metabolites produced by microorganisms) [1]. Therefore, a broader definition of xenobiotics includes 'all compounds that are released in any compartment of the environment by the action of man and thereby occur in a concentration that is higher than natural' [2]. This larger group also includes heavy metals, polycyclic aromatic hydrocarbons (PAHs), and oil derivatives such as toluene. In this review, we will limit our discussion on bacterial adaptation to organic xenobiotic compounds as defined by Leisinger [2] and simply refer to them as xenobiotics or xenobiotic compounds.

There are several mechanisms, or combinations thereof, by which microbial communities can adapt to the presence of xenobiotics in their environment. Firstly, there can be an increase in population size of those organisms that tolerate or even degrade the compound by induction of appropriate genes. Secondly, the cells can adapt through mutations of various kinds, such as single nucleotide changes or DNA rearrangements that result in resistance to or degradation of the compound. Thirdly, they may acquire genetic information from either related or phylogenetically distinct populations in the community by horizontal gene transfer (HGT) — also called 'lateral gene transfer' [3]. Eventually, the individual cells best suited to resist or degrade the xenobiotic will be selected and sweep through the population until they constitute a larger fraction of the total microbial community than before the presence of the xenobiotic.

HGT between bacteria in natural habitats is largely mediated by mobile genetic elements (MGEs), which together form the so-called 'horizontal gene pool' [4], also referred to as the 'flexible gene pool' [5]. These elements can be transferable plasmids, (conjugative) transposons, integrons, genomic islands or phage, which are all able to move within and/or between genomes, thus allowing 'evolution in quantum leaps' [5]. The ability of microbial communities to adapt to anthropogenic chemicals by HGT first became evident from the observation that rapid dissemination of antibiotic resistance determinants by means of MGEs accompanied the widespread use of antibiotics [6]. More recently, the importance of HGT in bacterial evolution has been confirmed by comparison of available sequence information for prokaryotic genomes, showing that as much as 24% of the *Escherichia coli* genome has been acquired due to HGT [7]. During the past 15 years, several groups have provided strong indications that MGEs also play an important role in the horizontal spread of existing catabolic pathways, as well as in the natural construction of novel ones. This transfer allows bacteria to rapidly adapt to new xenobiotic compounds entering their habitats (see also the review of van der Meer and Sentchilo

in this issue [8]). Here, we summarize the evidence by giving an overview of retrospective analyses of catabolic MGEs and of studies that have shown adaptation of communities to xenobiotics by means of HGT.

Retrospective evidence for HGT as a natural mechanism of bacterial adaptation

One approach to collect evidence about how gene transfer has contributed to the adaptation of bacterial populations to a specific xenobiotic is to characterize and compare the genes involved in degradation of identical or similar xenobiotic compounds in different closely or more distantly related bacterial isolates from geographically distinct locations. Four key findings have been made from such studies. Firstly, evolutionarily related catabolic genes and gene clusters are found in bacteria originating from very distant locations [9]. Secondly, in some cases, as shown for some 2,4-dichlorophenoxyacetic acid (2,4-D) degradation genes, the phylogeny of the catabolic genes is not congruent with that of the 16S rRNA genes of the corresponding hosts [10,11]. Thirdly, genes for the degradation of organic pollutants, both of natural and synthetic origin, are often associated with MGEs such as plasmids and transposons [12]. Finally, evolutionarily related catabolic genes and entire gene modules are involved in the degradation of structurally similar but different xenobiotic compounds [13–15]. These observations strongly indicate that horizontal gene transfer by MGEs plays a major role in the worldwide spread of catabolic pathways and in the formation of novel ones. Formation of novel pathways seems to occur by recruitment of catabolic genes or gene segments from different organisms into one suitable host. This was recently confirmed by the identification of relics of precursor genes encoding the degradation of toluene, flanking gene clusters for chlorobenzene degradation in two independently isolated bacterial strains [16,17]. The fact that synthetic pollutants did not appear and certainly did not accumulate in the environment until about half a century ago, suggests that the necessary pathway assembly events must have occurred quite recently.

Furthermore, it has been found that the same genes or gene clusters can be part of different replicons. For example, almost exact copies of the *xyl* operons encoding the enzymes for toluene/xylene degradation have been identified on plasmids of different incompatibility groups [18], and very similar gene modules encoding enzymes for chlorocatechol degradation have been identified on both plasmids and conjugative integrative elements [19••,20]. These findings indicate independent movement and acquisition of such operons, which in some cases can be explained by the fact that the genes are enclosed within transposons. All in all, these observations suggest that catabolic genes and entire gene clusters are being shuttled between different replicons, thereby facilitating their spread within and among microbial communities. As a consequence, it can be argued that *in situ* assembly of a xenobiotic catabolic pathway can occur independently by recruiting the same pre-existing catabolic genes or gene modules [16]. For example, the almost simultaneous detection of very similar atrazine degradative genes and gene combinations in different hosts on different continents [21] may be explained either by independent recruitment of the same genes or by long-distance transport of bacteria combined with HGT of the entire operon.

One important group of catabolic MGEs consists of the catabolic plasmids. Interestingly, among these, the IncP-2 and IncP-9 plasmids often encode degradation of naturally occurring compounds, whereas genes that encode degradation of mostly man-made compounds, such as several chloroaromatics, are often encoded by the well-known broad host range IncP-1 plasmids [22]. These plasmids are the most promiscuous self-transmissible plasmids characterized to date, with a host range that is much wider than that of IncP-2 and IncP-9 plasmids (see Table 1 for examples). The possible correlation between this high promiscuity of IncP-1 plasmids and their ability to acquire and transfer genes encoding degradation of recently introduced xenobiotics is very intriguing. Indeed, highly transferable and promiscuous plasmids such as those of the IncP-1 group that act as shuttle vectors for locally adapted genes may facilitate rapid local adaptation of both phylogenetically related and distinct populations present in the same bacterial community. More DNA sequence information for IncP-1 plasmids and a better understanding of their dynamics in response to recent pollution will be necessary to fully understand their role in bacterial adaptation to xenobiotics.

During the past decade, the types of MGEs that carry determinants for organic pollutant degradation have been extended to class I and class II transposons, which are often located on catabolic plasmids [23], and even to the increasing array of mosaic MGEs including genomic islands, conjugative transposons and integrative plasmids [8,19••,24–26]. An overview of some examples of the different types of catabolic MGEs, together with a brief description for each, is presented in Table 1.

Although the role of MGEs in the molecular adaptation to organic pollutants seems clear, some questions are still unanswered. For example, how are these genes recruited by MGEs? Only a few researchers have looked at the possibility of trapping genes with catabolic plasmids [27]. Interestingly, both on MGEs and in the chromosome, catabolic genes are often bordered by insertion sequences (IS). These IS elements may have played a role in recruitment of these genes by the replicon, but also increase the potential of further exchange of the genes between different hosts and replicons [28–30]. An intriguing case in this context is the identification of IS*1071*-like sequences, which flank many plasmid-borne catabolic genes in a diverse range of organisms including Gram-negative

Table 1

Examples of the different types of catabolic mobile elements.

Mobile element	Strain	Substrate(s)	Size of MGE (kb)	*Inc group	Relevant remarks and references
Plasmids					
pSS60	*Achromobacter* sp. LBS1C1	4-Chlorobenzoate	53	P1	Recruitment of a 4-chlorobiphenyl (4CBP) degradative gene cluster present on a chromosomally located mobile element by pSS60 has been observed, creating plasmid-encoded mineralization of 4CBP [24].
pBRC60	*Alcaligenes* sp. BR60	3-Chlorobenzoate	75	P1	Contains Tn*5271* carrying chlorobenzoate catabolic genotype *cba* (see below) [29].
pENH91	*Ralstonia eutropha* NH9	3-Chlorobenzoate	78	P1	Carries chlorocatechol catabolic composite transposon Tn*5707* (see below) with a gene module evolutionarily related to the chlorocatechol degradation gene modules present on the CB degradation plasmids pP51 and pPS12-1, on the *clc* element, and on the 2,4-D degradative plasmid pJP4 [20].
pJP4	*Ralstonia eutropha* JMP134	2,4-D	75	P1	Carries different gene modules the combined action of which leads to mineralization of 2,4-D. Chlorocatechol degradation genes are evolutionarily related to those on the *clc* element and pENH91, pP51 etc.; pJP4 contains a duplication of these genes and is prone to rearrangements. The plasmid contains IS elements flanking the chlorocatechol genes and IS*1071*-related elements. Other 2,4-D degraders carry similarly or differently organized genes [50].
pTSA	*Comamonas testosteroni* T-2	*p*-Toluenesulfonic acid	85	P1	Carries two copies of a *tsa* gene cluster encoding *p*-toluenesulfonic acid degradation. These are flanked by two IS*1071* elements, constituting a putative composite transposon; *tsa* genes were also found in other organisms from distant locations present on the same composite transposon, but only one strain contained a plasmid-encoded *tsa* gene [51].
pP51	*Pseudomonas* sp. P51	Chlorobenzene			Carries a chlorocatechol degradation gene module similar to that of pPS12-1, the *clc* element and pENH91. In addition, this plasmid encodes a composite transposon Tn*5280* (see below) carrying *tcbAB* genes for the degradation of CB into chlorocatechol. Thus, this pathway is a clear example of natural pathway assembly [17].
pPS12-1	*Burkholderia* sp. PS12	1,2,4,5- Tetrachlorobenzene	85	P1	Carries a chlorocatechol degradation gene module similar to that of pP51, the *clc* element and pENH91. In addition, it encodes a composite transposon carrying *tecAB* genes for the degradation of CB into chlorocatechol. These *tecbAB* genes are similar to the *tcbAB* genes of strain P51, but are flanked by different sized remnants of the toluene pathway [16].
Class I transposons					
Tn*5280*	*Pseudomonas* sp. P51	Chlorobenzene	8.5		Present on pP51 (see above), carries the *tcbAB* genes responsible for CB transformation into chorocatechol. The *tcbAB* genes are clearly derived from a toluene degradation pathway on the basis of the presence of gene relics belonging to the toluene degradation genes, which flank the *tcbAB* genes [17].
Tn*5271*	*Alcaligenes* sp. BR60 (pBRC60)	Chlorobenzoate	17		Present on pBRC60 (see above). Carries chlorobenzoate catabolic genotype *cba*. Homologous transposons have been found in other chlorobenzoate degraders from different geographical origins. In some cases these transposons carry gene relics of other pathways [29].
Tn*5707*	*Ralstonia eutropha* NH9 (pENH91)	3-Chlorobenzoate	15		Present on pENH91 (see above). Carries genes evolutionarily related to the chlorocatechol degradation genes on plasmids pP51 and pPS12-1, on the *clc* element and on pJP4 [20].
DEH	*Pseudomonas putida PP3*	Chlorinated aliphatic acids	9.74		This transposon contains a dehalogenase gene and is bordered by two copies of the IS element IS*Ppu12*, which has also been found flanking other catabolic genes [52].
Class II transposons					
Tn*4651*	*Pseudomonas putida* mt-2 (pWWO)	Toluene, xylene	56		Carries gene modules for toluene/xylene degradation via catechol, located between two IS elements. The transposon is located within another transposon Tn*4663*. The genes are similarly organized in many other TOL plasmids [18,53].
Tn*4653*	*Pseudomonas putida* mt-2 (pWWO)	Toluene, xylene	70		Carries Tn*4661* (see above) [53].

Table 1 Continued

Mobile element	Strain	Substrate(s)	Size of MGE (kb)	*Inc group	Relevant remarks and references
Tn*4655*	*Pseudomonas putida* G7 (NAH7)	Naphthalene	38		Carries three gene modules for naphthalene degradation via catechol. The module encoding catechol degradation is similar in organization to that of the pWWO plasmid [53].
Other elements					
Tn*4371*	*Ralstonia oxalatica* A5	Biphenyl/ 4-chlorobiphenyl	55		This element is an unusual combination of phage-like genes (including an integrase), catabolic genes and genes for plasmid transfer. Similar elements have been found in other polychorinated biphenyl degraders [24].
clc element	*Pseudomonas* sp. B13	Chlorocatechol	105		Carries a chlorocatechol degradation gene module similar to that of pP51, pPS12-1 and pENH91 [19••].

*Inc group, incompatibility group.

and Gram-positive bacteria [29,31]. Another study suggests integrase-dependent gene acquisition of catabolic genes [32]. New information on this topic will undoubtedly become available through analysis of whole plasmid genomes. Another interesting question is how the different genes constituting a complete pathway finally came together in one strain or even on one plasmid. Interestingly, for some xenobiotics, such as atrazine [33] and nitrotoluene [34], degradation is sometimes mediated by a combination of bacterial strains, which each carry different genes responsible for different parts of the pathway. The same genes are, however, in both cases also found combined in one bacterial strain, which is able to mineralize that same compound by itself. This might suggest that such gene combinations in one organism have evolved over time through independent gene exchange events between different strains, which were brought together in a synergistic community. HGT might have been facilitated by the formation of tight cell contacts (preferably on surfaces), which was required for efficient metabolic fluxes between the different cells of the consortia. It should be noted in this context that higher conjugative gene transfer frequencies have been reported in biofilms [35] and that natural conjugative plasmids can induce biofilm development [36]. Cell consortia and biofilms may therefore be very conducive to the assembly of new pathways by HGT and lead to final pathway assembly in one host if the community characteristics allow it. At first sight, it may seem more advantageous to any member of the consortium to contain the entire degradative pathway, instead of having to rely on neighboring cells. However, the fitness cost associated with maintaining this genetic information, often on a self-replicating self-transferable plasmid, may outweigh the benefit of being independent of the other members of the community for (part of the) resources. This delicate balance may explain why both bacterial consortia and single strains with very similar catabolic genes and phenotypes have been found.

These different pieces of information, although very informative, only provide retrospective evidence and questions still remain as to when, where, at what rates and between what range of 'species' these HGT events actually occur. We also need to address what the intensity of selection is for those catabolic genes under natural conditions. Our picture of the microbial populations involved in xenobiotic degradation may be very distorted by limitations such as poor culturability of important populations, selective enrichment for fast-growing bacteria under conditions of enrichment cultures in the laboratory, and possibly gene transfer during these enrichments. Therefore, these retrospective analyses, although very valuable, have to be complemented with studies that monitor the actual gene transfer processes and pathway assembly in natural habitats.

Direct evidence of microbial community adaptation by HGT

A direct way to examine if transfer of a degradative MGE among soil bacteria plays a role in bacterial adaptation to the corresponding organic contaminant is to monitor both the degradation of that xenobiotic compound and the dissemination of the degradative genes. Several studies have demonstrated that horizontal transfer of catabolic genes, mostly by means of plasmid-mediated conjugation, occurs in soil microcosms, bioreactors, lake mesocosms and so on after inoculation of a donor strain containing a natural catabolic MGE [12,37–39]. Some of these studies also showed that the number of transconjugants (i.e. indigenous bacteria that acquired the xenobiotic-degrading phenotype after uptake of the catabolic MGE) was higher when the corresponding xenobiotic was present in the habitat. Very few groups, however, have provided clear direct evidence of adaptation of the microbial community to this xenobiotic, that is, by showing that the overall degradative capacity improved due to this gene exchange. We will restrict our overview of these studies to the most recently published ones, and refer the reader to a recent minireview for a more complete and extensive description of case studies [12].

A series of studies on catabolic gene transfer in soils by Newby and coworkers [40–42] and Top and coworkers

[43••] have used the herbicide 2,4-D as a model compound, and the IncP-1β plasmid pJP4 or the non-IncP-1β plasmid pEMT1, both encoding 2,4-D degradation, as model plasmids. In some, but not all, cases a clear correlation was shown between transfer of the plasmids from inoculants to indigenous bacteria and adaptation of the soil community to degrade 2,4-D. Another study on gene transfer in soil showed adaptation of a rhizosphere bacterial community from pine seedlings mycorrhized with *Suillus bovinus* to a pollutant. The self-transferable TOL plasmid was acquired from an inoculated *Pseudomonas fluorescens*, thereby protecting the plant and fungus from meta-toluate [44]. *In situ* spread of the *tfdA* gene, encoding the first step in the degradation of 2,4-D and phenoxyacetic acid (PAA), from an inoculum into indigenous phenol degraders in soil was shown to result in vertical expansion of the phenol degradation pathway to now include PAA. Interestingly, a higher number of transconjugants was detected in the residue sphere (i.e. the interface between decaying plant material [barley leaves] and the soil matrix) than in the bulk soil, which was explained by an increased biomass of donors and recipients in this microhabitat [45••]. Similarly, Springael and coworkers [46••] also observed transfer of the *clc* element (see Table 1) in an inoculated model biofilm membrane reactor treating chlorobenzoate-containing influent. The original inoculum carrying the *clc* element was frequently found to be replaced by other chlorobenzoate-degrading bacteria. As control reactors without inoculum never developed a chlorobenzoate-degrading microbial community nor a detectable degradation activity, it was suggested that contaminant bacteria had acquired the *clc* element from the inoculum strain and became more competitive under the implied bioreactor conditions. This result is in agreement with earlier observations in a gnotobiotic biofilm, which showed that a new biochemical pathway offering a growth advantage can be introduced effectively in a biofilm community when carried on an MGE [47].

Several of these reports show that the spread of catabolic genes on MGEs enabled specific indigenous bacterial species to acquire new catabolic characteristics and thereby to become numerically dominant members of the community. In addition, habitats such as the rhizosphere residue sphere and biofilms might play a yet underestimated role in the adaptation of microbiota to pollutants. The disadvantages of these studies are the unnatural aspect of inoculation of high numbers of donor cells (often 10^6–10^7 colony-forming units [CFU]/g soil), and the relatively high concentrations of contaminants added. In addition, particularly in the case of soil, these manipulations almost always entail mixing and thus disturbing the samples. There is no direct evidence that HGT would be as clearly responsible for community adaptation without this disturbance and at lower, more environmentally relevant, xenobiotic concentrations. Another important aspect is the inclusion of all necessary control experiments to exclude the possibility that the detected 'transconjugants' are indigenous organisms that already contained the same or a similar catabolic gene or MGE as the one introduced via the donor strain. Often, transconjugants are only verified by limited screening methods to confirm the presence of the inoculated gene/MGE. It is not impossible that rare organisms with the same or similar catabolic MGEs become enriched upon manipulation of the ecosystem; for example, the inoculation of large cell numbers (often 10^6–10^8 CFU/g or ml) disturbs the system in an uncontrolled way and introduces nutrients once the cells die off. It may therefore be recommended to include a control microcosm inoculated with the donor strain without the catabolic MGE instead of the usual control without inoculum. Despite these shortcomings, overall these studies are very valuable, as they monitor a direct correlation between HGT and adaptation of soil bacterial populations to xenobiotics.

Field studies to assess microbial community adaptation by HGT

The most direct, non-disturbing and objective approach that could be used to evaluate whether MGEs have contributed to the adaptation of a microbial community to pollution, is to compare the degradation potential and the presence of degradative genes within bacterial communities in the field before and after a specific pollutant spill has occurred. A good indicator of gene exchange in response to a new pollutant entering the habitat would, for example, be the detection of a catabolic gene in only one population before the contamination, and in multiple phylogenetically distinct populations after contamination. Another valuable indication would be the assembly in one organism of two or more different catabolic genes or operons that were previously only found separately in distinct bacterial strains. To our knowledge there are no studies with such direct evidence, as data from the period before contamination are usually not available. There are, however, a few good examples of field studies that strongly suggest that the horizontal transfer of mobile catabolic genes has played an important role in community adaptation to the pollutant present in the site, in spite of the absence of data obtained before pollution. For example, six years after introduction of a plasmid-encoded *pheBA* operon (encoding enzymes for phenol degradation) for the purpose of bioremediation after an accidental fire in an Estonian oil shale mine, this gene cluster was detected in a few different *Pseudomonas* sp. isolates. These findings suggested that several bacterial populations had adapted to the polluted environment by acquiring and expressing the introduced genetic material that allowed them to use the pollutant as a substrate [48]. The authors were not able to completely exclude that this same *pheBA* gene cluster was already present in that site before the donor strain was released. They

argued, however, that this would have been very unlikely given the very high similarity of the detected *pheBA* operons with the unique features of the introduced one, which originated from a site in a different country and had been in a laboratory environment for a long time. In a coal tar contaminated field site in the US, strong evidence was provided that natural horizontal transfer of naphthalene dioxygenase genes had occurred between members of the soil bacterial community. A naphthalene catabolic plasmid may have played a role in adaptation of this community to the coal tar contamination [49] (see Update). Finally, a study by van der Meer *et al.* [15] found that chlorobenzene (CB)-degrading bacteria could be isolated from CB-contaminated groundwater, but not from the uncontaminated region outside. The study clearly showed that bacterial adaptation to the pollution, as demonstrated by enhanced CB removal, must have occurred by genetic recombination among bacteria in the aquifer, resulting in a novel CB degradation pathway. As pointed out above, important drawbacks in field studies that monitor the spread of a specific gene into various indigenous populations include the absence of perfect controls and problems with detection limits. The lack of detection of a specific gene or combination of genes in an organism before pollution or before inoculation of a specific donor strain, does not necessarily mean its absolute absence in that population. Therefore, detection of the gene afterwards is not per se due to HGT, but could also be a consequence of an increase in the population size of that previously undetectable catabolic gene(s)–host combination. Field studies that include the proper controls with sensitive detection methods will be indispensable to evaluate the relative importance of gene transfer as one of the mechanisms of local adaptation of bacterial populations to xenobiotics.

Conclusions

In conclusion, this review summarizes evidence that the catabolic operons known today have evolved in part thanks to MGEs, which brought diverse catabolic genes together in one host and thus allowed a wide range of organic compounds to be degraded through a few central metabolic pathways. Specific cases of clear correlations between the observed transfer of catabolic genes and microbial community adaptation to specific organic xenobiotics are still very limited. A few examples indicate that in cases where the limiting factor for biodegradation is the absence of the necessary genes, inoculated catabolic MGEs seem to spread and thereby allow rapid adaptation of the community, resulting in the accelerated removal of pollutants. It has become clear, however, that in spite of the many studies on gene transfer in soil and to some extent in bioreactors, our basic understanding of factors that determine the dissemination of genes in such systems needs to be improved. This includes understanding the conditions that stimulate conjugation and other gene transfer mechanisms in these ecosystems, and comprehending the role of IS elements such as IS*1071* in the assembly of catabolic pathways. In addition, we know very little about the natural host range of several catabolic MGEs and the expression range of their catabolic genes. Furthermore, we also need to learn more about the competitive fitness of transconjugants and the maintenance of MGEs, both in the absence and presence of selective pressure and at more environmentally relevant pollutant concentrations, which are often much lower than those applied so far in most studies.

Update

Recent work by Müller *et al.* [54] presents additional evidence on the involvement of HGT in the *de novo* construction of catabolic pathways in natural environments. A chlorobenzene catabolic pathway was shown to have been assembled in one bacterial strain. This was achieved by IS-mediated mobilization of a gene module that encodes conversion of chlorinated benzenes to chlorocatechol, onto an integrative element that encodes chlorocatechol degradation. In addition, very recent evidence for natural horizontal transfer of a naphthalene dioxygenase gene (*phnAc*) in a polycyclic aromatic hydrocarbon-contaminated site underlines the importance of HGT in bacterial adaptation to pollutants [55].

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The *clc* element was, together with Tn*4371*, the first conjugative integrative element or gene island to be shown to encode degradation of xenobiotics and employs phage-like integrases. These elements extend the types of xenobiotic degradative MGEs beyond plasmids and transposons and might indicate a 'yet to come' diversity of 'undiscovered' catabolic MGEs. This review gives an excellent overview of such MGEs, emphasizing their relationship with similar elements that encode different functions.

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