Class 1 Integrons Potentially Predating the Association with Tn402-Like Transposition Genes Are Present in a Sediment Microbial Community

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Integrons are genetic elements that contribute to lateral gene transfer in bacteria as a consequence of possessing a site-specific recombination system. This system facilitates the spread of genes when they are part of mobile cassettes. Most integrons are contained within chromosomes and are confined to specific bacterial lineages. However, this is not the case for class 1 integrons, which were the first to be identified and are one of the single biggest contributors to multidrug-resistant nosocomial infections, carrying resistance to many antibiotics in diverse pathogens on a global scale. The rapid spread of class 1 integrons in the last 60 years is partly a result of their association with a specific suite of transposition functions, which has facilitated their recruitment by plasmids and other transposons. The widespread use of antibiotics has acted as a positive selection pressure for bacteria, especially pathogens, which harbor class 1 integrons and their associated antibiotic resistance genes. Here, we have isolated bacteria from soil and sediment in the absence of antibiotic selection. Class 1 integrons were recovered from four different bacterial species not known to be human pathogens or commensals. All four integrons lacked the transposition genes previously considered to be a characteristic of this class. At least two of these integrons were located on a chromosome, and none of them possessed antibiotic resistance genes. We conclude that novel class 1 integrons are present in a sediment environment in various bacteria of the β-proteobacterial class. These data suggest that the dispersal of this class may have begun before the “antibiotic era.”

Integrons are a diverse family of genetic elements that possess a site-specific recombination system for the capture of genes that are contained within mobile gene cassettes (5, 12, 17, 40, 48). Class 1 integrons are the best-known examples of these elements and were the first to be characterized (28, 48). They are particularly important in clinical contexts, as their gene cassettes predominantly encode resistance to antimicrobial agents. They are a major contributor to the problem of multidrug-resistant pathogens (13), and the medical microbiology literature extensively cites examples of gram-negative and, more recently, gram-positive pathogenic bacteria that are intractable to antibiotic therapy as a consequence, at least in part, of possessing a class 1 integron (see references 40 and 44 for reviews). Class 1 integrons can also be recovered from commensal bacteria associated with humans and other animals outside a clinical context (9, 21). Most frequently, class 1 integron-containing strains are identified after antibiotic selection. However, at least one study has shown that class 1 integrons that include antibiotic resistance genes are sufficiently common that they can be recovered in the absence of antibiotic selection (2).

Class 1 integrons are generally embedded in mobile elements, including numerous plasmids and transposons. Consequently, unlike most other integron classes, they are amenable to lateral gene transfer (LGT) (22, 28) and, when present on a chromosome, they are presumed to have transposed there (27, 47). In contrast, “chromosomal” integrons are capable of recruiting a diverse assortment of novel mobile genes but are, nonetheless, confined to defined phylogenetic lineages (8, 17, 31, 46).

Antibiotic resistance as a problem arose soon after the onset of the clinical use of antibiotics. After that time, resistance genes were commonly observed to be present on mobile elements, such as plasmids and transposons; before that time, they appear to have been relatively rare on these elements (18). The antibiotic era also coincided with a time of rapid appearance of class 1 integrons carrying antibiotic resistance genes, with these integrons appearing in a number of independent locations in different transposons and plasmids (28, 48). The simplest explanation for this rapid appearance in diverse clinical isolates and on many different types of mobile elements is that the class 1 integrons were moving by transposition. If class 1 integrons were originally derived from a chromosomal integron, then association with transposition genes that facilitate their mobility presumably occurred in the preantibiotic era or very early after the onset of the antibiotic era.

Class 1 integrons are characterized by several features (Fig. 1). These include the presence of a 5′ conserved segment (CS). This region includes the DNA integrase gene, intI1, and the
genes inserted at position genes, when present, are located downstream of the type found in Tn\textsuperscript{402} and its relatives (23). These transposition genes, such as those in Tn\textsuperscript{402}, are not universally present. Consequently, the acquisition of such additional transposition functions had to occur through events postdating the linkage of the Tn\textsuperscript{402}-like genes to the site-specific recombination system.

The structure exemplified by Tn\textsuperscript{402} is likely to represent the ancestral mobile form of the mobile element that includes the class 1 integron. However, this structure is relatively rare, and most class 1 integrons are associated with an incomplete transposition (\textit{tni}) module. Instead, the class 1 site-specific recombination system is linked to another region, known as a 3'-CS (Fig. 1) (4, 48). This segment includes a truncated \textit{qacE} gene, the intact version of which is present in Tn\textsuperscript{402}, and it is presumed to have evolved from a Tn\textsuperscript{402}-like ancestor by events that include the incorporation of \textit{a sulI} gene conferring resistance to sulfonamides (23). This and other rearrangements have led to the loss of \textit{tni} functions (4) and to differences in the structure of the 3'-CS when different class 1 integrons are compared (14, 34, 35). Some class 1 integrons recovered from strains of \textit{Pseudomonas aeruginosa} also have a copy of an insertion sequence, IS\textit{Pa}7, inserted between \textit{IRi} and the end of \textit{intI1} (1, 25, 42, 49). Class 1 integrons, therefore, are found in association with considerable structural diversity, especially in relation to the makeup of the 3'-CS (4, 14, 34, 35). Despite these multiple rearrangements, all retain evidence of at least some Tn\textsuperscript{402}-like transposition features.

Whether the linkage of site-specific recombination functions with Tn\textsuperscript{402} transposition functions was an ancient event or one that occurred in historical times, it is nonetheless the case that it has become a powerful vehicle for the spread of resistance genes. The site-specific recombination system has allowed individual class 1 integrons to capture a diverse array of resistance determinants. The Tn\textsuperscript{402}-like transposition genes have, in turn, facilitated the incorporation of this element into a myriad of other mobile elements. These other elements can comprise plasmids into which the mobile class 1 integron has transposed directly or other transposons into which the class 1 integron/transposon has inserted (40). The latter situation, an “integron within a transposon within a transposon,” is perhaps best exemplified by the capture of a class 1 integron with Tn\textsuperscript{402} transposition functions by the Tn21 family of transposons (28).

As noted above, all class 1 integrons found to date possess at least some evidence of Tn\textsuperscript{402}-like transposon features and/or sequences that make up the 3'-CS (34, 35, 39). Other types of transposition genes, such as those in Tn21, are not universally present.

Here, we have screened forest soil and lake sediment environments removed from clinical settings for bacteria containing class 1 integrons. Sample culturing was done in a way that does not bias toward the recovery of antibiotic-resistant bacteria. All environments contained isolates that were positive for the \textit{intI1} gene. Class 1 integrons were characterized for four phylogenetically distinct bacteria. While they were identical or nearly identical to class 1 integrons from clinical settings, all lacked the Tn\textsuperscript{402}-like transposition genes that are presumed to have contributed to the dissemination of this class into pathogenic and commensal bacteria in historical times. In addition,
none included antibiotic resistance cassettes. The broad distribution of class 1 integrons in this environment, and the evidence for dispersal to numerous locations, demonstrates that extensive transfer of these integrons can occur across very disparate environments. This dispersal may have occurred via a process independent of that which brought about the dispersal of the class 1 integrons described up until now.

**Materials and Methods**

Sample site description. The class 1 integrons characterized in this study all came from reed-covered sediment sourced from Lake Yerbury, North Ryde, Australia. This is an artificial lake on the Macquarie University campus (33°46′4″S, 151°06′9″E) created by the construction of a small causeway across Mars Creek. Mars Creek forms part of the Lake Yerbury River catchment and drains a low-inhabited wetland area of Sydney. The creek collects storm water runoff but is not now within an area of agricultural or animal production. There is no hospital on the site or in the immediate vicinity. Prior to the construction of the university in the 1960s, adjoining land was used for small-plot market gardening. Information regarding the use of agricultural chemicals or antibiotics during this period is unavailable.

Strain recovery and characterization. Soil or shallow freshwater sediment was serially diluted in 100 mM sodium phosphate buffer (pH 7.0) and plated to plate count agar (PCA) medium (5 g tryptone, 2.5 g yeast extract, 1 g dextrose and 12 g agar per liter). Plates were incubated at 25°C for 5 days, after which 200 individual colonies from each environment were picked into 100 μl of PCA broth in sterile microtiter trays. Trays were incubated at 25°C for 48 h.

Crude DNA was prepared from all bacterial isolates (at least 180 for each environment) by harvesting 10 μl from each well into 30 μl sterile water, heating to 99°C for 10 min, and centrifuging to pellet cell debris. The 16S rRNA gene was also amplified for samples that tested positive for intI1 (24). The nitrogen fixation gene nifH was amplified from the DNA of isolate MUL2G9 using primers 19B and 407B (11). Amplification mixes consisted of 5 μl of template DNA, 200 nM dNTP, 50 pmol of each primer, 2 μM MgCl2, and 1 U of Hot DNA polymerase in the reaction buffer supplied with the enzyme. After initial thermal denaturation, PCR was performed for 30 cycles of 94°C for 1 min, 60°C for 45 s, and 72°C for 80 s. All putative gene products were sequenced using an ABI Prism 377 (PE Biosystems) and BigDye v3.1 chemistry. Table 1 shows a list of the PCR primers used to characterize and sequence class 1 integrons in this study.

Construction and screening of fosmid libraries. Fosmid libraries were constructed from the genomic DNA of Acidovorax sp. MUL2A2, Acidovorax sp. MUL2G8, Azoarcus communis MUL2G9, and Burkholderiales bacterium MUL2G11 fosmid libraries were not completely sequenced. Instead, their integrons were sequenced by walking away from the integron gene in both directions. Sequence (~5 kb) was obtained for each strain, including the complete integron as well as some flanking DNA.

Sequence alignments and phylogenetic analysis. 16S rRNA gene sequences for the MUL2A2, MUL2G8, MUL2G9, and MUL2G11 isolates were used as BLASTN queries to retrieve closely related sequences from GenBank and identify their taxonomic affiliations. Representative 18S rRNA genes from the taxonomic groups to which the isolates belonged were retrieved from GenBank and aligned to the sequences generated in this study using CLUSTALW (50). The alignment was subsequently edited manually to remove ambiguous characters. The 16S rRNA gene trees were constructed with PAUP* 4.0b4, applying the heuristic search option and using the tree bisection-reconnection branch-swapping algorithm. Maximum likelihood was used as the tree reconstruction method, with the nucleotide substitution model (general time reversible), gamma rate parameter α, proportion of invariable sites, and nucleotide frequencies determined using MODELTEST (37). The confidence of each node was determined by building a consensus tree of 100 maximum likelihood trees from bootstrap pseudoreplicates of the original data set.

Amino acid alignments of ORFs encoded in the intI1-containing genomic DNA fragments from the MUL2G8, MUL2G9, and MUL2G11 isolates were constructed using CLUSTALW and edited manually to remove ambiguous characters. Maximum likelihood phylogenetic analyses were applied to these datasets using PROML with the JTT amino acid substitution matrix, a rate heterogeneity model with gamma-distributed rates over four categories with the γ parameter being estimated using TREE-PUZZLE, global rearrangements, and randomized input order of sequences (10 jumbles). Bootstrap support values represent a consensus (obtained using CONSENSE) of 100 Fitch-Margoliash distance trees (obtained using PUZZLEBOOT and FITCH) from pseudo-replicates (obtained using SEQBOOT) of the original alignment. The settings of PUZZLEBOOT were the same as those used for PROML, except that no global rearrangements and randomized input order of sequences are available with this program. PROML, CONSENSE, FITCH, and SEQBOOT are from the PHYLIP package (obtained using PUZZLEBOOT and FITCH) from pseudo-replicates (obtained using SEQBOOT) of the original alignment. The settings of PUZZLEBOOT were the same as those used for PROML, except that no global rearrangements and randomized input order of sequences are available with this program.

**Table 1. Primers for the amplification and sequencing of class 1 integrons**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5′ to 3′)</th>
<th>Location or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS463a</td>
<td>CTGGATTCGATCCGGACGGC</td>
<td>16</td>
</tr>
<tr>
<td>HS464</td>
<td>ACATGCGTTAATACCTGTCG</td>
<td>16</td>
</tr>
<tr>
<td>HS458</td>
<td>GTGGTATGTTAGGACGAC</td>
<td>16</td>
</tr>
<tr>
<td>HS459</td>
<td>GCAAAGAGACGACATTATGAGC</td>
<td>16</td>
</tr>
<tr>
<td>HS549</td>
<td>ACTAAGCGTGCCCCCTTCCGGC</td>
<td>Start of u11 gene in 3′-CS</td>
</tr>
<tr>
<td>HS550</td>
<td>TTACGCTATGTAACCTCCGGG</td>
<td>End of u11 gene in 3′-CS</td>
</tr>
<tr>
<td>HS714</td>
<td>CCTTTCACAGAAGCGGCTGC</td>
<td>Within R1 of 5′-CS</td>
</tr>
<tr>
<td>HS715</td>
<td>GCAAGGCTCCAAAGATCG</td>
<td>Within intI1 gene</td>
</tr>
<tr>
<td>HS721</td>
<td>TCGAAAGCTTGTAGTGCAG</td>
<td>Within VP174 homolog of MUL2G8 and MUL2G11</td>
</tr>
<tr>
<td>HS722</td>
<td>CCTCAAGTGTCACAGTCAG</td>
<td>Within InP18orf1 of MUL2G11</td>
</tr>
<tr>
<td>HS723</td>
<td>AGCAATCTCAGCAGTCAG</td>
<td>Within m4 of gene of MUL2G11</td>
</tr>
<tr>
<td>HS724</td>
<td>GCAGCATTACCTGGAAGTGC</td>
<td>Within m4 of MUL2G11</td>
</tr>
<tr>
<td>HS725</td>
<td>AGGTIAAGGCATGGCCTGC</td>
<td>Within m4 of MUL2G11</td>
</tr>
<tr>
<td>HS726</td>
<td>GTGTCGCCTCACAATC</td>
<td>Within intI1, complementary to bases 5 to 24 of HS458</td>
</tr>
</tbody>
</table>
RESULTS

Detection of class 1 integrons in soil and sediment. To determine if class 1 integrons could be recovered from novel ecological niches, soil or lake sediment was recovered from three distinct locations in Sydney, Australia. Bacteria from each environment were cultured using PCA medium in the absence of antibiotic selection. Preliminary screening by PCR with primers HS463A and HS464 (Table 1 and Fig. 1) revealed that 2 to 4% of cultured cells were positive for intI1. One of these environments, Lake Yerbury (see Materials and Methods), was selected for more detailed analysis. In this case, 200 colonies that appeared on PCA medium were subcultured using the same medium. Four of the 192 colonies (2.1%) that regrew were positive by PCR for intI1. The presence of intI1 was confirmed by sequencing all four products. Molecular typing of the four isolates using 16S rRNA gene sequencing revealed four distinct profiles. Two of them, MUL2A2 and MUL2G8, were different but closely related and could be identified as belonging to the genus Acidovorax (Fig. 2A). A third isolate, MUL2G11, was most closely related to unclassified genera of the order Burkholderiales (Fig. 2A). The fourth isolate, MUL2G9, was relatively remote from the others phylogenetically and was identified as an example of Azotobacter communis from the order Rhodocyclales (Fig. 2B). The MUL2G9 16S rRNA gene was identical to its homolog from Azotobacter communis SWu3. MUL2G9 was also found to have a nifH gene identical to that of SWu3, strongly implying that the former can fix nitrogen and is able to form a symbiosis with plants, as is typical for nitrogen fixers of this species (30).

While integrons are defined functionally by the presence of an intI gene, a recombination site, attI, and a promoter for cassette genes (Pc), class 1 integrons from clinical environments also have other characteristic features. These include, in most cases, the presence of a 3′-CS; it is possible to recover cassette arrays from class 1 integrons by using primer pairs that target the two conserved regions (26) (Fig. 1). However, attempts to amplify cassette arrays by this approach using the primers HS458 and HS459 (Fig. 1) failed to generate a product in all four cases. In addition, primers HS549 and HS550 that target a region internal to the 3′/H11032-CS (Fig. 1) also failed to generate a product. Although some class 1 integrons lack most or all of the 3′-CS (19, 39), the lack of PCR products with these primer pairs was unusual. To further characterize these intI1-containing strains, large-insert fosmid libraries were constructed. These libraries were rescreened with the intI1-specific PCR primers, and at least one positive clone was identified for MUL2G8, for MUL2G9, and for MUL2G11. The presence of a class 1 integron was confirmed in all three cases, as determined by the presence of a complete intI1 gene, an attI1 site, and a Pc. In the case of MUL2A2, a screening of 1,920 clones failed to reveal any that were positive for intI1.

Characterization of a class 1 integron in Azotobacter communis strain MUL2G9. The isolate most extensively sequenced was MUL2G9, with sequence including a region of approximately 28 kb beyond the outer boundary of the 5′-CS and approximately 5 kb beyond attI1. The integron was missing the first 98 bases of the 5′-CS (Fig. 3). This deletion end point is within one base pair of the insertion point of the insertion sequence ISPa7 present in some class 1 integrons isolated from Pseudo-

FIG. 2. Best maximum likelihood trees for the 16S rRNA genes of the class 1 integron bearing isolates from Lake Yerbury sediments and their relatives. (A) Phylogeny of representative Burkholderiales and isolates MUL2A2, MUL2G8, and MUL2G11. (B) Phylogeny of representative Rhodocyclales and isolate MUL2G9.
monas aeruginosa (1, 42, 49). In these cases, the insertion element is an insertion into a conventional class 1 integron structure, as the first 99 bases are still present. In contrast, in MUL2G9, the first 98 bases of the 5′-CS are absent. These first 98 bases (measured from the outer boundary of IRi) (Fig. 3) that are lost include all of IRi and the two strong TnpA binding domains necessary for transposition (20). The region immediately adjacent to the 5′-CS in MUL2G9 includes a putative DNA integrase/recombinase (Fig. 4) with another 18 identified ORFs following. Of these 18 ORFs, seven predicted products showed very high matches to proteins located in the sequenced genome of the Azoarcus sp. EbN1, the closest relative of A. communis for which substantial sequence information is available (38) (Fig. 2B). All seven corresponding genes are located on the chromosome of EbN1, and at least three are housekeeping genes, including a sigma-54 transcriptional activator, an acyl coenzyme A synthetase, and an acetone carboxylase beta subunit (see database entry DQ372711). We conclude that the class 1 integron identified here is in the chromosome of A. communis MUL2G9.

The region immediately adjacent to attI in the MUL2G9 integron (Fig. 4) included two gene cassettes as determined by the presence of a 59-base element following an obvious open reading frame (ORF). The predicted protein in the second cassette had a low similarity to proteins of the cytochrome/ quinol oxidase (cox) family (BLASTP E value, 1E-05) and the predicted product encoded by the first cassette gene had no homologs in the databases. Neither are obvious antibiotic resistance genes. Beyond the second cassette, a transposase gene was identified. This gene closely matched three transposase genes located in the sequenced Azoarcus sp. EbN1 genome (ebA575, ebA2604, and p2A213). Beyond this transposase is a DNA gyrase subunit B gene (gyrB) which is not part of a gene cassette (Fig. 4). gyrB is an essential gene found only on chromosomes, reinforcing the observation that this class 1 integron is not extrachromosomal. None of the class 1 integron-assoc-
ated transposition functions were found for the MUL2G9 integron. Neither IRi nor IRt was present. Similarly, all of the Tn402-like transposition genes were absent.

Characterization of other class 1 integrons from lake sediment isolates. Sequence derived from a MUL2G8 fosmid clone also confirmed a class 1 integron (Fig. 4). Further, the integron showed structural similarities to the element in MUL2G9. First, it was missing the first 98 bases of the 5'-CS, with the breakpoint at the same location as seen for MUL2G9 (Fig. 3). While the DNA sequence beyond the breakpoint was unrelated to the corresponding sequence in MUL2G9, it was nonetheless the case that the predicted product of the gene immediately adjacent to the 5'-CS was a DNA integrase/recombinase homologous to the one found at the same position in MUL2G9. The amino acid identity between the two proteins was 72% over a region spanning the 165 C-terminal amino acids of these predicted proteins (Fig. 4). Thus, although the two insertion events are independent, a common underlying mechanism of insertion is implied. Two ORFs were found immediately beyond attI. The first of these is an ORF of unknown function that was followed by a putative transposase. The ORFs were not followed by an identifiable 59-base element, implying that neither gene was part of a gene cassette. The putative transposase was not closely related to the transposase adjacent to the cassette array in the MUL2G9 integron.

In addition to lacking Tn402 transposition functions, the MUL2G8 class 1 integron also lacked IRi and IRt. Overall, the sequence surrounding the MUL2G8 integron suggests that it is located in its host’s chromosome. Sequence comparisons of the 5'-CS present revealed that it was identical to the equivalent sequence in a number of class 1 integrons that include In2 of Tn21. In contrast, within the region common to the MUL2G8 and MUL2G9 integrons, 13 differences could be identified. In MUL2G9, these differences are represented by 2 substitutions in attI1 and 11 in intI1 (6 nonsynonymous and 5 synonymous) compared to MUL2G8 and In2. The 5'-CS sequence seen in MUL2G9 is not seen in extant class 1 integrons and is relatively divergent in the context of this region. Given the difference between MUL2G8 and MUL2G9, these two independent capture events are likely to be derived from different parent integrons if the events occurred in historical time.

Sequence analysis of a MUL2G11 fosmid clone revealed a class 1 integron that includes a complete 5'-CS (Fig. 3). That is, the first 98 bases of this region were present. The MUL2G11 5'-CS was also identical to the 5'-CS in In2. The sequence immediately beyond IRi in MUL2G11 included a DNA invertase/recombinase beyond which was DNA sequence that was closely related (>90%) to sequence found for several IncP1-β plasmids (Fig. 4). It was therefore concluded that this integron is located on a plasmid. The sequence beyond the attI1 site was identical to the equivalent region in the MUL2G8 integron. This sequence identity extended at least through the transposase (tnpA) gene, at which point the MUL2G8 clone ends (Fig. 4). Thus, while the locations of the class 1 integrons present in MUL2G8 and MUL2G11 are likely to be different, unlike the MUL2G9 example, they would appear to share a common immediate ancestor. Additional information on the MUL2G11 clone was derived by end sequencing from vector sequence. About 600 bases were sequenced from the right end (Fig. 4) of this clone. Interestingly, this region was about 89% identical to a region of Tn402 that includes the end of tniA and the beginning of tniB (Fig. 5).

We were unable to recover a fosmid clone containing the intI1 gene of MUL2A2. However, the general structure of its putative integron was investigated by PCR (Fig. 4). First, a PCR was carried out on MUL2A2 genomic DNA using the primers HS714 and HS463A. HS714 targets the region of 5'-CS that is present in MUL2G11 but absent in MUL2G8 or MUL2G9. This PCR generated a product of the same size as that for MUL2G11. Thus, it was concluded that MUL2A2 may be related to MUL2G11. Other primer combinations produced results as summarized in Table 2. MUL2A2 possesses the VP1784-like ORF, tnpA, sugE, and the tniAB region. Finally, a PCR using a primer pair that targets the 5'-CS and the IncP1-β origin of replication did not generate a product for MUL2A2, suggesting that the integron and immediate surroundings for this strain are inserted in a different location (on a chromosome or different plasmid). It is also noteworthy that MUL2G8 possesses a sugE gene but not tniAB, since HS724 and HS725 failed to generate a product when applied to genomic DNA of this strain. This demonstrates that the region of identity between MUL2G8 and MUL2G11 ends somewhere between sugE and tniAB. The fact that tniAB and the origin of replication (oriV) regions are both absent further supports the notion that the MUL2G8 integron is located in the chromosome.

DISCUSSION

Two broad types, as distinct from classes, of integrons are generally recognized (15). The first type of integron is that found in bacterial chromosomes and fixed in phylogenetic lines. The second type of integron is that associated with other types of mobile elements. As a consequence of their association with various plasmids and transposons, examples of the second integron type are routinely recovered from diverse
The descriptors “chromosomal” and “mobile” can be useful, but it
excess of 200 cassettes in the case of some
mosomal cassette arrays is that they can be quite large, in
unrelated to antibiotic resistance. A second feature of chro-
a potentially attributable function is found, it is almost always
(8, 17, 46, 51). For most, a function cannot be ascribed. Where
fined to phylogenetic lines, have different characteristics. The
chromosomal integrons, recognized as being generally con-
contains less than six or so cassettes (40). Cassette arrays of
integrons. A typical multicassette array in a class 1 integron
most of the clinically important antibiotics are found in class 1
resistance genes (40); collectively, resistance determinants to
be captured by integrons and any cassette-associated genes to
feature has not been experimentally demonstrated in every
Case. These features collectively allow mobile gene cassettes to
be expressed. Beyond these common features, however, the
two integron types differ in several aspects. The nature of their
respective cassette arrays is their most notable distinguishing
feature. Integrons associated with other mobile elements al-
be expressed. Beyond these common features, however, the
two integron types differ in several aspects. The nature of their
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most exclusively possess gene cassettes that carry antibiotic
resistance genes (40); collectively, resistance determinants to
most of the clinically important antibiotics are found in class 1
integrons. A typical multicassette array in a class 1 integron
contains less than six or so cassettes (40). Cassette arrays of
chromosomal integrons, recognized as being generally con-
fined to phylogenetic lines, have different characteristics. The
first of these is that the genes within cassettes are very diverse
(8, 17, 46, 51). For most, a function cannot be ascribed. Where
a potentially attributable function is found, it is almost always
unrelated to antibiotic resistance. A second feature of chro-
mosomal cassette arrays is that they can be quite large, in
excess of 200 cassettes in the case of some *Vibrio* species (29).

The distinction between integron types by the use of the
descriptors “chromosomal” and “mobile” can be useful, but it
does not alter the fact that both types are functionally equiv-
alent in that they capture mobile gene cassettes by site-specific
recombination. It has also been shown that cassette arrays
containing antibiotic resistance genes can be found in chromo-
smal integrons and that such arrays within integrons can be a
source of resistance genes for class 1 integrons (45).

Chromosomal integrons are very diverse and are broadly
distributed phylogenetically, so it is clear that these elements
have been in bacteria for a long period of evolutionary time
(17, 31, 32, 46). One hypothesis to explain the origin of the
mobilizable class 1 integron is that it is derived from a chro-
mosomal integron that became mobile by the acquisition of
Tn402-like transposition functions. This, together with the se-
lection for antibiotic resistance as a consequence of human
intervention, allowed the dispersal of class 1 integrons and
thereby greatly contributed to the clinical problem that we see
today.

Is the above hypothesis correct? Given the abundance and
distribution of chromosomal integrons, the mobilization of
such an integron by the addition of transposition genes is a
possibility. However, we observed with at least two cases
(MUL2G8 and MUL2G9) that class 1 integrons can be found
devoid of Tn402-like transposition functions and need not be
associated with antibiotic resistance (see Table 3) but can
counter be broadly dispersed among bacteria more likely to
be closely associated with plants than animals. The same is
nearly true for the remaining two (MUL2G11 and MUL2A2),
with the exception that IRi and two TnpA binding sites are
present. Our data can be interpreted in one of two ways. The
first of these is that the class 1 integrons isolated here were
derived from Tn402-like class 1 integrons that are beginning to

### Table 2. Determination of the structure of class 1 integrons of bacterial isolates from Lake Yerbury sediments by PCR amplification with primers targeting specific regions

<table>
<thead>
<tr>
<th>Target region</th>
<th>Primers</th>
<th>Size (bp) of product generated for:*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>intI</em></td>
<td>HS463a-HS464</td>
<td>470</td>
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<tr>
<td><em>5′</em>-CS-<em>3′</em>-CS</td>
<td>HS458-HS459</td>
<td>1,300</td>
</tr>
<tr>
<td><em>sulI</em></td>
<td>HS549-HS550</td>
<td>1,100</td>
</tr>
<tr>
<td><em>oriV</em>-intI1</td>
<td>HS722-HS715</td>
<td>NP</td>
</tr>
<tr>
<td><em>IRi</em>-att</td>
<td>HS714-HS726</td>
<td>1,320</td>
</tr>
<tr>
<td><em>intI</em>-VP1784</td>
<td>HS464-HS721</td>
<td>NP</td>
</tr>
<tr>
<td><em>tnpA-sugF</em></td>
<td>HS458-HS723</td>
<td>1,290</td>
</tr>
<tr>
<td><em>tniAB</em></td>
<td>HS724-HS725</td>
<td>1,490</td>
</tr>
</tbody>
</table>

* NP, no product generated.

### Table 3. Structural differences between previously identified class 1 integrons and those isolated in this study

<table>
<thead>
<tr>
<th>Characteristics of previously described class 1 integrons</th>
<th>Characteristics of Lake Yerbury class 1 integrons</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRi present</td>
<td>IRi absent in two examples*</td>
</tr>
<tr>
<td>IRi present or evidence of deletion-induced loss</td>
<td>IRi not present</td>
</tr>
<tr>
<td>Partial or complete suite of Tn402-like <em>tni</em> genes or evidence of deletion-induced loss</td>
<td>No Tn402-like <em>tni</em> genes or divergent genes in opposite orientation</td>
</tr>
<tr>
<td>Insertion in or near a <em>res</em> site</td>
<td>No <em>res</em> site at insertion point</td>
</tr>
<tr>
<td>3′*-CS commonly present</td>
<td>No 3′*-CS</td>
</tr>
<tr>
<td>Cassette arrays common; most contain antibiotic resistance genes</td>
<td>No cassettes or no resistance genes in cassette array</td>
</tr>
</tbody>
</table>

* Also absent are TnpA binding domains i1 and i2 (see text and reference 20).
radiate out of pathogenic and commensal bacteria. Implicit in this hypothesis is that this mobilization leads to the loss of most, or all, of the Tn402-like transposition functions and to the loss of antibiotic resistance genes. Further, the potential radiation of class 1 integrons out of pathogenic bacteria has probably occurred in historical times, that is, in the last 60 years or so, corresponding to the advent of the antibiotic era, since it was only at that time that they became prevalent. A second explanation for the observations made here is that class 1 integrons were already being mobilized prior to the acquisition of the Tn402-like transposition system and prior to the antibiotic era. Since the class 1 integrons found in the sampled lake sediment ecosystem have identical, or nearly identical, counterparts in multidrug resistant strains, it is not possible to say which way the lateral transfer events occurred. Intuitively, the second explanation is more likely. We have identified unusual class 1 integrons with respect to their lack of association with Tn402 transposition functions in at least four distinct bacteria. Also, the associated 5′-CS sequences are not all the same, suggesting either a common ancestor that predates the historical use on antibiotics or recent integration events that involve distinct immediate ancestors. It seems unlikely that multiple events involving simultaneous loss of the many features summarized in Table 3 could occur in historical times. Other anecdotal evidence also hints at the possibility that class 1 integrons may have been widespread in bacteria from sediment and soil environments from a time that predates the widespread use of antibiotics. In a metagenomic survey for integrons from the tailings of an abandoned gold mine in Colorado, 12 examples of class 1 integrons were identified, as determined by the sequencing of a region internal to the intI1 gene (32). It is not known how many of these samples were independent. In addition, we do not have sequence context information or know what the host organism(s) was. Although the possibility of recent anthropogenic disturbance cannot be ruled out, it is nonetheless an intriguing observation, since the mine itself has been abandoned for many decades (D. R. Nemergut, personal communication).

In contrast to the above findings, one of the class 1 integrons (MUL2G11) isolated in this study does have a complete 5′-CS, implying that it has a relative close to the Tn402-like integrons. The MUL2G11 integrase, however, lacks all of the other features normally associated with extant integrons. The possibility that this arrangement arose in historical times cannot be excluded, but in the context of the other independent examples recovered in this study, it seems less likely. It is interesting that the integron in MUL2G11 is also linked to transposition genes that are similar, but not identical, to those of intI1 and intI2 of Tn402. One interpretation of this may be that the MUL2G11 structure represents a linking of a class 1 integron to transposition genes of the Tn402 family independent of the linking event(s) that created the Tn402-like structure seen up until now. The experimental design employed targeted class 1 integrons in that the pumers used were specific for intI1 and would not detect other intI1 integrases. The strains recovered therefore reflect this bias, and it not possible to say whether class 1 integrons are uniquely dispersed among sediment and soil bacteria or whether this observation reflects a phenomenon of the dispersal of at least some other integron classes more generally. The question is particularly relevant, as the observation that many integron classes have been mobilized would make the movement of Tn402-like class 1 integrons into soil and sediment bacteria in recent times less likely.

Putting aside the question of whether class 1 integrons have moved into pathogenic and commensal bacteria or become dispersed from them, the widespread presence of this integron class in soil and sediment bacteria is remarkable. Although some degree of antibiotic contamination of the sampled ecosystem studied here is possible, it is nonetheless relatively remote from commensal populations, and it is certainly the case that the class 1-containing bacteria recovered are examples of neither commensal bacteria nor human pathogens. Nitrogen-fixing Azoarcus sp. strains, in particular, of which MUL2G9 is likely an example (it harbors a nifH gene), are best adapted to the highly specialized environment of the root interior of flood-tolerant grasses with large aerenchymatic air spaces in mature roots (41). The site from which MUL2G9 was recovered is consistent with this environment type in that it was sediment from a lake that had extensive reed growth. The environment type in which Azoarcus is found includes the rhizosphere and is an environment with a diverse microbial ecosystem and where the potential for LGT may be expected to be high. Indeed, the rhizosphere has been identified as an environment of prodigious rates of LGT (3); the sequenced Azoarcus strain EbN1, for example, shows considerable evidence of comprising an unusually plastic genome (38).

The fact that a single gene capture system is commonly present in commensals, pathogens, the rhizosphere, and aquatic environments further underscores the potential ease with which antibiotic resistance genes can transit from the common organisms that manufacture antibiotics to organisms detrimental to humans. In metagenomic studies, antibiotic resistance genes can be recovered from the general environment (43). The presence of class 1 integrons in disparate environmental niches and hosts effectively increases the pool of genes, including those encoding antibiotic resistance, that are accessible to these elements. Finally, it is noteworthy that one of the grasses most amenable to colonization by nitrogen-fixing Azoarcus species is rice (30). The presence of class 1 integrons in bacteria intimately associated with a crop production plant consumed worldwide arguably creates a major conduit for the frequent influx of new mobile genes into bacteria in close association with humans and other animals at a scale not previously appreciated.

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region of Salmonella enterica serovar Typhimurium DT104 or variants of it, is widely distributed in other S. enterica serovars. J. Bacteriol. 187:4401–4409.