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# Molecular Characterization of Class 3 Integrons from *Delftia* spp.[▽]

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**Two environmental strains, *Delftia acidovorans* C17 and *Delftia tsuruhatensis* A90, were found to carry class 3 integrons, which have seldom been reported and then only from pathogens in which they are associated with antibiotic resistance genes. The *Delftia* integrons comprised a highly conserved class 3 integrase gene, upstream and oppositely oriented from a set of three or four gene cassettes that encoded unidentified functions. The A90 integron had one more gene cassette than the C17 integron, but the two were otherwise the same; furthermore, they were located within regions of sequence identity in both strains and linked to chromosomal genes. A screen of other *Delftia* and related strains did not reveal the presence of additional class 3 integrons. The observations suggest that these integrons were horizontally transferred to *Delftia* as part of a larger region and reside as chromosomal elements that probably predate transposon dissemination, as has been proposed for certain class 1 integrons.**

Integrons constitute a diverse family of genetic elements that are found in many gram-negative and some gram-positive bacteria. As defined by Hall and Collis (18), these elements encode a site-specific recombination system that is able to capture gene cassettes in tandem arrays. Integron-associated gene cassettes are found on a variety of larger genetic elements such as transposons or plasmids that permit horizontal gene transfer between different bacterial genera; such elements may also be integrated into chromosomes. The most studied integrons are the resistance integrons (RIs), in which the cassettes are antibiotic resistance determinants. More than 80 different antibiotic-encoding cassettes have been identified in RI clusters (27). RIs are widely distributed in the *Enterobacteriacae*, where they are the major factors involved in the development of multidrug resistance (13, 17).

The key elements of all integrons are the integrase gene (*intI*), which encodes a tyrosine recombinase (2) responsible for the insertion and assortment of the gene cassettes, and an associated integration site (*attI*). The integrase gene incorporates a strong promoter sequence within its 5′-terminal sequence that is responsible for the transcription of gene cassettes that have been recombined into the *attI* site.

Three classes of RIs have been identified on the basis of the amino acid sequence of the integrase (14). Class 1 is widely distributed, is often associated with Tn*402*-like transposons, and is most commonly observed. The related but less frequently detected class 2 possesses a defective integrase gene and is usually embedded in a Tn*7* family transposon (36). Class 3 integrons are rare; to date, only two have been studied in detail (1, 7–9), although fragments of others have been detected by PCR in studies of clinical isolates from Japan (42, 43). The class 3 integrons that have been characterized are both RIs and encode a metallo-β-lactamase (1, 34) and an aminoglycoside acetyltransferase; they have essentially the same organization as class 1 and 2 integrons.

A number of related genetic elements have been found in bacteria, such as the superintegrons, which encode much larger clusters (~100) of gene cassettes that are almost exclusively unidentified open reading frames (28), and the resistance gene clusters found in strains of *Vibrio cholerae* (20). Recent metagenomic analyses of soils and sediments have revealed many integron-related gene cassettes encoding unidentified functions (32, 46). It has been proposed that integron-associated cassettes are genetic elements that played roles in bacterial chromosome evolution (19, 21, 27, 31).

During an investigation into the use of RIs for tracking the spread of resistant bacteria in aquatic environments, we detected class 3 integrase (*intI3*) sequences in DNA isolated from water samples, and by using colony hybridization (3), we isolated a strain of *Delftia tsuruhatensis* that carried the integrase within an integron-like structure. *Delftia* spp. are rod-shaped, nitrate-reducing, gram-negative bacteria with a G+C content in the range of 66% that are widely distributed in the environment and capable of degrading a variety of xenobiotics such as chlorinated aromatic compounds (10, 40). They were formerly considered to be members of the genus *Comamonas* or *Pseudomonas* (54). There have been no previous reports of integrons in *Delftia*; however, superintegrons are commonly found in related bacterial genera such as *Pseudomonas* (52). This prompted us to inspect other *Delftia* strains, and we identified a second class 3 integron, in *Delftia acidovorans*. The detailed genetic organization of the two *Delftia* integrons was determined by cloning and nucleotide sequencing and revealed two closely related chromosomal elements with gene cassettes but no known antibiotic resistance determinants.

## MATERIALS AND METHODS

**Detection of class 3 integrons in aquatic samples.** Water samples were collected from the Joint Abbotsford-Mission Environmental System Water Pollution Control Centre, Abbotsford, British Columbia, Canada, and filtered through a sterile 47-mm-diameter membrane (GVHP 04700; pore size, 0.22 μm; Millipore Corp.) to retain bacteria. Membranes were placed in 100 ml Luria-Bertani broth and incubated by shaking at 37°C overnight. Total DNA was extracted

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TABLE 1. Bacterial strains and sources

| Species | Strain[a] | Origin | Reference | 16S rRNA accession no. |
|---|---|---|---|---|
| *Delftia acidovorans* | NBRC14950$^T$ | The Netherlands[b] | 54 | AB021417 |
| *Delftia acidovorans* | SPH-1 | Germany[c] | 40 | AB021417[e] |
| *Delftia acidovorans* | C17 | United States[c] | 10[d] | This study |
| *Delftia acidovorans* | EEZ23 | Spain[b] | 38 | This study |
| *Delftia acidovorans* | WDL34 | Belgium[b] | 11 | AF538930 |
| *Delftia acidovorans* | NBRC13582 | United Kingdom[b] | 15 | AB020186 |
| *Delftia* sp. | EH2-1 | United States[b] | 51 | AY3677007 |
| *Delftia tsuruhatensis* | NBRC16741$^T$ | Japan[c] | 44 | AB075017 |
| *Delftia tsuruhatensis* | A90 | Canada[c] | This study | This study |
| *Delftia* sp. | AN3 | China[c] | 25 | AY052781 |
| *Delftia* sp. | 8 | Canada[b] | 24 | AF181575 |
| *Acidovorax avenae* subsp. *citrulli* | AAC00-1 | United States | 53 | AASX01000007[f] |
| *Acidovorax* sp. | JS42 | United States[b] | 16 | AASD01000022[f] |
| *Comamonas testosteroni* | I2gfp | Belgium[c] | 5 | This study |
| *Comamonas testosteroni* | KF-1 | Switzerland[c] | 40 | M11224[e] |

[a] NBRC strains were obtained from the National Biological Resource Center, Japan. Other strains were obtained from colleagues: C17, EEZ23, WDL34, and I2gfp from E. Top, University of Idaho; KF-1 and SPH-1 from D. Schleheck, University of New South Wales; EH2-1 from W. Hickey, University of Wisconsin; AN3 from S.-J. Liu, Research Center of Microbial Technology, Beijing, China; strain 8 from J. Lawrence, Environment Canada, Saskatoon; and AAC00-1 and JS42 from D. Stahl, University of Washington.
[b] Isolated from soil or groundwater.
[c] Isolated from wastewater or activated sludge.
[d] Isolated from material described in this reference, but not specifically identified by name (E. Top, personal communication).
[e] Sequence is identical to that of the type strain.
[f] Draft genome sequence.

using a QIAamp DNA minikit (QIAGEN Inc.) and used as a template for PCR amplification with primers that distinguish among the integrases. Primer pairs intI1F–intI1R (5′-GTTCGGTCAAGGTTCTGG-3′ and 5′-CGTAGAGACGT CGGAATG-3′, derived from GenBank accession no. Y18050), intI2F–intI2R (5′-CAAGCATCTCTAGGCGTA-3′ and 5′-AGAAGCATCAGTCCATCC-3′, from accession no. L10818), and intI3F–intI3R (5′-CATCAAGCTGCTCGA TCA-3′ and 5′-ACAACTCTTGCACCGTTC-3′, from accession no. D50438) were each used to amplify a portion of an integrase gene: 890 bp of *intI1*, 1,056 bp of *intI2*, or 878 bp of *intI3*, respectively. A fourth primer pair, int1.F–int1.R (5′-GGGTCAAGGATCTGGATTTCG-3′ and 5′-ACATGCGTGTAAATCAT CGTCG-3′ [29]), which generates a 484-bp amplicon from class 1 and class 3 integrons, was also used sometimes; in these cases, a secondary assay with intI3F–intI3R was also done. Thirty-five cycles of amplification were conducted (95°C for 20 s, 54°C for 30 s, and 70°C for 30 s) after 2 min at 95°C to denature template DNA. Plasmids with In1 (pJR88 [23, 55]), In2 (pIP1100 [4]), or In3 (pSMB731 [1]) were used as positive controls for PCR.

Cultures that were positive in the PCR assays were diluted to $10^{-6}$, and 100-μl aliquots were spread onto LB plates. After overnight incubation at 37°C, the plates were replicated onto nylon membranes (Nylon Membranes for Colony and Plaque Hybridization; Roche Diagnostics) and hybridized at 42°C with digoxigenin-labeled probes obtained by using *intI*-specific primers (described above) and a PCR DIG labeling kit (Roche Diagnostics) according to the manufacturer's instructions. The colonies detected by hybridization were then purified.

**Detection of class 3 integrons in other strains.** Strains (Table 1) were screened by PCR using the primers and conditions described above. DNA was extracted using a ChargeSwitch gDNA Mini Bacteria kit (Invitrogen Inc.). PCR for 16S rRNA genes (described below) was conducted as a positive control for DNA template quality during screening.

**Southern blot analysis.** Genomic DNA, extracted as for test strains, was either left untreated or digested with HindIII and then size fractionated by agarose gel electrophoresis. The DNA was blotted onto a nylon membrane (Roche Diagnostics), hybridized at 42°C to a digoxigenin-labeled *intI3* probe generated by PCR using primers intI3F and intI3R, and washed as directed by the manufacturer.

**Antibiotic sensitivity tests.** Disk diffusion assays to determine the antibiotic resistance of source strains and *Escherichia coli* hosts carrying library clones were conducted on Mueller-Hinton agar (Difco). All antibiotics were obtained from Sigma-Aldrich.

**16S rRNA analysis.** Molecular typing of A90, C17, and other strains was conducted using 16S rRNA. Primers 16S.0007F21 (5′-GAGAGTTTGATCCTG GCTCAG-3′) and 16S.1511R21 (5′-CGGCTACCTTGTTACGACTTC-3′) were used for PCR of the 16S rRNA gene from genomic DNA and for sequencing (MacroGen, Inc.). The PCR program comprised an initial incubation at 96°C for 3 min, followed by 35 cycles of 96°C for 30 s, 60°C for 45 s, and 72°C for 90 s. Sequence assembly, alignment of a ca. 1,300 nucleotide region, and dendrogram construction (neighbor-joining method, default parameters) were done with MacVector (Accelrys).

**Cloning and sequencing of integrons and flanking DNA.** Genomic DNA of A90 and C17 was isolated as for test strains, partially digested with Sau3A1, and cloned into SuperCos1 (Stratagene) as directed by the manufacturer. A90 genomic DNA was also completely digested with HindIII and cloned into pUC19. The libraries were screened by hybridization with the *intI3*-specific probe, and positive clones were wholly (pAV3.5, a pUC19 clone of A90) or partially (cosmids CA90-6 and CC17-15

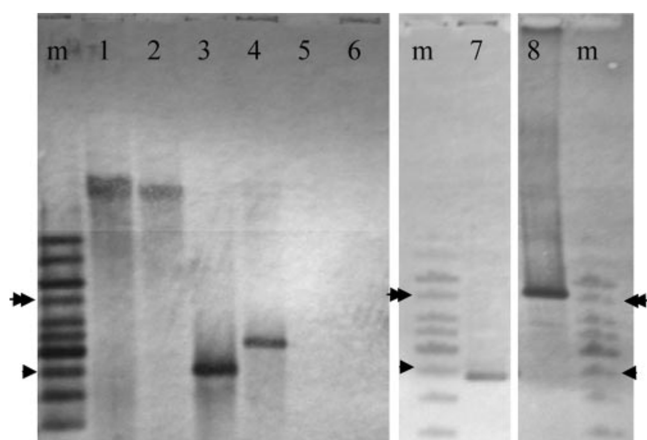


FIG. 1. Southern blot analyses of *Delftia* strains and cloned DNA using an *intI3*-specific probe. Lanes 1, 2, 5, and 6, uncut genomic DNA of *D. acidovorans* C17, *D. tsuruhatensis* A90, *D. acidovorans* NBRC14950$^T$, and *D. tsuruhatensis* NBRC16741$^T$, respectively. Lanes 3, 4, 7, and 8, HindIII-digested DNA of C17, A90, and cosmids CC17-15 and CA90-6, respectively. The hybridizing fragment in CA90-6 is 5.2 kb, larger than that in A90 genomic DNA (3.1 kb), because the latter is interrupted by cloning. A GeneRuler 1-kb ladder (Fermentas) in lanes m is used as a marker. Single arrowhead, 2.5 kb; double arrowhead; 5 kb.

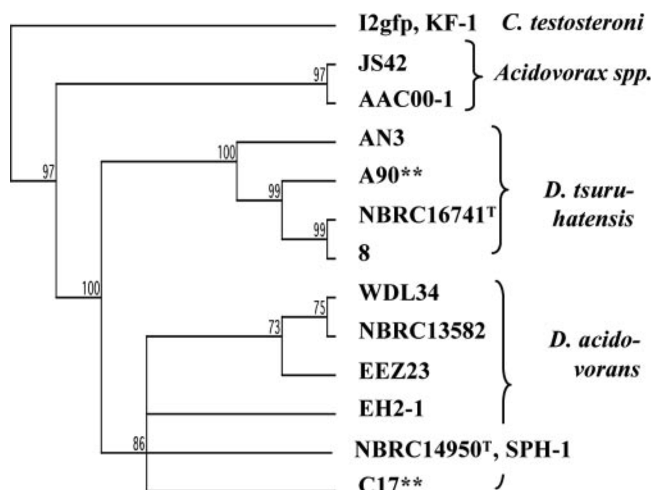


FIG. 2. 16S rRNA gene phylogeny of *Delftia* and related strains and distribution of integrons. Significant bootstrap support values for 500 replicates are shown. Class 3 *intI*-positive strains are indicated by asterisks. The 16S rRNA sequence of *C. testosteroni* I2gfp is the same as that of strain KF-1; the 16S rRNA sequence of *D. acidovorans* SPH-1 is identical to that of NBRC14959 $^{T}$ (40). A90 and C17 are 99.5% identical across a 1,315-nucleotide region.

from A90 and C17, respectively) sequenced to include the integron and adjacent DNA. The C17 contig was generated by primer-walking cosmid DNA, except for a 1.1-kb gap (nucleotides 2738 to 3843) closed by sequencing a PCR product. A90 sequences were derived from cosmid primer walking, and PCR products were generated to close gaps. A 2.7-kb region (nucleotides 1 to 2708) extending beyond CA90-6 was sequenced from amplicons obtained from A90 genomic DNA with primer pairs 5′-AGGCACTGGAKGCMGCYTCG-3′–5′-AACACCCGTGCGCT ATATGG-3′ and 5′-ATAGGTAGCTGTGAACGACG-3′–5′-CCTCGCACAGT CGCTGAACG-3′. These were determined from regions of identity between C17 and presumptive homologs. Sequences were compared to known genes by BLAST.

**Nucleotide sequence accession numbers.** New 16S rRNA sequences were deposited as GenBank accession no. EF421404 to EF421407. Sequences of contigs determined in this study were deposited in GenBank as accession no. EF467661 and EF469602.

## RESULTS AND DISCUSSION

**Isolation and characterization of strains.** A90, a strain containing class 3 integrase-encoding sequences, was isolated from a wastewater treatment plant in British Columbia by dilution plating of wastewater on a nonselective medium and colony hybridization with an *intI3*-specific probe. Sequencing of an amplicon generated by PCR with *intI3*-specific primers and probing of isolated genomic DNA with an *intI3*-specific probe confirmed that A90 was the source (Fig. 1). Hybridization of the probe to uncut genomic DNA suggested that the integron was located in the chromosome or on a large plasmid. Molecular typing by 16S rRNA gene sequencing indicated that A90 was most likely a strain of *Delftia tsuruhatensis (*Fig. 2).

To date, *intI3* sequences are known only from *Serratia marcescens*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Alcaligenes xylosoxidans* (1, 9, 42, 43). This prompted examination of available strains of *Delftia* and related genera in the *Comamonadaceae* to determine the prevalence of *intI3* in this new host group (Table 1). The only one of 14 strains tested that was positive for *intI3* by a PCR assay was another *Delftia* strain: *Delftia acidovorans* C17 was confirmed by Southern blot analysis to possess *intI3* sequences (Fig. 1 and 2). Strain C17 also originated from an activated-sludge community (10), but the source was a wastewater treatment facility in Idaho that was geographically distant from the source of A90 in Canada. Since class 3 integrons (1, 9), or portions thereof (42, 43), have previously been reported only from Japan and Portugal, strains A90 and C17 represent the first *intI3*-bearing strains from North America. The lack of association of *intI3* with a phylogenetic lineage among the representatives of the group and the geographic separation of the source habitats suggest that the *intI3* sequences were introduced into the two *Delftia* strains horizontally, on two occasions.

Integrons often have associated gene cassettes encoding resistance to a variety of antibiotics and play a role in the dissemination of resistance in hospitals. The two class 3 integrons characterized, one from *S. marcescens* AK9373 (1), designated In3-1 here for convenience, and one from *K. pneumoniae* FFUL 22K (9), referred to below as In3-2, were both isolated from clinical strains and were associated with gene cassettes for resistance to broad-spectrum β-lactams and other antibiotics. *Delftia* strains A90 and C17 are distinct from these in originating from an environmental rather than a clinical setting; it was therefore of interest to examine the organization of the integrons associated with the *intI3* sequences in *Delftia*.

**Isolation and characterization of *Delftia* class 3 integrons.** To isolate the entire integrons, genomic libraries of the two *Delftia* strains were constructed and screened by hybridization, and candidate clones were partially sequenced. An integron of 3,213 bp, designated In3-3, was identified in cosmid CC17-15 from *D. acidovorans* C17, and another integron, of 3,964 bp, designated In3-4, was found in cosmid CA90-6 from *D. tsuruhatensis* A90 (Fig. 3).

Detailed analyses showed that, except for the presence of an

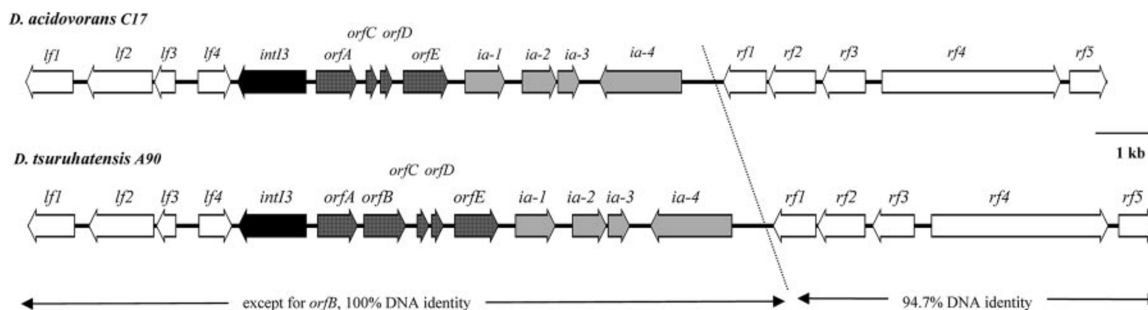


FIG. 3. Chromosomal regions containing class 3 integrons in *D. acidovorans* C17 and *D. tsuruhatensis* A90.

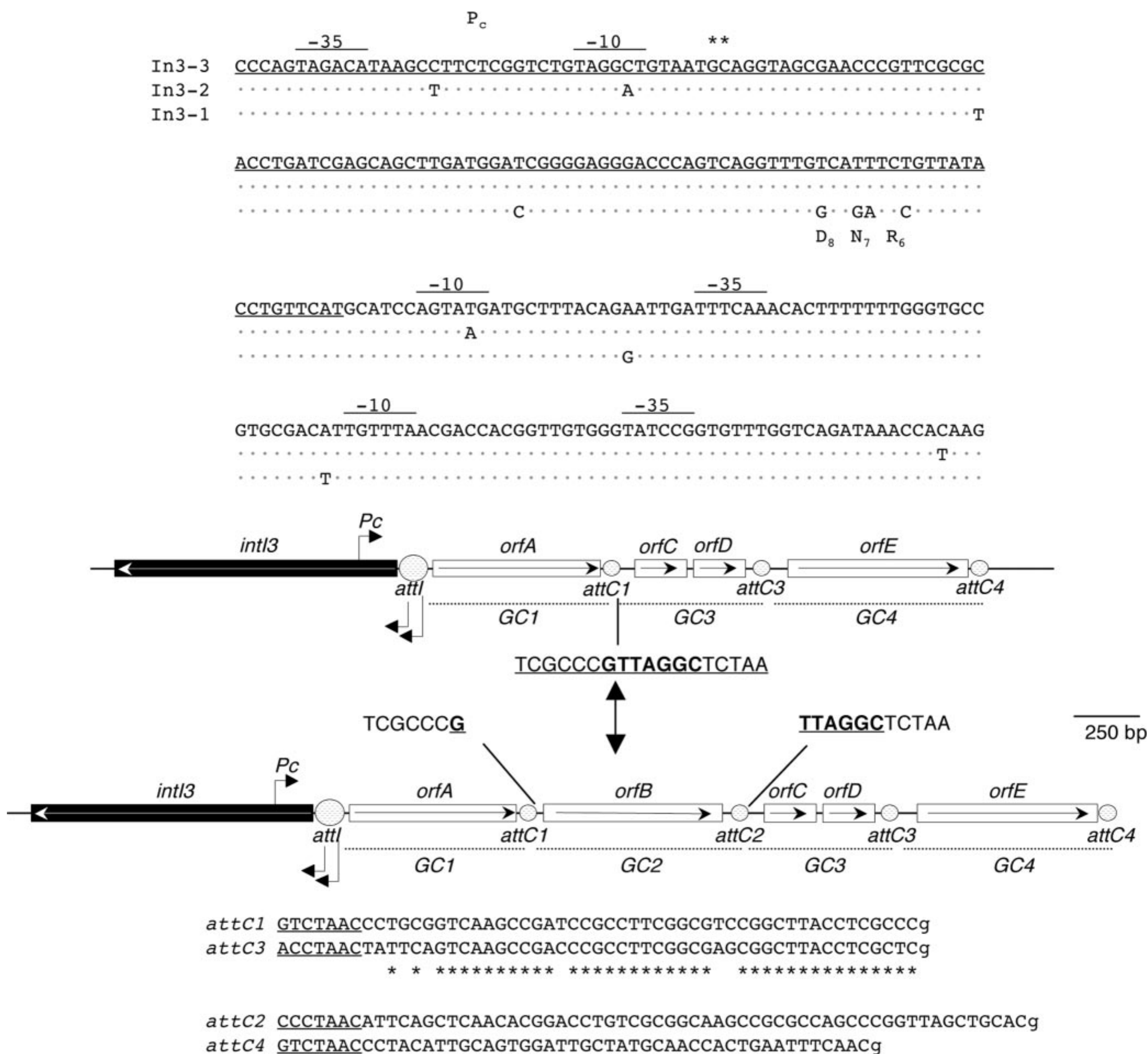


FIG. 4. Fine structure of integrons In3-3 (top) and In3-4 (bottom). Tandem gene cassettes (GC) are indicated by double-headed arrows; genes (filled arrows) in each GC are named as in Table 2. The *attI* and *attC* sites are represented by boxes. $P_c$ and two possible $P_{int}$ promoters (8) are indicated by angled arrows above or below the integron. (Upper box) Alignment of promoter and *attI* regions of In3-1 (GenBank accession AF416297), In3-2 (AY219651), and In3-3. The coding region (opposite strand) of *intI* is underlined. Features such as −10, −35, and transcription starts after $P_c$ (marked with double asterisks) as originally determined for In3-1 by Collis et al. (8) are labeled; the *attI* region as delimited in In3-1 by Collis and Hall (7) is highlighted. (Lower box) *attC* sites, starting from the inverse core (underlined) and including the first G at the possible sites of recombination ("GTTRRRY" motif) are shown; bases conserved between *attC1* and *attC3* are indicated by asterisks.

extra gene cassette, GC2, in In3-4, the integrons from the two *Delftia* strains were identical in structure and sequence (Fig. 3 and 4; Table 2). The key elements, *intI3*, $P_c$, and *attI3*, were highly similar to those from the only two previously characterized class 3 integrons. The *intI3* gene in *Delftia* is 99% identical to the two known *intI3* genes. Four of the differences in the *intI*3 gene of *Delftia* relative to *intI3* in In3-1 were associated with substitutions (G6R, S7N, and A8D), but none of them was in a position conserved among the tyrosine recombinases (30, 33). A region identical to the $P_c$ promoter of In3-1 studied by Collis et al., 2002 (8), including nucleotides identified as transcription starts, is found in *Delftia*. Next to *intI3*, in the *attI3* region, two differences were found, including a G/A transition in the more proximal of two possible $P_{int}$ promoters proposed for In3-1 (8) (Fig. 4). The *attI3* region in *Delftia* is identical to that of In3-1 (7) and is divided by the insertion of gene cassettes (see below) such that the remaining nine or ten 3′ nucleotides, including the remainder of the simple site, lie beyond the last gene cassette. In In3-1, the region beyond the last gene cassette can be aligned with the left end of Tn*402*-

TABLE 2. ORFs in class 3 integrons and flanking DNA

| ORF | % G+C | | No. of amino acids | Most similar CDSs[a] | GenBank accession no. | % Identity | |
|---|---|---|---|---|---|---|---|
| | A90 | C17 | | | | A90 | C17 |
| lf1 | 60 | 60 | 288 | Hypothetical protein, *Acidovorax* sp. strain JS42 (AJS228) | ZP_01384761 | 83 | 83 |
| lf2 | 59 | 59 | 329 | Hypothetical protein, *Acidovorax* sp. strain JS42 (AJS229) | ZP_01384762 | 96 | 96 |
| lf3 | 59 | 59 | 107 | Hypothetical protein, *Dechloromonas aromatica* RCB | YP_284873 | 50 | 50 |
| lf4 | 62 | 62 | 171 | Hypothetical protein, *Novosphingobium aromaticivorans* DSM 12444 | YP_497478 | 40 | 40 |
| *intI3* | 55 | 55 | 346 | IntI3 integrase, *Klebsiella pneumoniae* | AAO32355 | 99 | 99 |
| *orfA* | 49 | 49 | 208 | Hypothetical protein, *Vibrio splendidus* V12 | ZP_00990410 | 46 | 46 |
| *orfB* | 59 | | 217 | Hypothetical protein, *Comamonas testosteroni* KF-1 | ZP_01521201 | 79 | |
| *orfC* | 45 | 45 | 63 | Hypothetical protein, *Burkholderia vietnamiensis* G4 | ZP_00426308 | 39 | 39 |
| *orfD* | 48 | 48 | 65 | No significant hit | | | |
| *orfE* | 38 | 38 | 224 | Hypothetical protein, *Yersinia frederiksenii* ATCC 33641 | ZP_00827899 | 45 | 45 |
| ia-1 | 52 | 52 | 207 | Putative integrase/recombinase, *Acidovorax* sp. strain MUL2G8 | ABE73721 | 96 | 96 |
| ia-2 | 58 | 58 | 177 | Putative transcriptional regulator in pB8 (IncP-1β) | YP_358816 | 70 | 70 |
| ia-3 | 55 | 55 | 110 | Small multidrug resistance protein in pB8 (IncP-1β) | YP_358817 | 83 | 83 |
| ia-4 | 54 | 54 | 413 | Phage P4-type tyrosine recombinase, *Acidovorax* sp. strain JS42 (AJS3904) | ZP_01381320 | 95 | 95 |
| rf1 | 62 | 63 | 221 | Phosphoglycolate phosphatase, *Delftia acidovorans* SPH-1 (5344); phosphoglycolate phosphatase, *Acidovorax* sp. strain JS42 (AJS3903) | ZP_01578007, ZP_01381319 | 90, 63 | 100, 65 |
| rf2 | 61 | 62 | 240 | Ubiquinone biosynthesis OMTase, *Delftia acidovorans* SPH-1 (5343); ubiquinone biosynthesis OMTase, *Acidovorax* sp. strain JS42 (AJS3902) | ZP_01578006, ZP_01381318 | 97, 75 | 100, 77 |
| rf3 | 62 | 62 | 219 | OmpA/MotB, *Delftia acidovorans* SPH-1 (5342); OmpA/MotB, *Acidovorax* sp. strain JS42 (AJS3901) | ZP_01578005, ZP_01381317 | 98, 87 | 100, 88 |
| rf4 | 64 | 65 | 888 | DNA gyrase, A subunit, *Delftia acidovorans* SPH-1 (5341); DNA gyrase, A subunit, *Acidovorax* sp. strain JS42 (AJS3900) | ZP_01578004, ZP_01381316 | 99, 91 | 100, 92 |
| rf5 | 66 | 66 | 188 | Phosphoserine aminotransferase, *Delftia acidovorans* SPH-1 (5340); phosphoserine aminotransferase, *Acidovorax* sp. strain JS42 (AJS3899) | ZP_01578003, ZP_01381315 | 97, 88 | 100, 87 |

[a] Two database hits are shown for rf1 to rf5 to illustrate the conserved synteny (as indicated by consecutive locus tags in brackets) of this group of genes in related organisms and to emphasize the continuation of this series in *Acidovorax* sp. strain JS42 by an additional gene encoding a phage P4-type tyrosine recombinase. CDSs, coding sequences; OMTase, *o*-methyltransferase.

type class 1 integrons and terminates in an inverted repeat (IRi) (8) that provides part of the evidence that In3-1 has a Tn*402*-like backbone. The *Delftia* integrons lack the distal sequences and the IRi, but the proximal 44 bp of this region are completely conserved among all the class 3 integrons (Fig. 5). The far edge of this short tract of identity matches a "deletion endpoint" observed in some environmental class 1 integrons (47), so called because it marks a location beyond which conserved sequences that are typical for mobile class 1 integrons are missing. This location was noted as being within 1 bp of an insertion site for *P. aeruginosa* IsPa*7* in some class 1 integrons (47).

In3-3 has three (GC1, GC3, and GC4) and In3-4 has four (GC1 through GC4) tandemly arranged gene cassettes demarcated by potential *attC* (59-base element) sequences (Fig. 4). The *attC* sites associated with GC1 and GC3 are >80% identical to each other and to the *attC* site of a small, conserved gene cassette of unknown function in some class 1 integrons (26, 48). The three cassettes common to both In3-3 and In3-4 have G+C contents (38 to 49%) distinctly lower than that of

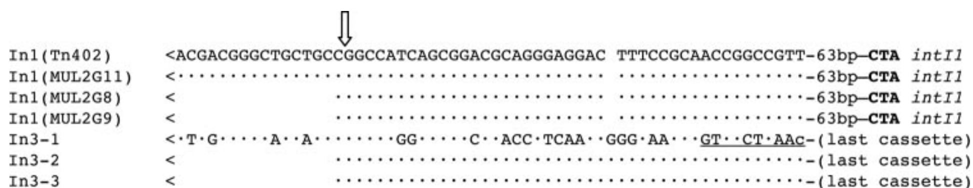


FIG. 5. "Left" boundaries of class 1 (In1) and class 3 (In3-X) integrons. The designation "left" follows the convention in references 8 and 47, from which the first five sequences are adapted; "<" at the start of each line indicates that additional upstream sequence, including the IRi, which is the actual left terminus in some cases, is not shown. Positions of identity with the sequence immediately above are shown as dots. The end of the *attI* site of the class 3 integrons is underlined, and the stop codon of *intI1* is boldfaced (opposite strand). A vertical arrow indicates where ISPa*7* is inserted in some class 1 integrons (47). Tn*402* and In3-1 sequences flanking the end of *intI1* or the last gene cassette, respectively, are related and terminate at the IRi (8). The class 1 integron in strain MUL2G11 has a complete left end like Tn*402*, but those in MUL2G8 and MUL2G9 are terminally deleted.

the *Delftia* host (~66%) (Table 2). GC3 is unusual in having two possible open reading frames (ORFs): *orfC* and *orfD* are separated by 23 nucleotides. Cassettes with two ORFs are not common. Notable exceptions are those encoding toxin-antitoxin gene pairs in superintegrons in *Vibrio* (49); however, the two ORFs in *Delftia* do not resemble these. None of the cassettes carried an obvious resistance gene: *orfD* has no orthologs in available databases, and the others had low similarities to various unidentified ORFs. The G+C content of *orfB* in GC2 was higher than that of the other ORFs, and its predicted product had significant similarity to a conserved hypothetical protein in *Comamonas testosteroni* KF-1.

**Characterization of flanking sequences.** Integrons are often components of mosaic structures including various recombination functions; they are themselves not capable of horizontal gene transfer but are frequently associated with mobile elements such as transposons and plasmids. Examination of the DNA context in which an integron is situated is therefore often useful in providing clues as to its history. A region of sequence identity was found to extend beyond the integrons on both sides (Fig. 3; Table 2). This suggests that the integrons shared a recent ancestor prior to the lateral gene transfer event that brought the segment of ancestral DNA, including the integrons, into *Delftia*. The border of the shared region on the left of the integrons remains to be determined but is at least 3.1 kb away from the end of *intI3*. In this left flank, lf1 and lf2 have strong similarity to two neighboring genes in *Acidovorax* sp. strain JS42 (Ajs_228 and Ajs_229) that are not found in related strains. On the right of the integrons, the region of identity includes four integron-adjacent/associated ORFs, ia-1 to ia-4, all of which have some association with transposons or some theoretical role in transposition. The deduced products of ia-1 and ia-4 resemble integrases/recombinases from strains of *Acidovorax*: in particular, ia-1 is most similar (88% DNA identity; 96% amino acid identity) to a putative integrase/recombinase in the environmental strain *Acidovorax* sp. strain MUL2G8 that is encoded by a gene adjacent to a class 1 integron (47). Short tracts around ia-1 also have similarity to DNA flanking the MUL2G8 gene: the 50 nucleotides upstream of both share 90% identity, and the 56 nucleotides immediately downstream of both share 87% identity. The ORF for ia-2 encodes a putative TetR family transcriptional repressor (37), and that for ia-3 encodes a putative QacF-like small multidrug efflux protein (35). The published genes most similar to these were found in a transposon inserted into IncP-1β plasmid pB8, where they were comparably arranged and conferred tolerance to quaternary ammonium compounds (41); one might speculate that these genes could have been introduced into the ancestral segment from a plasmid. The proximity of all these genes to In3 is very intriguing, but their functions remain to be demonstrated.

The regions of 100% identity in both A90 and C17 are followed on the right flank by rf1 to rf5, a group of housekeeping genes. They are overall 94.7% identical at the DNA level between the strains. These genes, including a *gyrA* homolog (rf4), are conserved and syntenic in strains from related genera that have been sequenced: *Acidovorax* sp. strain JS42, *Acidovorax avenae* subsp. *citrulli* AAC00-1, *D. acidovorans* SPH-1, and *Comamonas testosteroni* KF-1 (http://genome.jgi-psf.org/mic_home.html). Interestingly, Ajs_3904, the ia-4 ortholog in *Acidovorax* sp. strain JS42, is also part of the syntenic series in its native host. Colinearity with *gyrA* (rf), together with the results of Southern blot analysis, supports the conclusion that the *Delftia* class 3 integrons are chromosomally located, in contrast to In3-1 and In3-2, which are plasmid borne (1, 9, 22). The possibility of a preferred site for the transfer event is suggested by linkage of the integrons and their immediately surrounding regions to the same loci (rf1 to rf5) in two distinct *Delftia* strains, but examination of the sequence in the possible border regions has not yet revealed a particular mechanism. The transferred region might be of *Acidovorax*-related origin, given the similarities of some of the ORFs to the genome of *Acidovorax* sp. strain JS42, notably a putative phage P4-type tyrosine recombinase gene that is in the same location as ia-4 relative to the other JS42 chromosomal genes.

**Class 3 integrons in the environment.** The results of this study not only provide the first evidence of the presence of class 3 integrons in North America but also show that they have a wider distribution ecologically. The integrons and their contiguous DNA in *Delftia* have a number of similarities to certain environmental class 1 integrons that have been proposed as chromosomal elements that predate transposon dissemination (47). The resemblance of ORF ia-1 and its flanking DNA to the putative integrase/recombinase coding and intergenic regions in *Acidovorax* sp. strain MUL2G8 (47) adjacent to the integrase gene of a class I integron is striking. The fact that ia-1 follows the last gene cassette, GC4, rather than *intI3*, is consistent with the model that class 1 and class 3 integrons have opposite orientations (8). In addition, the *Delftia* integrons are bounded by precisely the same deletion endpoint after GC4 as are the integrase genes in the environmental class 1 integrons of strains MUL2G8 and MUL2G9 relative to Tn*402* (8, 47).

The organization of the new class 3 integrons includes elements that would allow the capture and assortment of gene cassettes. The possibility exists that they may be related to the superintegrons that are commonly found in microbial communities in soils, sediments, and aqueous environments. Superintegrons are chromosomally located, are associated with cassettes of varied organization and largely unknown function (6, 39, 52), and have been hypothesized to be the progenitors of mobile integrons (27). Although superintegrons typically contain many genes, the *Shewanella oneidensis* superintegron (12), with only three cassettes, provides a comparison to the *Delftia* integrons.

The discovery of In3-3 and In3-4 in wastewater treatment facilities may be important for understanding the ecology of both the host organisms and the integrons in future studies. Wastewater treatment plants have been proposed as important reservoirs of antibiotic resistance gene cassettes, e.g., on IncP plasmids (41, 50). Introduction of a new integron into a member of the local bacterial population such as *Delftia* spp. (some of which are known to carry IncP plasmids [45]) could not only lead to proliferation of the integron per se but also may add a new role for this group of organisms in the acquisition of resistance genes from antibiotic-resistant microbes in specialized wastewater input from hospitals or agriculture (10). Both effects will enhance the evolutionary and transmission dynamics of integron-associated antibiotic resistance and ultimately

contribute to its persistence and horizontal spread in the environment.


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