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The IntI-Like Tyrosine Recombinase of *Shewanella oneidensis* Is Active as an Integron Integrase

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We have found an integron-like integrase gene and an *attI* site in *Shewanella oneidensis* as part of a small chromosomal integron. We have cloned this gene and tested the ability of the integrase to excise cassettes from various integrons. Most cassettes flanked by two *attC* sites are readily excised, while cassettes in the "first" position, with an *attI1* or *attI3* site on one end, are not excised. An exception is a cassette with *attI2* on one end. The *attI2* site, from Tn*7*, has greater similarity to the *attI* site adjacent to the integrase of *S. oneidensis* than do *attI1* or *attI3*. We cloned the *attI* site of *S. oneidensis* and observed the integration of two different cassettes. We have, therefore, demonstrated the function of this integron-like integrase.

Integrons are gene capture systems that can acquire and disseminate elements known as gene cassettes. These systems are implicated in the dissemination of antibiotic resistance genes. The essential components of the integron are found within the 5′-conserved segment and include an integrase gene, *intI*, an adjacent recombination site, *attI*, and a promoter region from which integrated cassettes are expressed (4). The gene cassettes are located within the variable region and are integrated in tandem at the *attI* site. These cassettes contain a structural gene and an imperfect palindromic integrase-specific recombination site known as an *attC* site or 59-base element. The cassettes are mobile, nonreplicating, and generally lack a promoter region (10).

Several classes of integrons have been reported, all of which contain distinct but related integrase genes (12). Class 1 integrons are the most widespread, and most contain a 3′-conserved segment that contains a *qacEΔ1* gene encoding resistance to quaternary ammonium compounds and a *sulI* gene encoding resistance to sulfonamides (Fig. 1) (13, 14). Classes 2 and 3, which are rarer, are, like class 1, plasmid borne (9). The class 4 integron is a first example of a chromosomal superintegron and is located in the genome of *Vibrio cholerae* (5). Several other classes have been identified, but they are not fully characterized yet (7, 12).

The integron integrase is a member of the tyrosine recombinase family, a superfamily of site-specific recombination proteins (3). Among this family, there are also the well-known XerC/XerD, Cre, Flp, and λ integrase (2, 8). However, there is an extra domain, composed of an additional 36 amino acids near Patch III that distinguishes integron integrases from other tyrosine recombinases and which is implicated in the recognition of the *attI* and *attC* sites (6).

Sequence analysis. *Shewanella oneidensis* MR-1 partial genomic data was from The Institute for Genomic Research (TIGR). Sequence analysis was done using Sequencher (version 3.1) and Genetics Computer Group (version 10.1) software. By screening the partial genomic DNA sequences from TIGR, we found in contig 7833 of *S. oneidensis* MR-1 a sequence encoding a protein 45% identical and 54% similar to IntI1. The translated sequence also contained the extra domain of integron integrases. This bacterium was isolated from lake sediment and was previously named *Shewanella putrefaciens* (15). Figure 2 shows the alignment of some integron integrases with the tyrosine recombinase of *S. oneidensis* and with XerC/XerD from *Escherichia coli*.

We found a putative *attI* site adjacent to the tyrosine recombinase gene. The *attI* site is generally located upstream of the *intI* gene, at the right-hand end of the 5′-conserved segment of class 1 integrons. It is around 70 bp long, nonpalindromic, and ends with the consensus sequence GTTRRRY. The crossover site is between the G and the first T (11). Figure 3 shows the alignment of the partial *attI* site of *S. oneidensis* with its closest homologue, *attI2*. In this study, we characterized the ability of the tyrosine recombinase of *S. oneidensis* to excise cassettes at the *attC* sites and to integrate some cassettes at the *attI* site.

We found that the tyrosine recombinase and the *attI* site

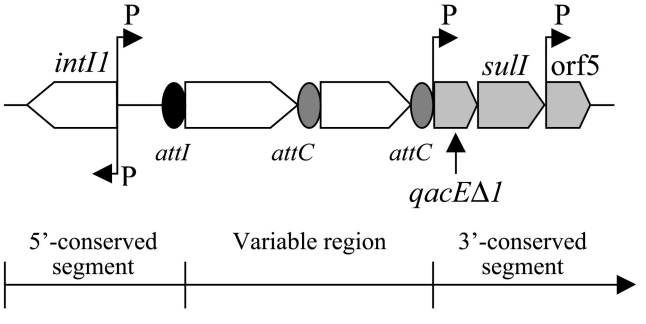

FIG. 1. General structure of class 1 integrons. Cassettes are inserted in the variable region by the integrase using a site-specific recombination mechanism. The *attI* and *attC* sites are shown by a black and a grey oval, respectively, and promoters are denoted by P. Genes are as follows: *intI1*, integrase gene; *qacEΔ1*, antiseptic resistance gene; *sulI*, sulfonamide resistance gene; *orf5*, gene of unknown function.

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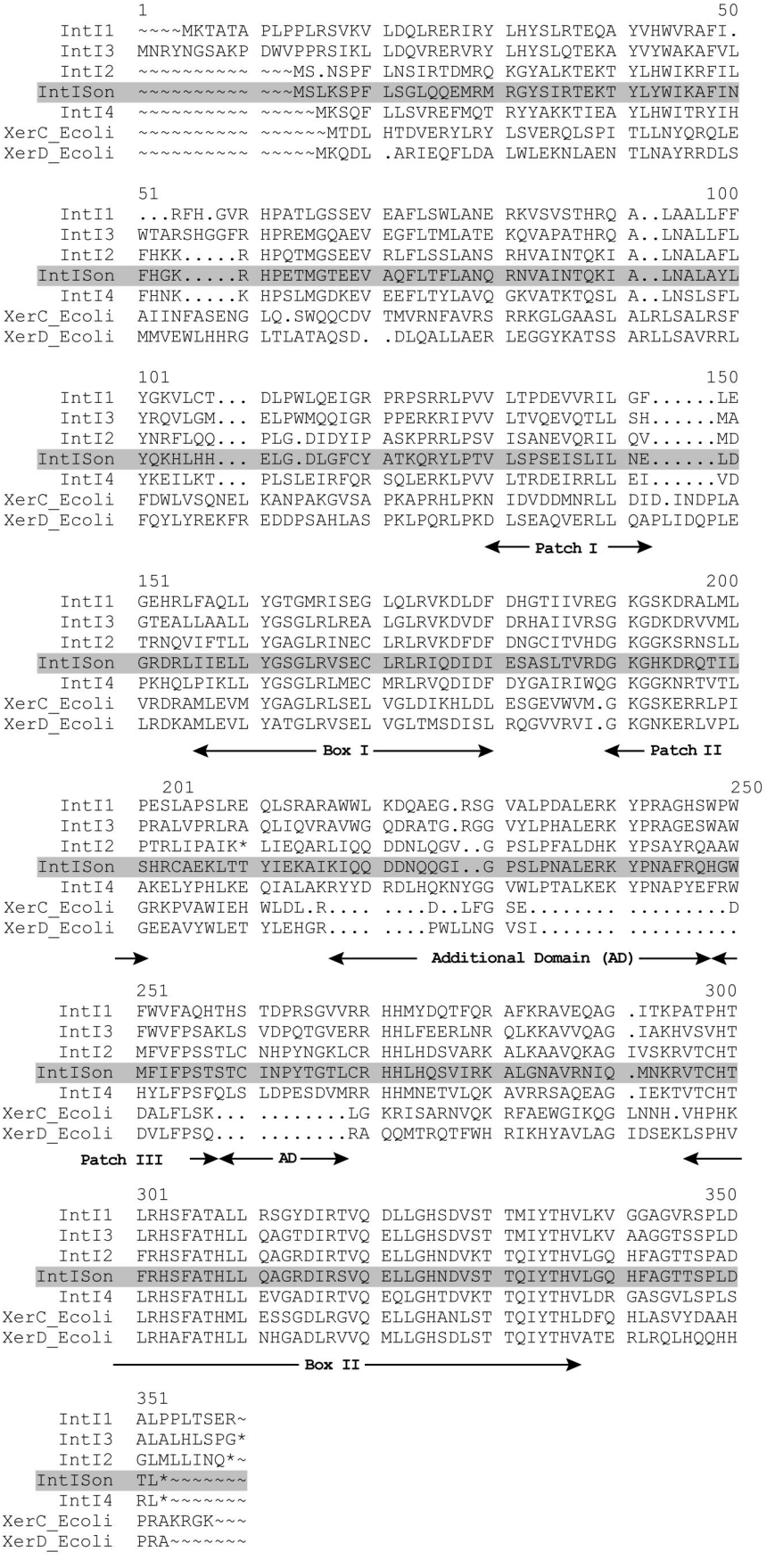

FIG. 2. Alignment of various integron integrases with XerC/XerD from *E. coli*. IntI1, class 1 integron integrase from plasmid pVS1; IntI3, class 3 integron integrase from a *Serratia marcescens* plasmid; IntI2, class 2 integron integrase from Tn7; IntISon, integron integrase from *S. oneidensis*; IntI4, class 4 integron integrase from the *V. cholerae* superintegron; XerC, recombinase from *E. coli*; XerD, recombinase from *E. coli*.

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attI2     AAACGAGCATGCTTACTAATAAAATGTT
          ||||| ||||||  || ||||||||||||
attISon   AAACGCGCATGCGCACGAATAAAATGTT
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FIG. 3. Alignment of the 3′ extremities of the *attI2* site from class 2 integrons with the *attI* site of *S. oneidensis.* The GTT is the crossover site.

were in fact part of an organized small chromosomal superintegron, shown in Fig. 4. On the left-hand side of *intI,* there are genes homologous to chromosomal genes of *E. coli*, namely *slt* (soluble lytic transglycosylase) and *rtn* (hypothetical protein). To the right of the *attI* site, there is a cassette containing an insertion sequence (IS). This IS is composed of a tyrosine recombinase, probably functioning as a resolvase, and a transposase. These two genes are homologous with those of the IS found linked to the *Acc*I restriction modification system of *Weeksella zoohelcum* (1). This cassette is also found at two other chromosomal locations in *S. oneidensis* MR-1. Downstream of this first cassette, there is a second cassette composed of an open reading frame (ORF) that shares some similarity to ORFs from *S. oneidensis* and *V. cholerae*. Its *attC* site resembles a VCR repeat (2). The third cassette is a short noncoding region followed by another *attC* site. This site is like *aadA*/*aadB attC* sites (≈60 bp) (10). After this cassette there is, on the bottom strand, an inverted repetition of the ORF lo-

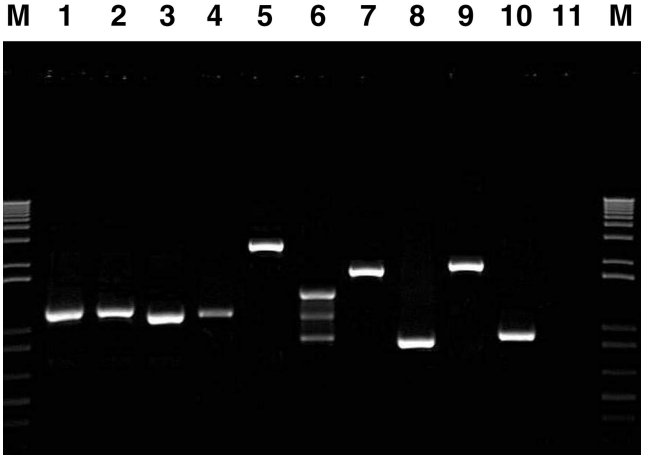

FIG. 5. Example of the results obtained for an excision test. Lane M, 1 Kb Plus (Gibco BRL) molecular weight marker; lane 1, *attI2-dfr1_attC* (pLQ 424) IntISon not induced; lane 2, *attI2-dfr1_attC* (pLQ 424) IntISon induced; lane 3, *attI1-dfrI_attC* (pLQ427) not induced; lane 4, *attI1-dfrI_attC* (pLQ427) not induced; lane 5, *aadA2_attC-aacA1a-orfG_attC* (pLQ428) not induced; lane 6, *aadA2_attC-aacA1a-orfG_attC* (pLQ428) induced; lane 7, *aadA2_attC-bla*$_{imp}$*_attC* (pLQ438) not induced; lane 8, *aadA2_attCbla*$_{imp}$*_attC* (pLQ438) induced; lane 9, *aadA2_attC-aadA1_attC* (pLQ443) not induced; lane 10, *aadA2_attC-aadA1_attC* (pLQ443) induced; lane 11, PCR negative control.

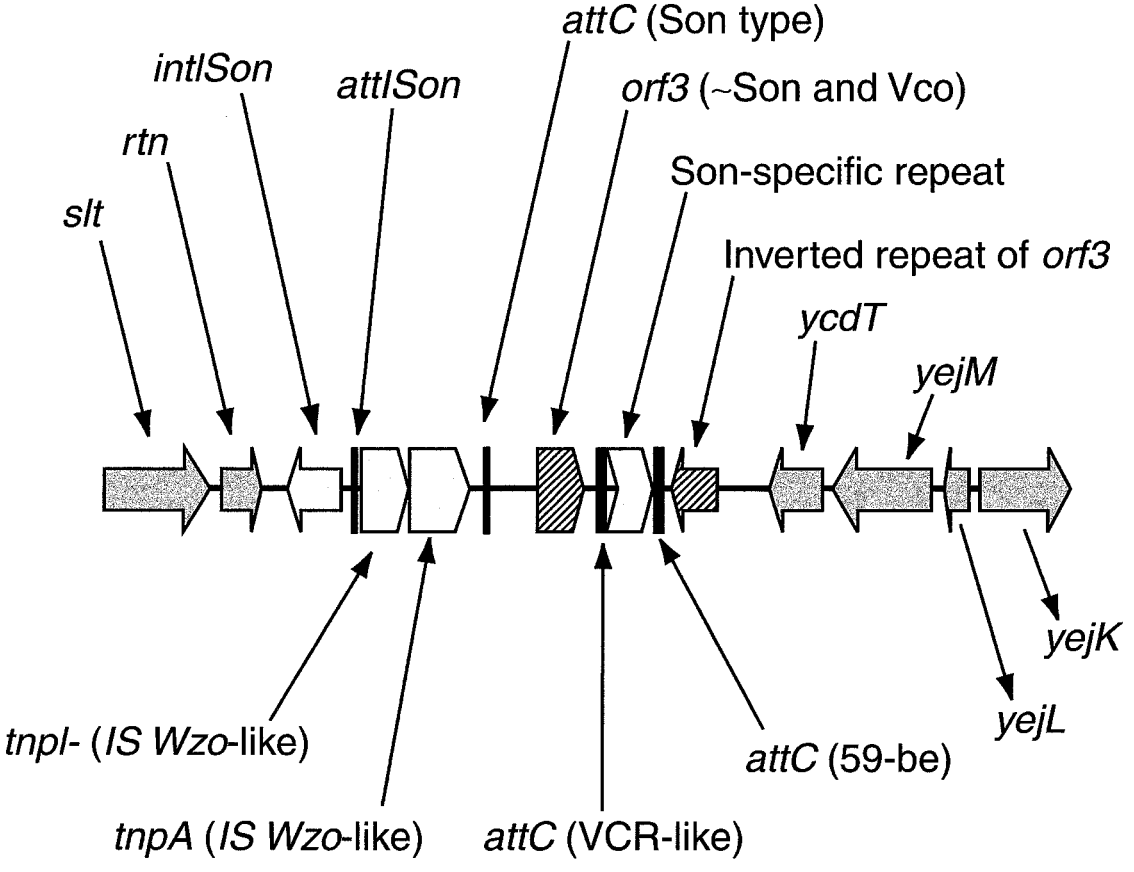

FIG. 4. Representation of the chromosomal superintegron of *S. oneidensis*. The chromosomal genes are in dark grey, the integrase and cassettes are in light grey, and *attC* sites are in solid black. The integron does not include the six chromosomal genes shown, which are identified by their similarities with *E. coli* chromosomal genes.

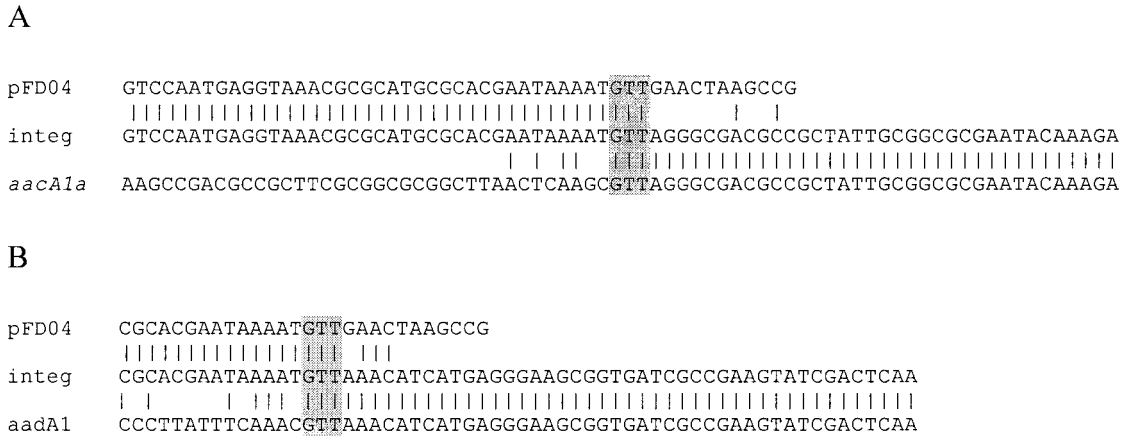

FIG. 6. Sequence obtained from the amplicon generated with excision-integration assays. (A) Integration of *aacA1a* cassette; (B) integration of *aadA1* cassette. Sequences are compared to the clone containing the *attI* site (pFD04, upper line) and to the *aacA1a* and *aadA1* cassettes in the context of the donor plasmids pLQ428 and pLQ443, respectively (lower line).

cated in the second cassette. The sequence afterwards returns to typical chromosomal genes with homologs in *E. coli*. These are *ycdT*, *yejM*, *yejL*, and *yejK*. The *yej* genes are in the same order in *E. coli.* Therefore, there appears to be a superintegron of about 6 kb in length that is flanked on either side by chromosomal genes.

Construction of the IntISon overproduction vector. PCR amplification was done on the genomic DNA of *S. oneidensis* MR-1 with primer sonint-*Bsp*HI (5′-GGCGCGTTTTCCAAA CCTATTATC-3′) and mutagenic primer sonint-*Bam*HI (5′-C AGTATTTCCCGGATCCCTATGGA-3′). Thereafter, the amplicon was digested with *Bsp*HI and *Bam*HI and it was then cloned into pTRC99a (Amersham-Pharmacia) digested with *Nco*I and *Bam*HI. Genes cloned using these sites are expressed from the *trc* promoter and use a vector-provided ribosome binding site. The clone, pFD03, was then transformed into *E. coli* JM109. This clone was used to overexpress the integrase in excision assays. The *attI* site of *S. oneidensis* was cloned into pFD03 by amplifying it with the mutagenic primers attIson-*Bam*HI (5′-ATTCCCGGATCCCAAGGAAAAT-3′) and attIson-*Hin*dIII (5′-TAGGAGTAAGCTTGGAGCAGCG-3′). The amplicon was then digested with *Bam*HI and *Hin*dIII and then cloned into pFD03 digested with *Bam*HI and *Hin*dIII to yield pFD04. This clone was used in the excision-integration assays.

Excision assays. We tried to excise several cassettes with different flanking sequences. The cells harboring the clone pFD03 were transformed by various clones harboring gene cassettes cloned into pACYC184. The double-transformants were then incubated overnight in 5 ml of Luria-Bertani medium with 0.2% glucose (wt/vol), subcultured (0.1 ml into 15 ml), and grown to an optical density at 600 nm of 0.6. Then, isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 0.4 mM and the culture was incubated at 37°C overnight; 5 ml was harvested and a miniprep (Qiagen) was done. PCR was done using primers pACYC184-5′ (5′-TGTA GCACCTGAAGTCAGCC-3′) and pACYC184-3′ (5′-ATAC CCACGCCGAAACAAG-3′) with the following cycles: 10 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 55°C, and 5 min at 72°C, and a final step of 10 min at 72°C. The length of the resulting amplicon indicated the presence or absence of excision.

Figure 5 shows an example of results obtained in excision assays. Table 1 shows a summary of the results. Some cassettes, like *aacA1a-orfG* and *orfH*, are always strongly excised and other cassettes, like *dfrA1*, are never excised by the integron integrase of *S. oneidensis.* This protein is able, like IntI1 but not IntI2 and IntI3 (F. Gagnon and P. H. Roy, unpublished results), to excise cassettes with a heterologous *attI* site as its left-hand neighbor, but this specificity is limited to *attI2*, with which *attISpu* shares sequence similarity (Fig. 3). The left-hand neighbor site seems to play a role in the ability of the tyrosine recombinase IntISon to excise cassettes, as the *aadA1* cassette is excised weakly to strongly, depending on the left-hand site of the cassette. The *attC* site of *aadA2* seems to be a very good left-hand neighbor site, since when this element is present, there is always a strong excision of the following cassette, with the exception of *dfrA1*. The integron integrase of *S. oneidensis* is in fact able to excise several cassettes associated with various *attC* elements, except for *dfrA1*. These results indicate that this integrase should be able to excise cassettes from various classes of integrons.

Excision-integration assays. Using two clones for which excision was particularly strong, namely pLQ428 and pLQ443, coupled excision-integration assays were done. The protocol was the same as for the excision assays, except that the integrase clone was pFD04, which also contained the *attI* site. We tried two cassettes known to be efficiently excised, namely *aacA1a* and *aadA1*. For the detection of integration, PCR was done using primers for the cassette tested. For the *aacA1a* cassette, the primers sonint1143 (5′-TTCACATCTGCTCCAT AGGATC-3′) and aacA1a-NH2 (5′-TAATTGCTGCATTCC GC-3′) were used. For the *aadA1* cassette, primers sonint1143 and aadA-Tn21 (5′-TCGATGACGCCAACTAC-3′) were used.

Figures 6A and B show the integration results obtained for pLQ428 and pLQ443, respectively. The upper sequence (pFD04) is that of the clone pFD04. The middle sequence (integ) is that obtained from the amplicon generated by sonint1143 and aacA1a-NH2 or aadA-Tn21 primers. The

TABLE 1. Results obtained from the excision tests[a]

Clone name (pLQ)	Left-hand neighbor site	Crossover site sequence	Gene cassette with its *attC* site	Crossover site sequence	Right-hand neighbor site	Excision[b]
423	*attI1*	GTTAAAC	*aadA1*	GTTAGAT	*qacEΔ1*	−
424	*attI2*	GTTAACC	*dfrA1*	GTTAGGC	*orf1*	−
425	*attI3*	GTTAGAA	bla_{imp}	GTTAGGC	*aacA4*	−
427	*attI1*	GTTAACC	*dfrA1*	GTTAAAC	*aadA1*	−
429	*attI1*	GTTAGAC	*aadA2*	GTTAGGG	*aacA1a*	−
439	*attI1*	GTTAGAA	bla_{imp}	GTTAGGC	*aacA4*	−
440	*attI1*	GTTAGGG	*aacA1a-orfG + orfH*	GTTAGGC	*orfI*	−
441	*attI2*	GTTAGGG	*aacA1a-orfG + orfH*	GTTAGGC	*orfI*	+
442	*attI3*	GTTAGGG	*aacA1a-orfG + orfH*	GTTAGGC	*orfI*	−
443	*aadA2_attC*	GTTAAAC	*aadA1*	GTTAGAT	*qacEΔ1*	+++
428	*aadA2_attC*	GTTAGGG	*aacA1a-orfG + orfH*	GTTAGGC	*orfI*	+++
437	*aadA2_attC*	GTTAACC	*dfrA1*	GTTAAAC	*aadA1*	−
438	*aadA2_attC*	GTTAGAA	bla_{imp}	GTTAGGC	*orfI*	+++
426	*dfrA1_attC*	GTTAAAC	*aadA1*	GTTAGAT	*qacEΔ1*	+
444	*dfrA1_attC*	GTTAGGG	*aacA1a-orfG + orfH*	GTTAGGC	*orfI*	+++
430	*dfrA1_attC*	GTTAGGC	*sat*	GTTAAAC	*aadA1*	++
445	*aacA1_attC*	GTTAAAC	*aadA1*	GTTAGAT	*qacEΔ1*	++
446	bla_{imp}*_attC*	GTTAGGG	*aacA1a-orfG + orfH*	GTTAGGC	*orfI*	+++
431	*aacA4_attC*	GTTAGCC	*pse1*	GTTAGAC	*aadA2*	++

[a] The cassette *aacA1-orfG* is always cloned in tandem with *orfH*. This is why there are two *attC* sites in clones with *aacA1a-orfG* cassette.
[b] −, no excision; +, weak excision (<20%); ++, medium excision (20 to 75%); +++, strong excision (>75%).

lower sequence (pLQ428 or pLQ443) is that of the pLQ clone from which the excised cassette originates. These results show that integration has occurred at the predicted crossover point, namely G/TT (11) and, thus, that the tyrosine recombinase of *S. oneidensis* is a fully active integron integrase. The fact that this integrase can recognize any type of *attC* site explains why there are three types of *attC* sites in the chromosomal super-integron of this strain. Class 1, 2, and 3 integrons are antibiotic resistance integrons (13). Although antibiotic resistance has emerged in the middle of the last century, its mechanisms must have evolved from ancestral forms found in antibiotic-producing organisms before the introduction of the antibiotics (12). By being able to excise and integrate antibiotic resistance gene cassettes, *S. oneidensis* has a potential for evolution of antibiotic resistance integrons similar to that seen for Class 1 integrons.

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REFERENCES

1. **Brassard, S., H. Paquet, and P. H. Roy.** 1995. A transposon-like sequence adjacent to the *Acc*I restriction-modification operon. Gene **157:**69–72.
2. **Clark, C. A., L. Purins, P. Kaewrakon, and P. A. Manning.** 1997. VCR repetitive sequence elements in the *Vibrio cholerae* chromosome constitute a mega-integron. Mol. Microbiol. **26:**1137–1138.
3. **Esposito, D., and J. J. Scocca.** 1997. The integrase family of tyrosine recombinases: evolution of a conserved active site domain. Nucleic Acids Res. **25:**3605–3614.
4. **Hall, R. M., and C. M. Collis.** 1995. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. Mol. Microbiol. **15:**593–600.
5. **Mazel, D., B. Dychinco, V. A. Webb, and J. Davies.** 1998. A distinctive class of integron in the *Vibrio cholerae* genome. Science **280:**605–608.
6. **Messier, N., and P. H. Roy.** 2001. Integron integrases possess a unique additional domain necessary for their activity. J. Bacteriol. **183:**6699–6706.
7. **Nield, B. S., A. J. Holmes, M. R. Gillings, G. D. Recchia, B. C. Mabbutt, K. M. Nevalainen, and H. W. Stokes.** 2001. Recovery of new integron classes from environmental DNA. FEMS Microbiol. Lett. **195:**59–65.
8. **Ouellette, M., and P. H. Roy.** 1987. Homology of ORFs from Tn *2603* and from R46 to site-specific recombinases. Nucleic Acids Res. **15:**10055.
9. **Recchia, G. D., and R. M. Hall.** 1995. Gene cassettes: a new class of mobile element. Microbiology **141**(Pt. 12)**:**3015–3027.
10. **Recchia, G. D., and R. M. Hall.** 1997. Origins of the mobile gene cassettes found in integrons. Trends Microbiol. **5:**389–394.
11. **Recchia, G. D., H. W. Stokes, and R. M. Hall.** 1994. Characterisation of specific and secondary recombination sites recognised by the integron DNA integrase. Nucleic Acids Res. **22:**2071–2078.
12. **Rowe-Magnus, D. A., A. M. Guerout, P. Ploncard, B. Dychinco, J. Davies, and D. Mazel.** 2001. The evolutionary history of chromosomal super-integrons provides an ancestry for multiresistant integrons. Proc. Natl. Acad. Sci. USA **98:**652–657.
13. **Sallen, B., A. Rajoharison, S. Desvarenne, and C. Mabilat.** 1995. Molecular epidemiology of integron-associated antibiotic resistance genes in clinical isolates of enterobacteriaceae. Microb. Drug Resist. **1:**195–202.
14. **Stokes, H. W., and R. M. Hall.** 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol. Microbiol. **3:**1669–1683.
15. **Venkateswaran, K., D. P. Moser, M. E. Dollhopf, D. P. Lies, D. A. Saffarini, B. J. MacGregor, D. B. Ringelberg, D. C. White, M. Nishijima, H. Sano, J. Burghardt, E. Stackebrandt, and K. H. Nealson.** 1999. Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. *nov*. Int. J. Syst. Bacteriol. **49**(Pt. 2)**:**705–724.