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Excision and Integration of Cassettes by an Integron Integrase of *Nitrosomonas europaea*

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We found in the environmental strain *Nitrosomonas europaea* a chromosomal integron-like structure with an integrase gene, $intI_{Neu}$. We have tested the capacity of the IntINeu integrase to excise and integrate several resistance gene cassettes. The results allow us to consider IntINeu a new functional integron integrase.

Integrons are a gene capture system in which gene cassettes are mobile elements. They consist of an integrase gene (*intI*) (18), a member of the tyrosine recombinase family, followed by a nonpalindromic *attI* site specific to each integrase, and one or more integrase-dependent mobile cassettes expressed from a common promoter upstream of this site (4, 13). Excised cassettes in their free circular form consist of a structural gene and a palindromic *attC* site and are unable to replicate (5). Cassettes are preferentially integrated at the *attI* site, containing the GTTRRRY core site.

Four classes of multiresistance integrons have been identified to date. The first three classes are usually located on plasmids, while the fourth is chromosomal, in the SXT(ET) constin of *Vibrio cholerae*. Several new classes of integrons have been found to be chromosomally located in environmental bacteria, and their cassettes code for proteins with as yet unidentified functions (17, 23, 25).

Class 1 integrons (Fig. 1A), which constitute the most widespread and studied class in the resistant bacterial population, are composed of two conserved segments and one variable region. The 5′-conserved segment (5′-CS) contains the integrase gene (*intI1*), a promoter region, and the IntI1-specific integration site *attI1*. The 3′-conserved segment (3′-CS) contains a quaternary ammonium resistance gene (*qacEΔ1*), and most segments also contain a sulfonamide resistance gene (*sul1*) (9, 19, 20) and an open reading frame (ORF5). Between the two conserved segments, the variable region (14) can contain from zero to eight cassettes.

Chromosomal integrons, named superintegrons (15, 23, 24), have been found in *V. cholerae* isolates from the 19th century and clearly predate the antibiotic era (Fig. 1B). This discovery indicates that the first function of integrons was probably a general, but not essential, gene capture system for bacterial genome evolution, which encodes adaptive rather than indispensable functions. Superintegrons differ from antibiotic resistance integrons by several characteristics: most of their cassettes encode unknown functions, their *attC* sites are more uniform, some cassettes possess their own promoters, and some carry ORFs in the orientation inverse to that of their recombination sequence.

Chromosomal superintegrons have been found in other *Vibrionaceae* species (*Vibrio mimicus* [3], *Vibrio salmonicida* [H. Sørum, K. Dommarsnes, K. Sandersen, L. Sundström, M. Gullberg, and A. Solberg, unpublished data {GenBank accession number AJ277063}], and *Listonella pelagia* [23]) as well as in *Pseudomonas alcaligenes* (25) and uncultivated environmental bacteria (17). The integrases of *V. cholerae* (12) and *Shewanella oneidensis* (7) have been shown to be functional. Other chromosomal integrases from environmental bacteria, like IntIGsu from *Geobacter sulfurreducens* and IntITde from *Treponema denticola*, have been identified from genome projects (17).

Nitrosomonas europaea is an environmental gram-negative β-proteobacterium that plays a central role in the availability of nitrogen to plants. Its 2.81-Mbp genome has been completely sequenced by the U.S. Department of Energy Joint Genome Institute (JGI) (http://www.jgi.doe.gov/JGI_microbial/html/index.html) in order to explore the role of microorganisms in global carbon sequestration. Using the *intI1* and the additional domain sequences characteristic to integron integrases (16), we identified in the genome of *N. europaea* two integrase-like genes. One lacks an identifiable initiation codon, and the second, named $intI_{Neu}$, is associated with a chromosomally located integron. In this study, we have tested the ability of the $intI_{Neu}$ integrase to excise gene cassettes—resistance genes cloned into pACYC184—and integrate them by site-specific recombination into the $attI_{Neu}$ site. We showed that in the presence of IntINeu most of the cassettes tested in this study, preceded by either *attI* or *attC* sites, could be excised from pACYC184 and integrated into the $attI_{Neu}$ site cloned in pTrc99A. These results were presented at the 102nd Annual Meeting of the American Society for Microbiology (G. Leon and P. H. Roy, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. H-92, 2002).

Sequence environment of the *N. europaea* integron. The integron of *N. europaea* is flanked by typical chromosomal genes. The *metX* and *metW* genes are found on the left-hand side as represented in Fig. 1C, while on the right-hand side an *ampG* gene is present. The *metX* and *metW* genes are involved in the biosynthetic pathway of methionine from homoserine and are homologous to *Escherichia coli* chromosomal genes. The

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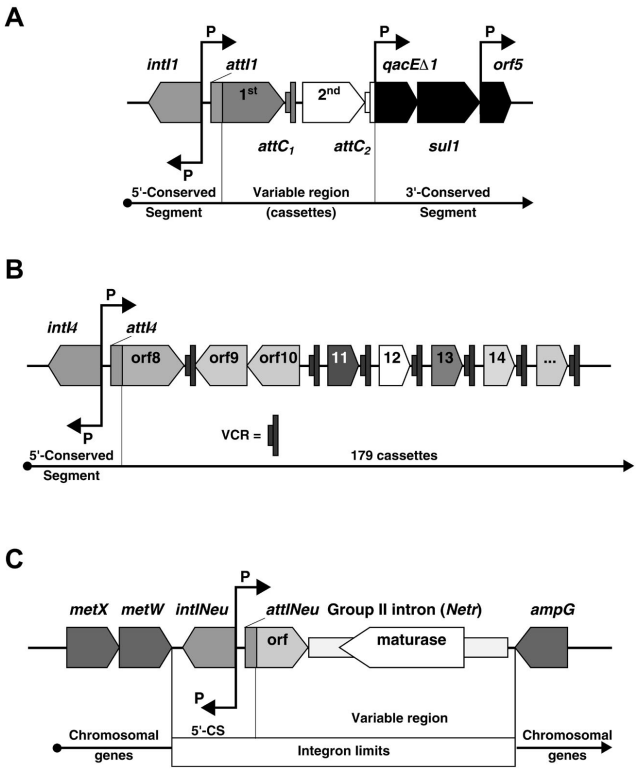

FIG. 1. Structures of integrons. (A) General structure of class 1 integrons, composed of two conserved segments flanking a variable region where cassettes containing an *attC* site are integrated at the *attI1* site by the integron integrase by a site-specific recombination mechanism. P, promoters; *intI1*, integrase gene; *qacEΔ1*, antiseptic resistance gene; *sul1*, sulfonamide resistance gene; *orf5*, gene of unknown function. (B) Structure of the class 4 integron (superintegron of *V. cholerae*). P, promoters; *intI4*, integrase gene; *attI4*, integration site; *orf*, genes of unknown functions; VCR, *V. cholerae* repeat (*attC* site). (C) Structure of *N. europaea* chromosomal integron. P, promoters; $intI_{Neu}$, integrase gene; $attI_{Neu}$, integration site; *orf*, gene coding for a 123-amino-acid *qacE*-like protein; *metX* and *metW*, chromosomal genes involved in methionine biosynthetic pathway; *ampG*, chromosomal permease gene involved in β-lactamase induction and recycling of peptidoglycan.

ampG gene is also homologous to an *E. coli* chromosomal gene and codes for a permease essential to β-lactamase regulation and recycling of peptidoglycan. The integrase gene is adjacent to an *attI* site that is followed by an ORF. This integron possesses only one ORF coding for a 123-amino-acid protein, possibly related to *qacE*, inserted into the $attI_{Neu}$ site and a group II intron (Netr), coded in the same orientation as the integrase gene. The group II intron (Netr) is integrated in the inverted core site of the single cassette's *attC*. However, the major part of the *attC* site was not present, as expected, on the right-hand extremity of the intron (Fig. 1C) but was found adjacent to a second copy of Netr 92 kb away. The cassette-intron and intron-*attC* assemblies may represent intermediates in cassette formation (2). Netr is a 2.0-kb element, with ribozyme activity and an ORF coding for a putative maturase/reverse transcriptase. A similar group II intron has been observed in *Serratia marcescens*, also integrated at the *attC* inverse core site in an integron cassette (2).

Construction of plasmids. *N. europaea* was obtained from the ATCC collection (ATCC 25978) and cultured under aerobic conditions at 26°C with agitation, in a special *Nitrosomonas* medium (ATCC medium no. 221 broth). Total DNA isolation was done by using a phenol-chloroform purification method (1). The integrase gene $intI_{Neu}$ was amplified by PCR on the genomic DNA with Herculase polymerase (New England BioLabs, Beverly, Mass.) and two primers, one of which is mutagenic and creates an *Nco*I site (Table 1) designed by using the OLIGO software package (version 4.1; National Biosciences, Plymouth, Minn.). The conditions for the PCR with mutagenic primers were as follows: 10 min at 95°C; 3 cycles consisting of 1 min at 95°C, 1 min at 42°C, and 1 min per kb at 72°C; 30 cycles consisting of 1 min at 95°C, 1 min at the appropriate annealing temperature (Table 1), and 1 min per kb at 72°C; and a final elongation step of 10 min at 72°C. For conventional primers, PCR conditions were the same except that the three cycles at a lower annealing temperature (42°C) were omitted. The amplicon was then digested with *Nco*I and *Pst*I and cloned into the expression vector pTrc99A (Amer-

TABLE 1. PCR primers[a]

Primer pair	Nucleotide sequence (5′ to 3′)	Annealing temp. used (°C)	Use
INTINEU-5′	TAT CAA CTT TCC ATG GGA AAT AC	60	$intI_{Neu}$ cloning (*Nco*I-*Pst*I)
INTINEU-3′	CGT CCA CAA CTT TCT GAA TAC GG	60	
ATTINEU-5′	TTG ATA CAG ATG CAT AGG CGT GAT	57	$attI_{Neu}$ cloning (*Nsi*I-*Hin*dIII)
ATTINEU-3′	GAC AAC AAA GCT TGC GCT AGA GAC	57	
PACYC184-5′	TGT AGC ACC TGA AGT CAG CC	55	Excision tests
PACYC184-3′	ATA CCC ACG CCG AAA CAA G	55	
ATTINEU[b]	AGG CGT GAT AGA TTC TC	48	
AADA1[c]	TCG ATG ACG CCA ACT AC	48	
BLA_{IMP}[c]	TGC GGT AGC AAT GCT GC	48	Integration tests
AACA1a[c]	TAA TTG CTG CAT TCC TCC GC	48	
PSE-1[c]	CGG ATG GTA TTA AAA GC	44	

[a] For the first two pairs, mutagenic primers (restriction sites underlined) were used to clone the integrase $intI_{Neu}$ and its recombination site $attI_{Neu}$. Excision of several cassettes by the integrase was verified by using the primer pair PACYC184-5′ and PACYC184-3′ for PCR amplifications. Integration of the excised cassettes was detected by using ATTINEU and a cassette-specific primer.
[b] Upper primer.
[c] Lower primer.

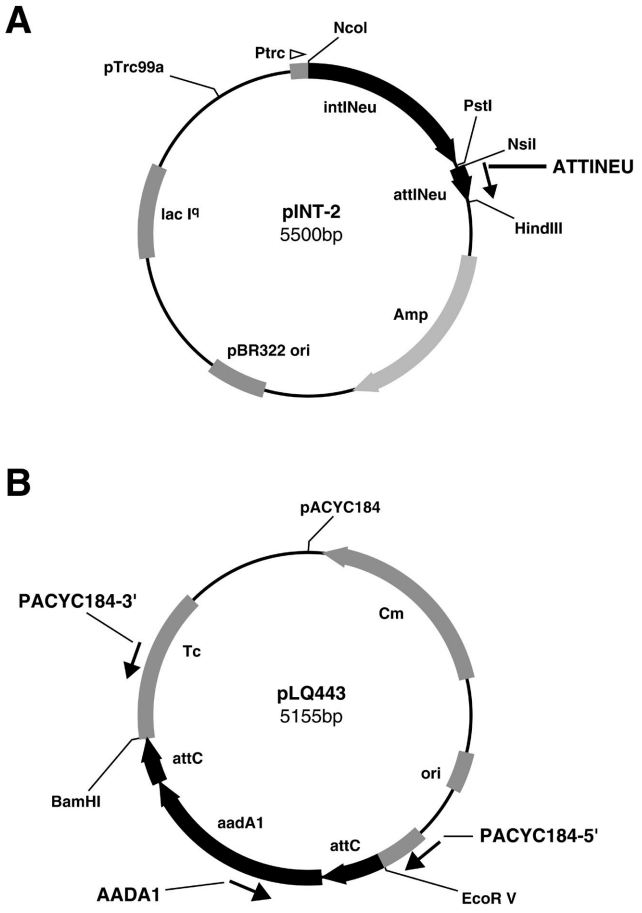

FIG. 2. Vector constructions. (A) Clone pINT-2 used for the integration test, which differs from the pINT-1 clone by addition of the $attI_{Neu}$ site. $IntI_{Neu}$, integrase gene cloned as an *Nco*I-*Pst*I fragment; $attI_{Neu}$, recombination site for integration of cassettes cloned as an *Nsi*I-*Hin*dIII fragment; *amp*, ampicillin resistance gene; pBR322 ori, replication origin; $lacI^q$, constitutive repressor; Ptrc, inducible promoter. (B) Clone pLQ443 is the donor clone containing the *aadA1* cassette used for excision and integration tests. Ori, replication origin; *Cm*, chloramphenicol resistance gene; *attC-aadA1-attC*, streptomycin-spectinomycin resistance cassette cloned as an *Eco*RV-*Bam*HI fragment into the tetracycline resistance gene (*Tc*) of pACYC184.

sham-Pharmacia) digested with the same enzymes to yield clone pINT-1. The $attI_{Neu}$ site was also amplified by PCR from genomic DNA by using the same protocol with mutagenic oligonucleotides (Table 1) containing the restriction sites *Nsi*I (upper primer) and *Hin*dIII (lower primer), and then it was cloned into pINT-1 digested with *Pst*I and *Hin*dIII to yield clone pINT-2 (Fig. 2A).

Excision and excision-integration assays. Nineteen clones containing resistance gene cassettes integrated in different recombination sites were used to test the activity of IntINeu. The integrase was induced in the presence of one or two excisable cassettes cloned in pACYC184 (F. Gagnon and P. H. Roy, unpublished results). Each resistance gene, bounded by two recombination sequences—the ORF-associated *attC* site downstream and a second recombination element (*attI* or *attC*) upstream—was susceptible to excision as a circular intermediate by the integrase and then to integration by a site-specific event into the $attI_{Neu}$ site of pINT-2 (Table 2).

In vivo tests were done by double transformation of pINT-1 (for the excision test) or pINT-2 (for the excision-integration test) into *E. coli* DH5α [*supE44* Δ*lacU169* (ϕ*80lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*] with one of the cassette-containing pLQ clones (Table 2). Double transformants were grown overnight at 37°C in a modified Luria-Bertani medium (10 g of Bacto Tryptone, 5 g of yeast extract, 5 g of NaCl, and 2 g of glucose) with selective antibiotics (50 μg each of ampicillin and chloramphenicol per ml). Then, 15 ml of a new medium (without antibiotic) was inoculated with 2% of the overnight culture and grown until the optical density at 600 nm reached 0.6 at 37°C before adding isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.4 mM for an overnight induction. Plasmids were purified from 5 ml of the overnight culture with the Miniprep kit (Qiagen) and used for detection by PCR of excision or integration by using the appropriate primer pair (Table 1). For each positive integration, the $attI_{Neu}$ site was sequenced to determine the exact point of integration.

The results of the excision tests using PACYC184-5′ and PACYC184-3′ primers for PCR amplifications showed that IntINeu is unable to excise cassettes integrated into a class 1, 2, or 3 *attI* site, except for the clones containing the *aacA1a-orfG* and *orfH* cassettes, from clones pLQ440, 441, and 442.

In clones pLQ443, 428, 437, and 438, cassettes downstream of the *aadA2 attC* site showed good levels of excision by IntINeu integrase (Fig. 3A), except for the trimethoprim resistance cassette *dfrA1* (pLQ437). This cassette has previously been shown to be less frequently excised by IntI1 than the *aadA1* cassette (10). For pLQ443, excision of the *aadA1* cassette yields a PCR amplicon of 900 bp whereas the amplicon is 1,800 bp when the cassette is not excised. In each test, excision by IntINeu was not totally efficient and there were still unexcised cassettes in each induced reaction. We observed in the induced excision of the pLQ428 cassettes (Fig. 3A) PCR products of 889, 1,341, and 2,047 bp, corresponding to the excision of both cassettes (*aacA1a-orfG+orfH*) and of either of the two cassettes independently (*aacA1a-orfG* and *orfH*), respectively. Excision of *orfH* alone had not been previously observed with the IntI1 integrase (8). Excision results were confirmed by excision-integration tests, and the amplicons obtained by using ATTINEU and several cassette-specific primers (Table 1) were around the expected 200 bp in length (Fig. 3B).

Clones pLQ426, 444, and 430 possess cassettes downstream of the *dfrA1 attC* site. The *dfrA1 attC* site is an unusual element (95 bp) and is not in one of the usual three groups (22). When this *attC* site was the left-hand neighbor, excisions of the aminoglycoside resistance gene *aadA1* (pLQ426) and the streptothricin acetyltransferase gene *sat* (pLQ430) (result not shown in Fig. 3A) were not observed. Only the *aacA1a-orfG* and *orfH* cassettes in pLQ444 were excised. Finally, clones pLQ445, 446, and 431 have different cassettes, each with a different left-hand neighbor: a 117-bp $attC_{aacA1a\text{-}orfG}$ site for pLQ445, a group 3 $attC_{blaimp}$ site for pLQ446 (127 bp), and a group 2 $attC_{aacA4}$ site for pLQ431 (70 bp) (21). In all cases, excision of the cassettes was observed but a greater efficiency was obtained for the pLQ445 cassette.

These results have also been confirmed by subsequent inte-

TABLE 2. In vivo site-specific excision and integration by IntINeu[a]

pLQ clone	Left-hand neighbor	Gene cassette and associated *attC*	Right-hand neighbor		Excision[b]	Integration
			Crossover site sequence	Gene		
423	*attI1*	**GTTAAAC** + *aadA1*	**G**TTAGAT	*qacEΔ1*	−	No
424	*attI2*	**GTTAACC** + *dfrA1*	**G**TTAGGC	*orfI*	−	ND
425	*attI3*	**GTTAGAA** + bla_{imp}	**G**TTAGGC	*aacA4*	−	ND
427	*attI1*	**GTTAACC** + *dfrA1*	**G**TTAAAC	*aadA1*	−	ND
429	*attI1*	**GTTAGAC** + *aadA2*	**G**TTAGGG	*orf1*	−	ND
439	*attI1*	**GTTAGAA** + bla_{imp}	**G**TTAGGC	*orf1*	−	ND
440	*attI1*	**GTTAGGG** + *accA1a-orfG* + *orfH*	**G**TTAGGC	*orf1*	+	Yes
441	*attI2*	**GTTAGGG** + *accA1a-orfG* + *orfH*	**G**TTAGGC	*orf1*	+	Yes
442	*attI3*	**GTTAGGG** + *accA1a-orfG* + *orfH*	**G**TTAGGC	*orf1*	+	Yes
443	$attC_{aadA2}$	**GTTAAAC** + *aadA1*	**G**TTAGAT	*qacEΔ1*	+++	Yes
428	$attC_{aadA2}$	**GTTAGGG** + *accA1a-orfG* + *orfH*	**G**TTAGGC	*orf1*	++	Yes
437	$attC_{aadA2}$	**GTTAACC** + *dfrA1*	**G**TTAAAC	*aadA1*	−	No
438	$attC_{aadA2}$	**GTTAGAA** + bla_{imp}	**G**TTAGGC	*orf1*	+++	Yes
426	$attC_{dfrA1}$	**GTTAAAC** + *aadA1*	**G**TTAGAT	*qacEΔ1*	−	No
444	$attC_{dfrA1}$	**GTTAGGG** + *accA1a-orfG* + *orfH*	**G**TTAGGC	*orf1*	+++	Yes
430	$attC_{dfrA1}$	**GTTAGGC** + *sat*	**G**TTAAAC	*aadA1*	−	ND
445	$attC_{aacA1a\text{-}orfG}$	**GTTAAAC** + *aadA1*	**G**TTAGAT	*qacEΔ1*	+++	Yes
446	$attC_{blaimp}$	**GTTAGGG** + *accA1a-orfG* + *orfH*	**G**TTAGGC	*orf1*	+	Yes
431	$attC_{aacA4}$	**GTTAGCC** + *pse1*	**G**TTAGAC	*aadA2*	+	Yes

[a] Gene cassettes and their associated *attC* sites (core site in bold type) were cloned into pACYC184 and contained the GTTRRRY recombination consensus sequences of various *attI* or *attC* sites. One cassette, *aacA1a-orfG*, was always cloned in tandem with *orfH.* Right-hand neighbors (*qacEΔ1*, *orfI*, *aacA4*, *aadA1*, *aadA2*) were not complete cassettes and were not excised by the integrase.

[b] −, no excision; +, weak excision (<20%); ++, moderate excision (20 to 75%); +++, strong excision (>75%); ND, not determined.

grations of the excised cassettes into the $attI_{Neu}$ site. Sequencing amplicons from some of the integration tests and comparison with pINT-2 and the donor clones confirm that the excised cassettes were integrated specifically into the G/TTRRRY consensus site of the $attI_{Neu}$ site (Fig. 4).

IntINeu is able, like IntI1, to excise cassettes integrated in a heterologous *attI* site, but this ability is limited to easily excisable cassettes such as *aacA1a-orfG* and *orfH*. In accordance with the excision results from IntISon (7) and with the first three classes of integrases (6, 11), our data confirmed that the efficiency of excision depends not only on the cassette's own *attC* site but also on the neighboring left-hand site (*attC* or *attI*). Certain *attC* sites have a negative effect on excision when in either position. The $attC_{dfrA1}$ to the left of several cassettes (in pLQ426, 444, and 430) and as part of its own *dfrA1* cassette (clone pLQ437) is an example of this effect, as only one clone, with the *aacA1a-orfG+orfH* cassettes bounded by this $attC_{dfrA1}$, was excised. Excision of the *dfrA1* cassette itself was not observed. The first few bases of the right-hand neighbor of the cassette may also influence the level of excision. These bases correspond to the last few bases of the core site (TTRRRY) of the *attC* site of the excisable cassette and vary according to the right-hand neighbor (Gagnon and Roy, unpublished).

Sequence comparison with other tyrosine recombinases. A characteristic of integron integrases, permitting them to form a specific clade within the tyrosine recombinase family, is an extra domain, implicated in the recognition of the *attI* and *attC* sites, near Patch III of the family (16). This domain is absent from the XerC and XerD recombinases of *E. coli* and from other tyrosine recombinases. Comparison with 16 integron integrase sequences identified to date allowed us to construct a dendrogram (Fig. 5A). IntINeu is not closely related to chromosomal integron integrases such as IntI4 (from the superintegron of *V. cholerae*), IntISon (from *S. oneidensis*), IntIGsu (from *G. sulfurreducens*), or IntITde (from *T. denticola*). However, several superintegron integrases, like IntIPal (from *P. alcaligenes*) (25), IntIXca (from *Xanthomonas campestris* pathovar campestris) (23), and IntI6, IntI7, and IntI8 (from uncultured bacteria) (17), are the closest relatives of IntINeu, with up to 60% amino acid identity. Also, other integron integrases form separate branches within the integron integrase clade.

Excision tests on IntI1, IntI2, and IntI3 (Gagnon and Roy, unpublished) on IntISon (7) and on IntINeu show similar patterns of excision levels for IntINeu, IntI1, and IntI3. A distinct pattern was observed for IntI2 and IntISon, which are closely related in sequence, and which also possess similar *attI* sites (7).

The IntINeu sequence is closest to IntIPal, which is associated with the superintegron of *P. alcaligenes*. This superintegron possesses a cluster of ORFs, but each ORF is associated with smaller recombination elements (*P. alcaligenes* repeats [PAR] of 75 to 89 bp in length) than V. cholerae repeats (25).

Sequence comparison with other *attI* recombination sites. For excision and integration of cassettes, integrases act preferentially at specific sequences designated *attI* attachment sites. The sequences of *attI* sites are not closely related to each other. A multiple sequence alignment of the *attI* sequences identified to date, using the 70 bp up to the consensus site, including the GTT, allowed us to construct a dendrogram of 13

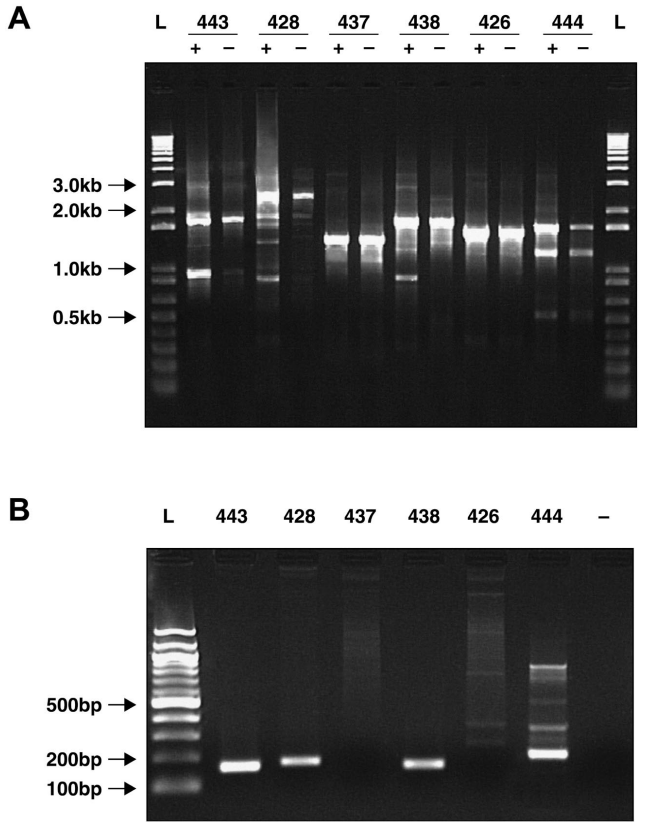

FIG. 3. PCR amplification results after induced excision or integration of cassettes by the IntINeu integrase. (A) Example of results obtained from the excision tests. Six clones were tested with the IntINeu integrase induced (+) or not induced (−) (see Table 2 for description). A positive excision gives, in the induced sample, one or more bands that are smaller than in the noninduced sample. L, DNA molecular weight marker (1kb plus; Gibco BRL). (B) The same clones from the excision test used in integration tests. Integration was confirmed by a positive amplification. L, DNA molecular weight marker (100 bp; Gibco BRL); −, negative PCR control (see Table 1 for the primer pairs used in these two tests).

attI sequences (Fig. 5B). Interestingly, the results obtained with the *attI* sites yield a dendrogram comparable to that of the tyrosine recombinases, except that with the former there are three major groups formed. The IntINeu recombination site ($attI_{Neu}$) is most closely related to the IntIPal recombination site ($attI_{Pal}$), with which it forms a separate group within the chromosomal integron *attI* sites ($attI_{Pal}$, $attI_{Xca}$, and $attI_{Gsu}$). A distinct group is formed by the *attI* sites of integrons and superintegrons of *Vibrio* and related species (*attI4*, $attI_{Lpe}$, *attI9*, and $attI_{Vsa}$). As with the IntI2 and IntISon integrases,

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attINeu       CCTAGAAAGGTGCCTACTTAACGTTAGGCTTACAGAGTCAGGAGCAGATATGAGC
              ||||||||||||||||||||||||||||||  ||  |  |           |
integration   CCTAGAAAGGTGCCTACTTAACGTTAGGGCGACGCCGCTATTGCGGCGCGAATAC
              |   |  ||         | ||||||||||||||||||||||||||||||||||
aacA1a-orfG   CGCGGCGCGGCTTAACTCAAGCGTTAGGGCGACGCCGCTATTGCGGCGCGAATAC
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FIG. 4. Sequence alignment confirming site-specific integration of the *aacA1a-orfG* cassette from pLQ428 into the consensus $attI_{Neu}$ site GTTRRRY. A sequence from the amplicon obtained from the integration test is compared to the sequences of the $attI_{Neu}$ site (pINT-2) and the donor clone (containing the cassettes).

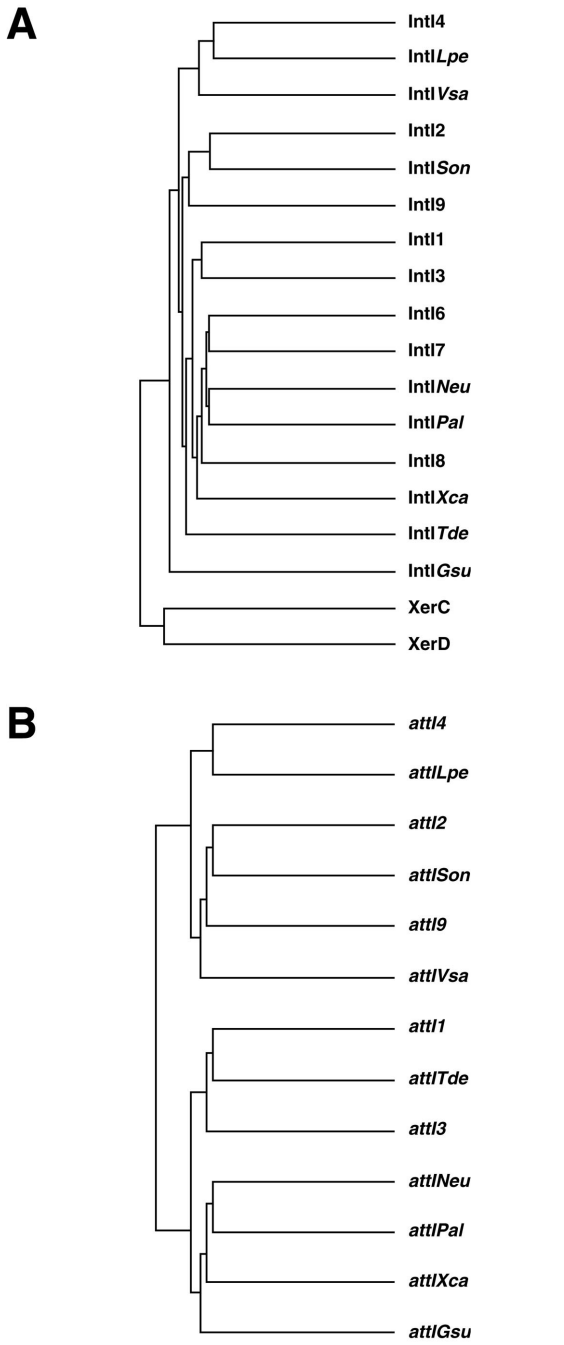

FIG. 5. Phylogenetic relationships between integron integrase genes (A) and between *attI* sites (B) among the proteobacteria. Dendrograms are based on known *intI* gene and *attI* site sequences. IntI1 and *attI1*, class 1 integrase and *attI* site; IntI2 and *attI2*, class 2 integrase and *attI* site; IntI3 and *attI3*, class 3 integrase and *attI* site; IntI4 and *attI4*, class 4 integrase and *attI* site; IntI6, 7, and 8, class 6, 7, and 8 integrases (uncultured bacteria); IntINeu and $attI_{Neu}$, integrase of *N. europaea* and *attI* site; IntISon and $attI_{Son}$, integrase of *S. oneidensis* and *attI* site; IntIGsu and $attI_{Gsu}$, integrase of *G. sulfurreducens* and *attI* site; IntITde and $attI_{Tde}$, integrase of *T. denticola* and *attI* site; IntIXca and $attI_{Xca}$, integrase of *X. campestris* pv. campestris and *attI* site; IntIPal and $attI_{Pal}$, integrase of *P. alcaligenes* and *attI* site; IntIVsa and $attI_{Vsa}$, integrase of *V. salmonicida* and *attI* site; IntILpe and $attI_{Lpe}$, integrase of *L. pelagia* and *attI* site; XerC and XerD, recombinases from *E. coli*.

their respective recombination sites, *attI2* and $attI_{Son}$, are very similar, correlating with the similar excision-integration results obtained with these two integrases (7).

The finding of superintegrons in environmental strains such as *V. cholerae* (15), *P. alcaligenes* (25), and *S. oneidensis* (7) supports the hypothesis of an environmental origin of multiresistant integrons (23) and is consistent with the hypothesis that integrons are a common feature of bacterial populations. However, small chromosomal integrons, such as those of *N. europaea*, *S. oneidensis*, and *G. sulfurreducens*, rather than superintegrons may have been a source of the integrases and *attI* sites of multiresistance integrons.

We thank the Joint Genome Institute (JGI) for the genome sequence of *N. europaea.* For the phylogenetic analysis, the integrase and the *attI* sequences were obtained from EMBL or GenBank, except for *G. sulfurreducens*, *S. oneidensis*, and *T. denticola* (obtained from The Institute for Genomic Research) and *N. europaea* (obtained from JGI). The sequences were compared by using the Genetics Computer Group program Pileup.

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