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Isolation and Characterization of a Novel Insertion Sequence Element, IS*1248*, in *Paracoccus denitrificans*

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A new suicide vector, pRVS3, was constructed to facilitate gene replacements in the genome of *Paracoccus denitrificans*. In control experiments, incorporation of this suicide vector into the genome did not depend on the presence of homologous DNA. Using appropriate restriction enzymes, the suicide vector and flanking DNA were recovered from the genomic DNA. Sequence analysis demonstrated that both up- and downstream of the ex-integrant vector there was an element that showed high homology with bacterial insertion sequences (IS). Southern blot analysis of wild-type and integrant strains revealed that at least four copies of this IS element reside in the *P. denitrificans* genome, one of which, designated IS*1248*, had been involved in the transpositional event described here. IS*1248* is 830 bp long, has 13-bp imperfect inverted repeats at the borders, and contains five open reading frames. With respect to the organization and primary sequences of the open reading frames, IS*1248* closely resembles IS*869* and IS*427* of *Agrobacterium tumefaciens*, IS*402* of *Pseudomonas cepacia*, and IS*myco* found in *Mycobacterium tuberculosis*.

Paracoccus denitrificans is a gram-negative aerobic soil bacterium able to grow under a variety of conditions. During aerobic heterotrophic growth, its respiratory chain strongly resembles that of mitochondria in terms of the enzymes involved in electron transport (John and Whatley, 1977; Vignais *et al.*, 1981). This feature has made *P. denitrificans* an attractive organism for studies on the elucidation of the fundamental process of electron transport-driven free-energy transduction in biological systems. The organization of several electron transport routes in *P. denitrificans* has been elucidated, and the control of environmental signals on the expression of the different operating branches is beginning to become understood. One of the most successful methods has been the biochemical and physiological analysis of respiratory mutants, generated through gene replacement (Gerhus *et al.*, 1990; Raitio *et al.*, 1990; Van Spanning *et al.*, 1990, 1991; De Boer *et al.*, 1994; De Gier *et al.*, 1994). The construction of suicide vectors, able to introduce marked and unmarked mutations into the genome of *P. denitrificans*, has been described (Van Spanning *et al.*, 1990, 1991). However, in most cases suitable for the cloning of mutated target genes, these vectors have a limited number of cloning sites. In order to facilitate cloning strategies, a new suicide vector derived from the pBluescript II (KS) phagemid, was constructed.

We here report that this vector integrated into the genome of *P. denitrificans* by itself through the action of an insertion sequence (IS)[2] element. IS elements have been described for a number of organisms (Galas and

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[2] Abbreviations used: IS, insertion sequence; BHI, brain–heart infusion broth; IHF, integration host factor.

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TABLE 1

BACTERIAL STRAINS AND PLASMIDS

Strain or plasmid	Relevant characteristics[a]	Source of reference
Bacteria		
E. coli		
S17-1	Sm^r, *pro*, r$^-$, m$^+$, RP4-2 integrated (Tc::Mu)(Km::Tn7)	Simon *et al.* (1983)
TG1	*supE, hsdΔ5, thi, Δ(lac-proAB)*, F′, (*traD36 proAB lacIq, lacZ ΔM15*)	Sambrook *et al.* (1989)
P. denitrificans		
Pd1222	DSM 413, Rif^r, Spec^r, enhanced conjugation frequencies	De Vries *et al.* (1989)
PdX13	Pd1222, pLOT1 integrated in IS*1248* locus	This study
Plasmids		
pBluescript II (KS$^+$)	*oriV* (colEI), Amp^r, *lacZ′*	Stratagene
pBSorII	pBluescript II (KS$^+$), *oriT* (RK2)	This study
pUC4K	Km^r (Tn*903*)	Pharmacia
pGRPd1	*oriV* (colEI), *oriT*, Amp^r, Sm^r (Tn*1831*)	Van Spanning *et al.* (1990)
pRVS3	*oriT* (RK2), *Km^r* (Tn*903*), pBSII (KS) derivative	This study (Fig. 1)
pLOT1	*cyo* locus *E. coli*, pRVS3 derivative	This study (Fig. 1)
pXSP13	IS*1248 Sph*I region, pLOT1 derivative	This study (Fig. 3)
pXSS13	IS *1248 Sst*I region, pLOT1 derivative	This study (Fig. 3)
pXBC13	IS*1248 Bcl*I region, pLOT1 derivative	This study (Fig. 3)

[a] Sm, streptomycin; Km, kanamycin; Rif, rifampicin.

Chandler, 1989). These elements are small, their sizes ranging from 800 to 2000 bp, have inverted repeats at the borders, and their target sites are duplicated upon transposition. Most IS elements contain overlapping open reading frames, one of which encodes the transposase function. Generally, more than one copy of the same IS resides on the genome. Of the host factors that can affect transposition, the involvement of integration host factor has been well documented (Galas and Chandler, 1989).

This paper describes the isolation and sequence of a new IS element, IS*1248*, from *P. denitrificans* and its role in integration of foreign DNA, most probably via cointegrate formation.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Growth Conditions

The strains and plasmids used are listed in Table 1. *Escherichia coli* and *P. denitrificans* strains were grown aerobically at 34°C in batch cultures on brain–heart infusion broth (BHI). When necessary, antibiotics were added to final concentrations of 40 μg of rifampin per milliliter, 25 μg of kanamycin per milliliter, 25 μg of streptomycin per milliliter, and 50 μg of ampicillin per milliliter.

DNA Manipulations

Cloning was carried out essentially as described by Maniatis *et al.* (1982). Plasmid DNA was isolated from *E. coli* by the cleared-lysate method (Van Embden and Cohen, 1973) and purified by the use of Qiagen. For rapid screening, plasmid DNA was isolated by the alkaline lysis method (Maniatis *et al.*, 1982). Chromosomal DNA of *P. denitrificans* was isolated as described (Van Spanning *et al.*, 1990). DNA restriction fragments were purified from agarose gels by using GeneClean (Bio 101, Inc., San Diego, CA). Digested chromosomal DNA (5 μg per lane) was electrophorezed on 1% agarose gels, denatured, and transferred to posi-

tively charged nylon membranes (Boehringer-Mannheim) according to the method of Southern (1975). Southern analysis of chromosomal restriction fragments involved random-primed DNA labeling of cloned sequences with digoxigenin and subsequent detection of hybrids by an enzyme immunoassay according to the protocol of the manufacturer (Boehringer GmbH, Mannheim, Federal Republic of Germany). Conjugations were carried out by streaking cells of donor and recipient strains on BHI plates. After 1 day of incubation at 30°C, cells were collected and plated on selective plates. Sequence reactions were performed on single-stranded M13mp18 and mp19 clones using the dye-primer and dye-terminator cycle kits from ABI and loaded on a ABI 373A fluorescent sequencer (Applied Biosystems, Perkin–Elmer).

Analyses

The Macintosh computer programs used were DNA Strider 1.0 and GeneWorks 2.2.1. The e-mail servers of NCBI running the BLAST program (Altschul *et al.*, 1990), the FASTA and BLITZ servers at Heidelberg, and the BLOCKS server were used for sequence comparisons.

Nucleotide Sequence Accession Number

The nucleotide sequence of IS*1248* and its flanking regions is part of a sequence that has been assigned GenBank Accession No. U08864.

RESULTS

Construction of Suicide Vector pRVS3 and Integration of pRVS3 Derivatives into the Genome of P. denitrificans

A map of suicide vector pRVS3 is shown in Fig. 1. During transfer experiments, it was observed that the vector alone, i.e., without insert, integrated into the genome of *P. denitrificans*. The molecular mechanism respon-

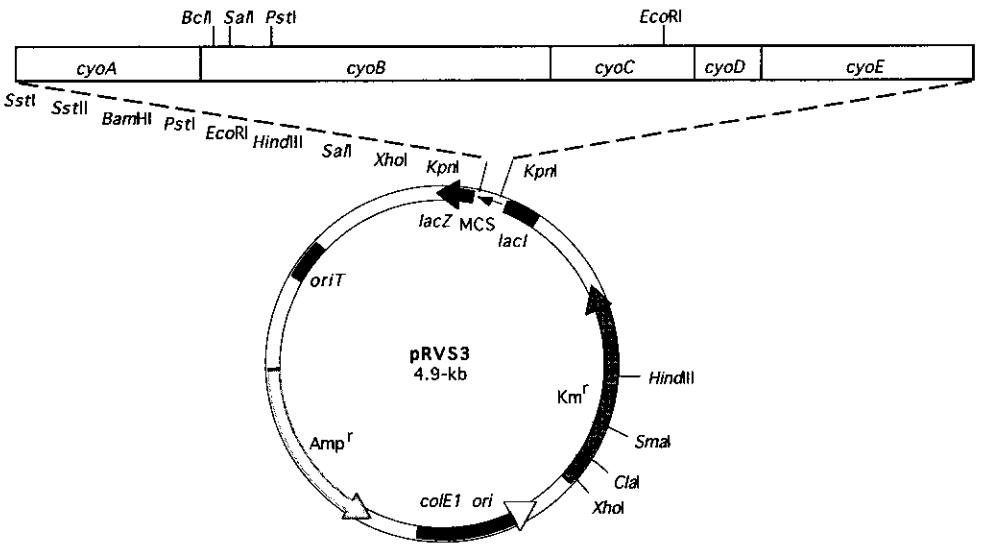

FIG. 1. Map of suicide vector pRVS3. For construction of pRVS3, the pBluescript II (KS$^+$) vector was modified by replacement of the *Ssp*I fragment by a 0.6-kb *Hin*cII–*Sma*I fragment of pGRPd1, bearing the origin for conjugative transfer (*oriT*) from the broad-host-range plasmid RK2 (Van Spanning *et al.*, 1990). The resulting plasmid, pBSoriT was then restricted at the unique *Afl*III site, the sticky ends were filled in, and the 1.4-kb fragment of pUC4K, which contains the kanamycin resistance gene from transposon Tn*903*, was inserted. Plasmid pRVS3 has a multiple cloning site with unique *Sst*I, *Xba*I, *Bam*HI, *Pst*I, *Eco*RI, *Eco*RV, *Hin*dII, *Acc*I, *Sal*I, and *Kpn*I restriction sites. A map of the *cyo* insert as it is cloned into pRVS3, resulting in vector pLOT1, is shown at the top.

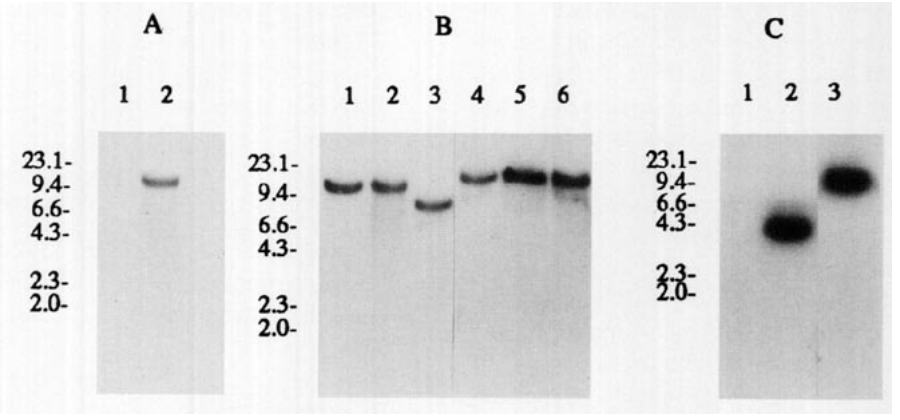

FIG. 2. (A) Southern analysis of *Sph*I-digested chromosomal DNA from Pd1222 (lane 1) and PdX13 (lane 2). DNA was hybridized with labeled pRVS3. (B) Southern analysis of pXSP13 (lanes 1 to 3) and pXSS13 (lanes 4 to 6) digested with *Sph*I (lanes 1 and 4), *Sst*I (lanes 2 and 5), and *Pst*I (lanes 3 and 6) with labeled pRVS3 as a probe. (C) Southern analysis of *Eco*RI-digested DNA of plasmids pLOT1 (lane 1), pXSP13 (lane 2), and pXSS13 (lane 3). The 0.3-kb *Hin*dIII–*Sph*I fragment of IS*1248* was used as a probe. The positions of the markers are indicated by their relative sizes in kb.

sible for this integration was not obvious as pRVS3 should not contain sequences that allow integration via homologous recombination. In a first approach to understand this unexpected process, pRVS3 was equipped with a 4.5-kb *Kpn*I fragment containing the major part of the *E. coli cyo* locus (Chepuri *et al.*, 1990). The resulting vector was designated pLOT1. The rationale behind this was to introduce extra DNA into the vector, such that its integration could occur outside the essential vector functions. After conjugation of *P. denitrificans* and *E. coli* S17-1 harboring pLOT1, and plating the conjugation mixture on selection plates, kanamycin-resistant colonies of *P. denitrificans* appeared at frequencies of about 10^{-6}. One of these strains, designated PdX13, was isolated for further analysis. Chromosomal DNA from PdX13 was restricted with *Sph*I for Southern analysis with the labeled vector as probe. Hybridization was observed with an 11-kb *Sph*I chromosomal DNA fragment. Since pLOT1 itself does not have a site for *Sph*I, this result demonstrated that pLOT1 had indeed been incorporated into the genome of *P. denitrificans* (Fig. 2A). In order to isolate the integration zone for further analysis, chromosomal DNA from PdX13 was restricted with either *Sst*I, *Sph*I, or *Bcl*I. After circularization, these fragments were used to transform competent *E. coli* TG1. Plasmids isolated from the resulting transformants were designated pXSS13, pXSP13, and pXBC13, respectively. Southern analyses of these plasmids with labeled pRVS3 as a probe showed that these plasmids are indeed pRVS3 derivatives (Fig. 2B). On the basis of restriction enzyme analysis of the three plasmids, the plasmid-borne integration region of PdX13 was mapped in detail (Fig. 3A). As expected, integration of pLOT1 into the *P. denitrificans* genome had occurred at a site in the cloned *E. coli cyo* locus, since this locus was split into two portions flanking the original suicide vector pRVS3. A 0.35-kb *Hin*dIII–*Sph*I fragment of plasmid pXSP13, which is located immediately downstream of integrated pLOT1, was isolated and used as a probe for Southern analysis of *Eco*RI-digested plasmids pXSP13 and pXSS13. As expected, pXSP13 hybridized with a 4.5-kb fragment, but surprisingly hybridization was also observed with a 12-kb fragment of pXSS13, which only contains *P. denitrificans* DNA located upstream of integrated pLOT1 (Fig. 2C).

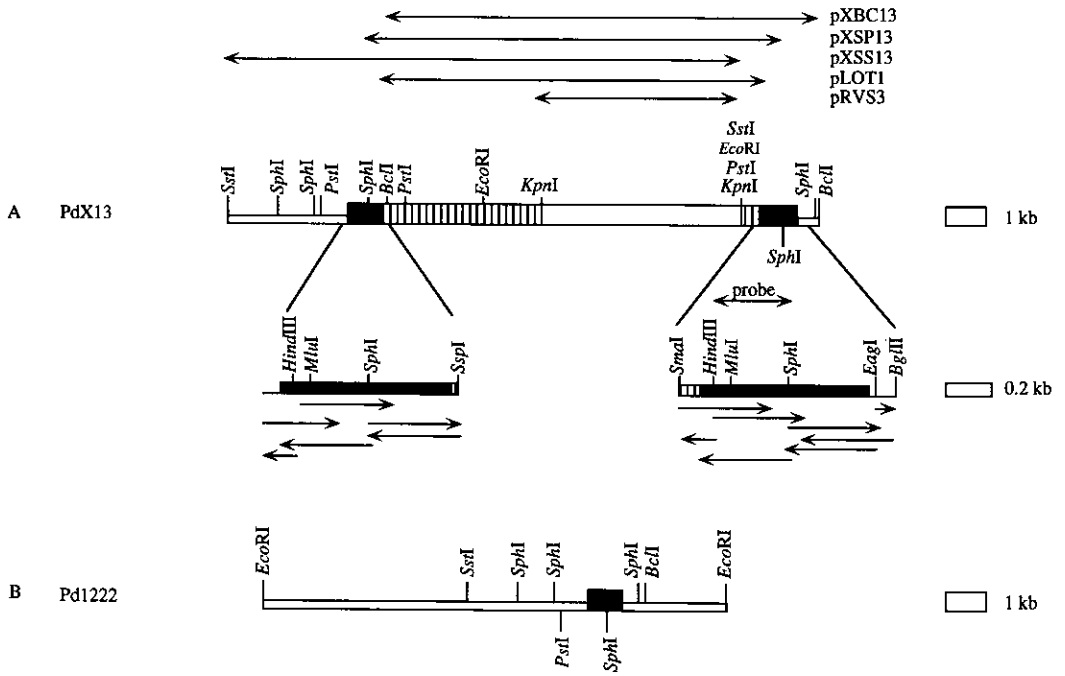

FIG. 3. (A) Map of the IS*1248* integration region of PdX13 and the sequencing strategy. Positions of plasmids pRVS3, pLOT1, pXBX13, pXSP13, and pXSS13 are shown above the map. The location of the probe fragment is as indicated. (B) Map of the wild-type genomic region showing the residence of IS*1248*. Black bars represent IS*1248*, hatched bars represent the *E. coli cyo* locus, and the white bar located between the two IS elements represents vector pRVS3.

Identification and Characterization of IS1248 of P. denitrificans

Fragments of pXSS13, pXSP13, and pXBC13 were subcloned into M13 for sequence analysis. The sequencing strategy is presented in Fig. 3A. A sequence that showed high similarity to a number of IS elements was found both up- and downstream of integrated pLOT1. Apparently, the integration of pLOT1 into the genome of *P. denitrificans* yields donor backbone and vector fused by two copies of the IS element. This is reminiscent of a one-step IS-mediated cointegration. With this in mind, a physical map of the *P. denitrificans* wild-type locus with the resident IS element was deduced (Fig. 3B). The sequence of part of this locus as well as the IS element itself are presented in Fig. 4. The IS element was designated IS*1248*.

The size of IS*1248* is 830 bp. Five partially overlapping open reading frames (ORFs) are each preceeded by possible Shine and Dalgarno sequences (Shine and Dalgarno, 1975). The coding direction of ORFs 1, 2, and 3 is opposite to that of ORFs 4 and 5. The translation start of ORFs 1 and 4 is GUG, while the others start with AUG. The putative proteins encoded by the ORFs are hydrophilic and lacked recognizable structure motifs. The codon usage of ORFs 1 and 3 corresponded with the codon preference established for *P. denitrificans* genes (Steinrücke and Ludwig, 1993), while that for ORFs 2, 4, and 5 deviated strongly. ORF 1 encodes a putative protein of 189 residues with a size of 22.0 kDa and a theoretical isoelectric point (p*I*) of 11. Its primary sequence shows 59 and 63% similarity, respectively, to ORF 1 of IS*869* and ORF 3 of IS*427*, found in *Agrobacterium tumefaciens* (De Meirsman *et al.*, 1990; Paulus *et al.*, 1991). The IS*1248* ORF 1 was also similar to

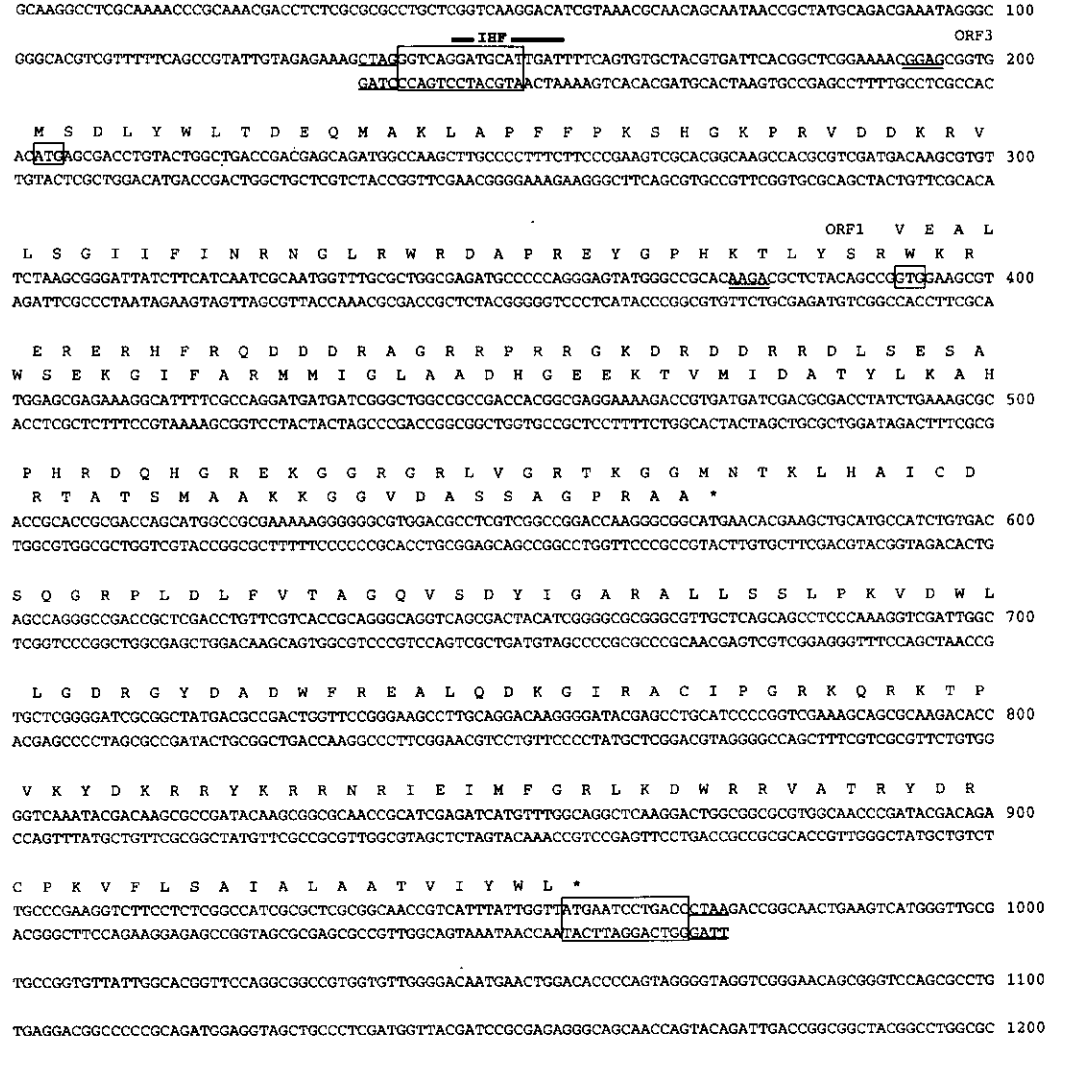

FIG. 4. DNA and deduced amino acid sequences of the chromosomal region containing IS*1248* of *P. denitrificans*. DNA of IS*1248* is presented double stranded. For both strands, the possible target site duplication is underlined. Inverted repeats at either end are boxed. A putative site for binding IHF is presented with a line above the sequence. Shine Dalgarno sequences are doubly underlined. Positions of start codons for the ORFs are boxed for one strand. ORF, open reading frame; IHF, integration host factor.

ORF 2 of IS*402* from *Pseudomonas cepacia* (Ferrante and Lessie, 1991), to the C-terminal part of the single ORF of IS*myco* found in *Mycobacterium tuberculosis* (Mariani *et al.*, 1993), and to ORF 2 of IS*1106* from *Neisseria meningitidis* (Knight *et al.*, 1992), with similarity scores of 69, 56, and 44%, respectively. ORF 3 encodes a putative protein of 123 residues with a size of 13.9 kDa and a p*I* of 10.67. The protein has high similarity to ORF 3 of IS*869* and ORF 4 of IS*427* from *A. tumefaciens* (De Meirsman *et al.*, 1990; Paulus *et al.*, 1991), with similarity scores of 66 and 67%, respectively. Similarity of IS*1248* ORF

3 was also found with respect to the ORF 1 of IS*402* from *Ps. cepacia* (Ferrante and Lessie, 1991), to the N-terminal part of the single ORF of IS*myco* from *M. tuberculosis* (Mariani *et al.,* 1993), to ORF 3 of Tn*4811* from *Streptomyces lividans* (Chen *et al.,* 1992), to the single ORF of IS*Rm4* of *Rhizobium meliloti* (Soto *et al.,* 1992), and to the single ORF of IS*1031* of *Acetobacter xylinum* (Coucheron, 1993). No apparent sequence similarities with the international data bases were found for ORFs 2, 4, and 5. With respect to the size of the elements and the organization and primary sequences of the ORFs, IS*1248* strongly resembles IS*869* from *A. tumefaciens.* To a lesser extent, resemblance is found with IS*427* from *A. tumefaciens,* IS*402* from *P. cepacia,* and IS*myco* from *M. tuberculosis.* An alignment of the amino acids of ORFs 1 and 3 of IS*1248* with those of the corresponding ORFs from IS*869* and IS*427* is shown in Fig. 5. An overview of the organization of ORFs in these elements is presented in Fig. 6.

At the 5′ and 3′ ends of IS*1248,* 13-bp inverted repeats with a single mismatch were found. Comparison of the inverted repeats with those present in the closely related elements IS*869,* IS*427,* and IS*402* (De Meirsman *et al.,* 1990; Ferrante and Lessie, 1991; Paulus *et al.,* 1991) revealed the motif GNNTCATAA. The inverted repeats of IS*1248* are flanked by 4-bp imperfect direct repeats with up- and downstream sequences 5′-CTAG-3′ and 5′-CTAA-3′, respectively. At the 5′ border of IS*1248,* the sequence GANNNNTTGAT, which has only one mismatch with the consensus sequence of the binding site for the integration host factor (IHF), was found.

From the sequence of the PdX13 integration region it was deduced that vector pLOT1 had integrated at the site 5′-CTAG-3′ at position 1562 of the *cyo* sequence as published by Chepuri *et al.* (1990). A comparison of this integration region with the one deduced for the *P. denitrificans* genome revealed the presence of short stretches of similar sequence and several areas of high AT enrichment. A search in the *E. coli cyo* locus revealed a sequence located 58 bp downstream of the putative target site that has only one mismatch with the consensus sequence of the IHF binding site 5′-AANNNNTTGAT-3′ (Gamas *et al.,* 1987).

Multiple Copies of IS1248 Reside in the Genome of P. denitrificans

Southern analysis of genomic DNA both of the wild-type strain and of PdX13 is shown in Fig. 7. The internal 0.35-kb *Hin*dIII–*Sph*I fragment of IS*1248* was used as a probe. Both with *Eco*RI and *Sph*I, hybridization was observed with at least four restriction fragments from the wild-type strain. IS*1248* is located on a 12-kb *Eco*RI fragment in the wild-type strain. Probably as a result of the insertion of pLOT1, the 12-kb *Eco*RI fragment has disappeared and two new bands of 4 and 11 kb are found in PdX13. The *Sph*I pattern of PdX13 reflects the appearance of pLOT1 in the genome. Compared to Pd1222, the integrant strain contains an additional 10.5-kb segment composed of pLOT1 and the left- and right-hand side portions of two copies of IS*1248.*

DISCUSSION

The results described in this paper demonstrate the presence of an IS element, IS*1248,* in the genome of *P. denitrificans.* At least four copies of this element reside in the genome. A newly constructed suicide vector pLOT1 integrated into the *P. denitrificans* genome, presumably via IS*1248,* promoted cointegrate formation. As a result, the foreign DNA was trapped between two IS*1248* copies in a transposon-like organization. IS*1248* is relatively small and with respect to the organization of ORFs and primary sequences of the deduced proteins, it appears to be a member of a family of IS elements that include IS*869* and IS*427* of *A. tumefaciens* (De Meirsman *et al.,* 1990; Paulus *et al.,* 1991), IS*402* of *Ps. cepacia* (Ferrante and Lessie, 1991), and IS*myco* of *M. tuberculosis* (Mariani *et al.,* 1993).

ORFs 1 and 3 of IS*1248* have the potential to encode proteins with high p*I* values. Since many transposase proteins characterized thus

A

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ORF3 IS1248     MS-DLYWLTDEQMAKLAPFFP-KSHGKPRVDDKRVLSGIIFINRNGLRWR    48
ORF3 IS869      MTRRRFDLTDFEWTVIQPLLPNKPRGVPRVDDRRVINGILWRFRTGSPWA    50
ORF4 IS427      MS--RYDLTDFEWRVIEPLLPNKPRGVPRVDDRRVLNGIFWVLRSGAPWR    48
Consensus       M*...*.LTD.*...*.P..P.K**G.PRVDD*RVL.GI.*..R.G.PW.    50

ORF3 IS1248     DAPREYGPHKTLYSRWKRWSEKGIFARMMIGLAADHGEEKTVMIDATYLK    98
ORF3 IS869      DVPDRYGPYTTCYNRFVRWRKAGVWDHVLGEISKAFDGD-IVMIDSSCVR    99
ORF4 IS427      DLPERYGPRTTCYNRFIRWRKAGVWDRMMDAITAAYDGD-IQMIDSTSVR    97
Consensus       D.P..YGP..T.Y.R*.RW...G**.***..**.....*...MID**.**   100

ORF3 IS1248     AHRTATSMAAKKGGVDASSAG-PRAA                            123
ORF3 IS869      VHQHAAT--GKRGINTMAAWDVPVAA                            123
ORF4 IS427      AHQQAAT--AKRGIEIIVSA-VPEAGSRPKSTRSSMGRVSRSG           137
Consensus       .H..A**..*K*G.....*...P.A*                            142
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B

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ORF1 IS1248     VEALERERHFRQDDDRAGRRPRRGKDRDDRRDLSESAPHRDQHGREKGGR    50
ORF1 IS869         MAKGGCLGSCSGRDFQGFRR-RYRYDRQLLCPCSSTCG-HGKKGDKH    45
Consensus          ..*.........R.....RR-*.R.DR..L...**...-HG*....*    50

ORF1 IS1248     GRLVGRTKGGMNTKLHAICDSQGRPLDLFVTAGQVSDYIGARALLSSLPK   100
ORF1 IS869      DGCMGRSRGGLTTKIHAVVDADGRPIRLALTAGQAHDGRMAEPLLQTISK    95
ORF3 IS427                                                    VGA     3
Consensus       ...*GR**GG*.TK*HA*.D**GRP*.L.*TAGQ..D...A..LL.***.   100

ORF1 IS1248     VDWLLGDRGYDADWFREALQDKGIRACIPGRKQRKTPVKYDKRRYKRRNR   150
ORF1 IS869      GAILLADKAYDTNAIRAFAKQRQAWANIPAKSNRKGSFPFSQWVYRQRNL   145
ORF3 IS427      GTIVLADKAYDADRIRASLREKGAFANIPPKANRRSKPYFSTWLYRERNL    53
Consensus       ...*L*D**YD**..R....**...A.IP**.*R**...F....Y*.RN.   150

ORF1 IS1248     IEIMFGRLKDWRRVATRYDRCPKVFLSAIALAATVIYWL               189
ORF1 IS869      VERFFSKLKQFRGIATRYDKDPLNFLAAVKLAAVRIWIRSL             186
ORF3 IS427      IERFFSKLKHFRRVATRYDKLAENFLAMVQLASMRLWLRAYESTA          98
Consensus       *E..F**LK.*R.*ATRYD*.*..FL*.*.LA*..*W........         196
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FIG. 5. (A) Alignment of proteins deduced from ORF 3 of IS*1248*, ORF 3 of IS*869*, and ORF 4 of IS*427*. (B) Alignment of proteins deduced from ORF 1 of IS*1248*, ORF 1 of IS*869*, and ORF 3 of IS*427*. The consensus sequence shows the identical residues (one-letter symbols) and chemically related residues (marked by an asterisk). Assumed amino acid relations were as follows: STPAG, NDEQ, HRK, MLIV, FYW, C.

far have high isoelectric points (Galas and Chandler, 1989), these two ORFs are the best candidates for the transposase function in IS*1248*. Interestingly, the related IS element IS*myco* from *M. tuberculosis* (Mariani *et al.*, 1993) contains only a single ORF, which is

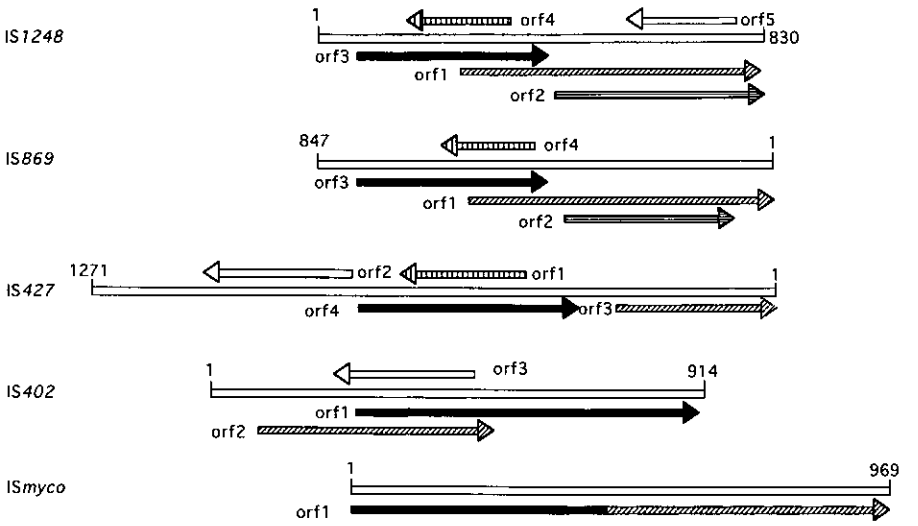

FIG. 6. Comparison of ORF organizations of related IS elements. IS*1248* is from *P. denitrificans* (this study), IS*869* and IS*427* are from *A. tumefaciens* (De Meirsman *et al.*, 1990; Paulus *et al.*, 1991), IS*402* is from *Ps. cepacia* (Ferrante and Lessie, 1991), and IS*myco* is from *M. tuberculosis* (Mariani *et al.*, 1993). The elements are aligned with respect to the start of ORF 3 of IS*1248* and its counterparts. Similar fill patterns are used for homologous ORFs. ORFs with no homology are white. The direction of the arrows reflects the direction of transcription. Numbers at the starts and ends of the elements denote the first and last nucleotides of the respective sequences.

most likely the transposase protein essential for transposition of this element. Part of the deduced protein sequence of IS*myco* is highly homologous with the N-terminus of ORF 3, and a second part with the C-terminus of ORF 1. Apparently, in this element the two ORFs are fused into a unique ORF. A comparable situation is found for IS*1031* of *A. xylinum* (Coucheron, 1993). Whereas the transposase protein thus seems to be encoded by a unique ORF in the latter two IS elements, in the case of IS*1248* of *P. denitrificans*, IS*869* and IS*427* of *A. tumefaciens*, and IS*402* of *Ps. cepacia* this function seems to reside in a joint action of two proteins. Alternatively, a single protein may be translated due to the ribosome slippage. The latter phenomenon occurs in a number of IS elements (Galas and Chandler, 1989).

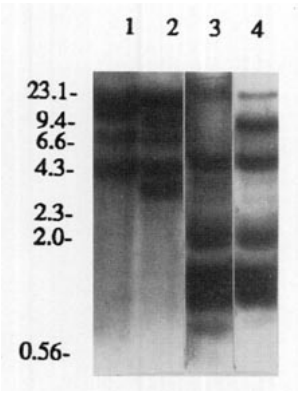

FIG. 7. Southern analysis of chromosomal DNA from the wild-type strain (lanes 1 and 3) and PdX13 (lanes 2 and 4) restricted with *Eco*RI (lanes 1 and 2) or *Sph*I (lanes 3 and 4). The 0.3-kb *Hin*dIII–*Sph*I fragment of IS*1248* was used as a probe. The positions of the markers are indicated by their sizes in kb.

Transposition of IS elements often involves specific features of the donor backbone and of the target DNA (Galas and Chandler, 1989). The DNA in the *P. denitrificans* integration region exhibits some of these features. First, in PdX13 the sequence 5′-CTAG-3′ is found adjacent to either copy of IS*1248* at three of the four positions. This sequence may represent

the duplication of the target site. The sequence 5′-CTAA-3′ is found at the fourth position. Apparently, the original sequence was changed by a mutation and stabilized. Second, sequences within IS*1248* itself, and in the vicinity of its target site, resemble the IHF binding site. IHF has been shown to be involved in stimulation of transposition of a number of IS elements (Galas and Chandler, 1989). Third, within the inverted repeats of IS*1248* a sequence is present that is identical to the sequences found in related IS elements. This sequence may be a recognition site for their transposases.

In terms of amino acid homology, IS*1248* of *P. denitrificans* is closely related to IS elements found in organisms that are evolutionarily remote. *P. denitrificans* itself belongs to the α-3 cluster of the eubacterial phylum of purple bacteria, while *A. tumefaciens* belongs to the α-2 cluster, *Ps. cepacia* to the β-2 cluster, and *M. tuberculosis* even to the eubacterial phylum of gram-positive bacteria (Woese, 1987). Especially the similarity of IS*1248* to IS*869* is striking, suggesting that these IS elements have a common ancestor. IS*869* resides on the conjugative Ti plasmid of *A. tumefaciens*, a plasmid involved in plant tumor induction (Paulus *et al.*, 1991).

Several methods for entrapment of IS elements in bacteria have been developed. One of these concerns a positive selection procedure involving the *B. subtilis sacB* gene (Gay *et al.*, 1985). Another method makes use of the inactivation of the β-galactosidase gene after entrapment of IS elements (Cirillo *et al.*, 1991). Both methods allow the cloning and analysis of transposed copies of IS elements, but lack the possibility of studying the original residences of the elements. Insertion of suicide vector pRVS3 with or without an additional DNA segment into the genome via IS-mediated cointegrate formation allows the rapid cloning and characterization of IS elements and the chromosomal loci where they reside.

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