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Identification of a transposable genomic island of *Paracoccus pantotrophus* DSM 11072 by its transposition to a novel entrapment vector pMMB2

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A novel shuttle entrapment vector, pMMB2, was used to identify a large transposable element, Tn*Ppa1* (44·3 kb), of *Paracoccus pantotrophus* DSM 11072. Tn*Ppa1* has a composite structure with divergently oriented copies of a cryptic transposon, Tn*3434* (Tn*3* family), located at both termini. The core region of the element contains a large set of putative genes, whose products show similarity to enzymes involved in central intermediary metabolism (e.g. tricarboxylic acid cycle or 2-methylcitrate cycle), transporters, transcriptional regulators and conserved proteins of unknown function. A 4·2 kb DNA segment of Tn*Ppa1* is homologous to a region of chromosome cII of *Rhodobacter sphaeroides* 2.4.1, which exemplifies the mosaic structure of this element. Tn*Ppa1* is bordered by 5 bp long directly repeated sequences and is located within a mega-sized replicon, pWKS5, in the DSM 11072 genome. Spontaneous inversion of the core region of Tn*Ppa1* was detected in the host genome. Analysis of the distribution of Tn*Ppa1* in three other strains of *P. pantotrophus* revealed that this element was present exclusively within DSM 11072, which suggests its relatively recent acquisition by lateral transfer. The identification of Tn*Ppa1* (which may be considered a transposable genomic island) provides evidence for the transposition and lateral transfer of large DNA segments of chromosomal origin (carrying various housekeeping genes), which may have a great impact on the evolution of bacterial genomes.

INTRODUCTION

Sequencing projects have revealed that bacterial genomes are not monolithic structures. They can contain integrated transposable elements (TEs), integrons, plasmids and bacteriophages, as well as genomic islands (GEIs) and islets, acquired by different lateral transfer events. Identification of these foreign components of the genome mosaic provides evidence supporting genome plasticity and enables prediction of the direction and frequency of lateral gene transfer in the environment.

TEs [insertion sequences (ISs) and transposons (Tns)] are the most abundant mobile elements in bacterial genomes. The majority of ISs and non-composite Tns have been identified as a result of various sequencing projects. However, it is much more difficult to identify functional composite Tns through DNA sequence analysis. Theoretically, all DNA is potentially mobile (Campbell, 2002), because any DNA fragment bordered by two, even different (Ramírez-Romero *et al.*, 2001), TEs, could form a composite Tn. However, whether such DNA segments actually are mobile has to be verified experimentally. Several alternative strategies have been developed for the identification of functional TEs and the detection of their transposition activity. These strategies employ various entrapment vectors, which are convenient tools enabling the direct identification of even phenotypically silent elements (reviewed by Solyga & Bartosik, 2004).

In previous studies we have performed analyses aimed at the identification and characterization of TEs in several strains (DSM 11072, DSM 11073, DSM 65 and LMD 82.5) of a facultative chemolithoautotroph *Paracoccus pantotrophus* as well as in *Paracoccus solventivorans* DSM 11592 (Bartosik *et al.*, 2003a, b). These strains show great physiological heterogeneity, which might result from the presence of various metabolic Tns. Through the application of positive-selection entrapment vector pMEC1 (which carries a *cI–tetA* selective cartridge) we showed that TEs are abundant in *Paracoccus* spp. We identified eight novel ISs: IS*Ppa1*, IS*Pso1* (IS*256* family), IS*Ppa2*, IS*Ppa3*, IS*Ppa4*, IS*Pso2*, IS*Pso3* (IS*5* family) and IS*Ppa5* (IS*66* family), as well as two closely related Tns of the Tn*3* family, cryptic Tn*3434* (DSM 11072) and streptomycin-resistant Tn*5393* (LMD 82.5) (Bartosik *et al.*, 2003a, b).

Abbreviations: GEI, genomic island; IPCR, inverse PCR; IS, insertion sequence; TE, transposable element; Tn, transposon.

The GenBank/EMBL/DDBJ accession number for the sequence reported in this paper is DQ149577.

The size of the identified TEs [400 tetracycline-resistant (Tc^R) clones analysed] did not exceed 5·3 kb (Tn*5393*) (Bartosik *et al.*, 2003a). This raised the question whether the studied strains contained Tns of larger size and whether the tool used was not suitable for their capture. We speculated that the replicator region of pMEC1, derived from a small plasmid pWKS1 (2·7 kb) of *P. pantotrophus* DSM 11072, might be unable to maintain larger plasmid genomes carrying inserted Tns. To circumvent this potential limiting factor we have constructed a modified version of pMEC1 (pMMB2), carrying a megaplasmid-specific *repABC*-type replicon.

In the present study, we describe the identification and characterization of a novel large composite TE, Tn*Ppa1* (44·3 kb), isolated from *P. pantotrophus* DSM 11072 by its transposition to the entrapment vector pMMB2.

METHODS

Bacterial strains, plasmids and culture conditions. The bacterial strains and plasmids used in this study are listed in Table 1. All strains were grown in Luria–Bertani (LB) medium at 30 °C (*Paracoccus* spp.) or 37 °C (*Escherichia coli*). Where necessary, the medium was supplemented with antibiotics at the following concentrations: kanamycin 50 μg ml^{-1}, rifampicin 50 μg ml^{-1} and tetracycline 0·5 μg ml^{-1}.

DNA manipulations. Plasmid DNA was isolated as described by Birnboim & Doly (1979) and when required, purified by CsCl–ethidium bromide gradient centrifugation. Megaplasmid visualization was achieved by in-gel lysis and DNA electrophoresis, as described by Wheatcroft *et al.* (1990). Total DNA from *P. pantotrophus* strains was isolated by phenol extraction (Williams *et al.*, 1998). Common DNA manipulation methods were performed as described by Sambrook & Russell (2001). For Southern hybridization, DNA probes were labelled with DIG (Roche). Hybridization and visualization of bound probes were carried out as recommended by the supplier.

Construction of entrapment shuttle vector pMMB2. The construction of the shuttle entrapment vector was performed in *E. coli* TG1 in two steps. The *E. coli*-specific mobilizable vector pABW1 (Bartosik *et al.*, 1997) was digested with *Pst*I and ligated with the 2·9 kb entrapment cartridge from pGBG1 (Schneider *et al.*, 2000). The resulting plasmid (pMMB1) was digested with *Xba*I and *Kpn*I and ligated with a 5·6 kb linear form of the *repABC*-containing mini-replicon pTAV320 (Bartosik *et al.*, 1998), recovered from shuttle plasmid pABW22A (Table 1). The resulting positive selection vector was designated pMMB2 (Fig. 1). The selective cartridge of pMMB2 is composed of a silent Tc^R gene (*tetA*) under the control of the pR promoter of bacteriophage λ and the gene encoding the λ cI repressor. Inactivation of the repressor gene or operator (e.g. through insertion of a TE) results in the constitutive expression of Tc^R.

Introduction of plasmid DNA into bacterial cells. Transformation of *E. coli* TG1 was performed as described by Kushner (1978). Triparental mating experiments were conducted as previously described (Bartosik *et al.*, 2003a). Briefly, overnight cultures (pelleted by centrifugation and washed to remove antibiotics) of the donor strain *E. coli* TG1 carrying the mobilizable vector, the recipient strain *P. pantotrophus* DSM 11072R, and *E. coli* DH5α carrying

Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Characteristics	Reference or source
Strains		
*P. pantotrophus**		
DSM 11072	Contains pWKS1 (2·7 kb), pWKS3 (>400 kb)	Jordan *et al.* (1997)
DSM 11072R	Rif^R derivative of DSM 11072	Bartosik *et al.* (2002)
DBB1	Derivative of DSM 11072R deprived of pWKS3 (megaplasmid removed by incompatibility with a mini-derivative of related replicon pTAV3 of *P. versutus* UW1)	This study
DSM 11073	Contains pKLW1 (~100 kb) and pKLW2 (>400 kb)	Jacobs *et al.* (1996)
DSM 65	Contains pHG16-a (~70 kb) and pHG16-b (>400 kb)	Gerstenberg *et al.* (1982)
LMD 82.5	Contains pPAN1 (~110 kb) and pPAN2 (>400 kb)	Ludwig *et al.* (1993)
E. coli		
TG1	Host strain for plasmids	Gibson (1984)
DH5α	Host strain for pRK2013	Hanahan (1983)
Rhizobium etli CE3	Sm^R	Noel *et al.* (1984)
Plasmids		
pABW1	Km^R; mobilizable cloning vector; ColE1 origin; *oriT* RK2	Bartosik *et al.* (1997)
pTAV320	Km^R; mini-replicon of a composite plasmid pTAV1 of *P. versutus* UW1, containing *repABC* replication/partitioning module	Bartosik *et al.* (1998)
pGBG1	Cm^R; contains a selective cartridge composed of *cI* gene of bacteriophage λ and Tc^R gene under control of *pR* promoter	Schneider *et al.* (2000)
pABW22A	Km^R; derivative of pABW1 carrying *Stu*I linearized pTAV320 cloned within MCS†	This study
pMMB2	Km^R; shuttle vector composed of pABW1, pTAV320 and selective cartridge of pGBG1	This study
pMMB10	Km^R, Tc^R; derivative of pMMB2 with integrated Tn*Ppa1*	This study
pRK2013	Km^R; helper plasmid carrying RK2 *tra* genes	Ditta *et al.* (1980)

*All *P. pantotrophus* strains were shown to carry an additional mega-sized replicon of similar size, >640 kb (data not shown).
†Multiple cloning site.

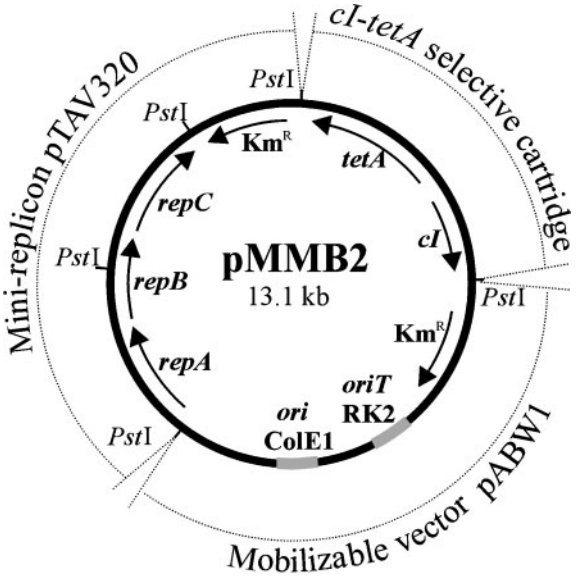

Fig. 1. Structure of the mobilizable shuttle entrapment vector pMMB2.

the helper plasmid pRK2013, were mixed at a ratio of 1 : 2 : 1. A 100 µl aliquot of this mixture was spread on a plate of solidified LB medium. After overnight incubation at 30 °C, the bacteria were washed off the plate and suitable dilutions were plated on selective media containing rifampicin (selective marker of the recipient strain) and kanamycin to select transconjugants. Spontaneous resistance of the recipient strains to kanamycin was undetectable under these experimental conditions.

Isolation of insertion mutants. The entrapment vector pMMB2 was introduced by triparental mating into recipient strain *P. pantotrophus* DSM 11072R. An overnight culture of a kanamycin-resistant (Km^R) transconjugant carrying pMMB2 was spread on plates of solidified LB medium supplemented with tetracycline. Appropriate dilutions of the culture were also spread on tetracycline-free, solidified LB medium in order to determine the frequency of transposition. One hundred and eighty Tc^R colonies were picked and further analysed for plasmid content and restriction pattern. Spontaneous resistance to tetracycline was undetectable under these experimental conditions.

PCR amplification. For identification of the target site of Tn*Ppa1* integrated within pMMB2, five nested pairs of cartridge-specific forward and reverse oligonucleotide primers (ALIS/ARIS, BLIS/BRIS, CLIS/CRIS, DLIS/DRIS, ELIS/ERIS) were used as described by Bartosik *et al.* (2003a). The chromosomal DNA sequences adjacent to Tn*Ppa1* within the *P. pantotrophus* genome were amplified with the following primers: RTN3434 (5′-TCCTCGCCGCCATCATCAT-3′), LTN3434 (5′-GCAGATTGACACAACGACTG-3′) and 147LCD2 (5′-ATCGGCAAGGCAGATTGACC-3′) (see Fig. 3 and Results for details). For detection of the inverted form of Tn*Ppa1*, the following primers were used: RINV (5′-GAGGCCGTCCATGCTCTTGT-3′), LINV (5′-AGAGAGGTCACGTCACGGTC-3′) and LNUC (5′-CGTGTCACCATCAACGAGGC-3′) (see Fig. 3 and Results for details). For amplification of the internal fragment of Tn*3434* (used as a probe in hybridization) the primers L3434 and R3434, previously described by Bartosik *et al.* (2003a), were used. Amplification was performed in a Mastercycler (Eppendorf) using the above synthetic oligonucleotides, *OptiTaq* polymerase (Eurx) (with supplied buffer) and appropriate template DNAs. PCR products were analysed by electrophoresis on 0·8 % agarose gels and, where necessary, purified with a Gel Out kit (A&A Biotechnology) and cloned into the pGEM-T Easy Vector (Promega).

DNA sequencing and analysis. Nucleotide sequences were determined using a dye terminator sequencing kit and an automatic sequencer (ABI377; Perkin Elmer). A combination of vector-derived primers and primer walking was used to obtain the entire nucleotide sequence of Tn*Ppa1*. Sequence analysis was performed using programs included in the UWGCG Package (Devereux *et al.*, 1984). Similarity searches were performed using the BLAST programs (Altschul *et al.*, 1997) provided by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast). The G+C plot was created with the program Artemis (Rutherford *et al.*, 2000) with a window setting of 150 nt.

RESULTS

Identification of Tn*Ppa1*

To facilitate the identification of large Tns of *Paracoccus* spp., a novel mobilizable entrapment vector, pMMB2, was created (Fig. 1; see Methods for details). This plasmid was a fusion of: (i) an *E. coli*-specific vector, pABW1 (Bartosik *et al.*, 1997); (ii) mini-replicon pTAV320, carrying a *repABC*-type replicon originating from low–copy-number plasmid pTAV1 (107 kb) of *Paracoccus versutus* UW1 (Bartosik *et al.*, 1998); and (iii) a *cI–tetA* selective cartridge (Schneider *et al.*, 2000). The functionality of pMMB2 was tested in *P. pantotrophus* DSM 11072R. This strain was chosen for two reasons: (i) it is rich in TEs, and carries IS*Ppa1*, IS*Ppa2*, IS*Ppa3*, IS*Ppa5* and Tn*3434*, elements entrapped by pMEC1 (Bartosik *et al.*, 2003a); and (ii) its natural plasmids are compatible with the *repABC* replicon of pMMB2 (data not shown).

A pool of Tc^R mutants of strain DSM 11072 carrying pMMB2 with inserted TEs of various sizes was identified and analysed. The frequency of Tc^R clones and of transposition was similar to that previously observed with pMEC1 ($2{\cdot}1 \times 10^{-6}$). Among 180 tested Tc^R clones, several classes of plasmids were distinguished carrying: (i) point mutations; (ii) putative co-integrates; (iii) potential ISs and small Tns (inserts of 1–4 kb); and (iv) large Tns (inserts of ~45 kb) (Table 2). Of the tested plasmids, 15 % carried a very large element (~45 kb), which was not previously identified in this strain using pMEC1 (Table 2). DNA restriction analysis revealed that all the clones carried the same element, which was designated Tn*Ppa1* (data not shown). One randomly selected Tc^R clone, carrying a pMMB2-derivative with inserted Tn*Ppa1* (pMMB10), was subjected to further detailed study. PCR analysis with the use of five pairs of nested, cartridge-specific primers confirmed that Tn*Ppa1* had integrated within the *cI* gene. This enabled precise localization of Tn*Ppa1* within the cassette by sequencing of the flanking regions of the element using appropriate primers.

Nucleotide sequence of Tn*Ppa1*

Detailed hybridization, PCR and sequencing analyses revealed the structure of Tn*Ppa1*. Tn*Ppa1* (44 286 bp) was composed

Table 2. Comparison of Tc^R mutants isolated from *P. pantotrophus* DSM 11072 using pMEC1 and pMMB2

Entrapment vector	Frequency of mutations of particular type (%)			
	Putative point mutations*	Putative co-integrates†	Insert 0·8–4 kb	Insert 45 kb
pMEC1	21	0	79	0
pMMB2	7	23	55	15

*Plasmids of the size of the entrapment vector.
†Lacks the autonomous form of the plasmid.

of an ~37 kb DNA segment, placed between two identical, divergently oriented copies of a cryptic, non-composite Tn*3434* (3·7 kb) related to Tn*3* (Bartosik *et al.*, 2003a) (Fig. 2). These terminal elements were designated Tn*3434*L and Tn*3434*R (Fig. 2a). Tn*3434* carried two genes, in divergent orientation, encoding a transposase (*tnpA*) and a resolvase (*tnpR*) bordered by terminal inverted repeats of 35 bp. The two ORFs were separated by a putative recombination site (*res*), which in Tn*3*-like elements is involved in co-integrate resolution and regulation of the *tnpA* and *tnpR* genes (Grindley, 2002) (Fig. 3). Sequencing of the integration site of Tn*Ppa1* revealed that the element had been inserted into an AT-rich sequence within the selective cartridge of pMMB2 (Fig. 2a), which is typical for non-composite members of the Tn*3* family (Grindley, 2002). Upon insertion, it generated a 5 bp duplication of the target sequence 5′-GTGTT-3′.

The overall G+C content of Tn*Ppa1* (62 mol%) was similar to that of *P. pantotrophus* (64–68 mol%), in contrast to the G+C content of Tn*3434*, which was 59 mol% (Fig. 2a). Analysis of the Tn*Ppa1* nucleotide sequence indicated the presence of 38 ORFs (Fig. 2b). Sequence database comparisons identified: (i) a 4·2 kb DNA segment of Tn*Ppa1* (encoding ORFs 32–36) similar to part of chromosome cII of *Rhodobacter sphaeroides* (Table 3); and (ii) two ORFs (18 and 19) homologous to hypothetical genes of *Bordetella bronchiseptica* (Table 3) as well as to the sequences within the GEI of *E. coli* O157 : H7 EDL933 (z0891 and z0890; accession numbers AAG55060 and AAG55059, respectively; data not shown). The presence of these elements exemplifies the mosaic structure of Tn*Ppa1*.

Tn*Ppa1* encodes a number of putative genes (including housekeeping genes) conserved in chromosomes of various bacterial species. The predicted coding regions were dominated by enzymes involved in central intermediary metabolism [e.g. tricarboxylic acid cycle (ORFs 17, 20, 22, 23 and 34–36) or 2-methylcitrate cycle (ORFs 13 and 24)], transporters, transcriptional regulators and conserved proteins of unknown function. A summary of the predicted ORFs of Tn*Ppa1*, including their position, the size of the encoded proteins and their closest homologues, is presented in Table 3.

Identification of the Tn*Ppa1* integration site

The target site of Tn*Ppa1* within the DSM 11072 genome was determined by inverse PCR (IPCR). For this purpose, two Tn*3434*-specific, divergently priming, oligonucleotide primers LTN3434 and RTN3434, were designed (Fig. 3). The template DNA was prepared by *Nru*I digestion of total DNA of strain DSM 11072 followed by ligation of the mixture of DNA fragments. The *Nru*I endonuclease does not cleave Tn*3434*, therefore ligation should result in the circularization of DNA fragments containing entire copies of the Tn, together with adjacent sequences. This template was used in IPCR reactions with primers LTN3434 and RTN3434. Two IPCR products were expected (one for each copy of Tn*3434*), but only one of size 1 kb was obtained. DNA sequencing of the amplified fragment (with the primers used for IPCR) established the presence of the terminal Tn*3434* sequences as well as: (i) an internal sequence of Tn*Ppa1* adjacent to the right copy of Tn*3434*; and (ii) a short genomic sequence, separated by the *Nru*I site created by ligation (Fig. 3).

Possibly the *Nru*I site in genomic DNA flanking Tn*3434*L is located far away from the Tn, which would preclude obtaining a PCR product. Therefore, an additional IPCR experiment was performed, employing a second set of divergently priming primers, RTN3434 and 147LCD2 (Fig. 3), and template DNA of strain DSM 11072 prepared by *Sph*I digestion and ligation. As shown in Fig. 3, a *Sph*I site was present within Tn*3434*. Therefore, the desired restriction fragment contained terminal parts of Tn*3434* together with the adjacent target sequence. This revised strategy allowed amplification and determination of the genomic sequence (277 bp) flanking the left copy of Tn*3434*.

TBLASTX comparison of the amplified genomic DNA sequence with the GenBank protein sequence databases revealed the presence of a truncated ORF (disrupted by insertion of Tn*Ppa1*) with similarity to a number of bacterial nucleases. The highest similarity (~60 %) was observed with a 104 aa product encoded by gene *yci* present on an octopine-type Ti plasmid of *Agrobacterium tumefaciens* (accession no. NP_059691) (data not shown).

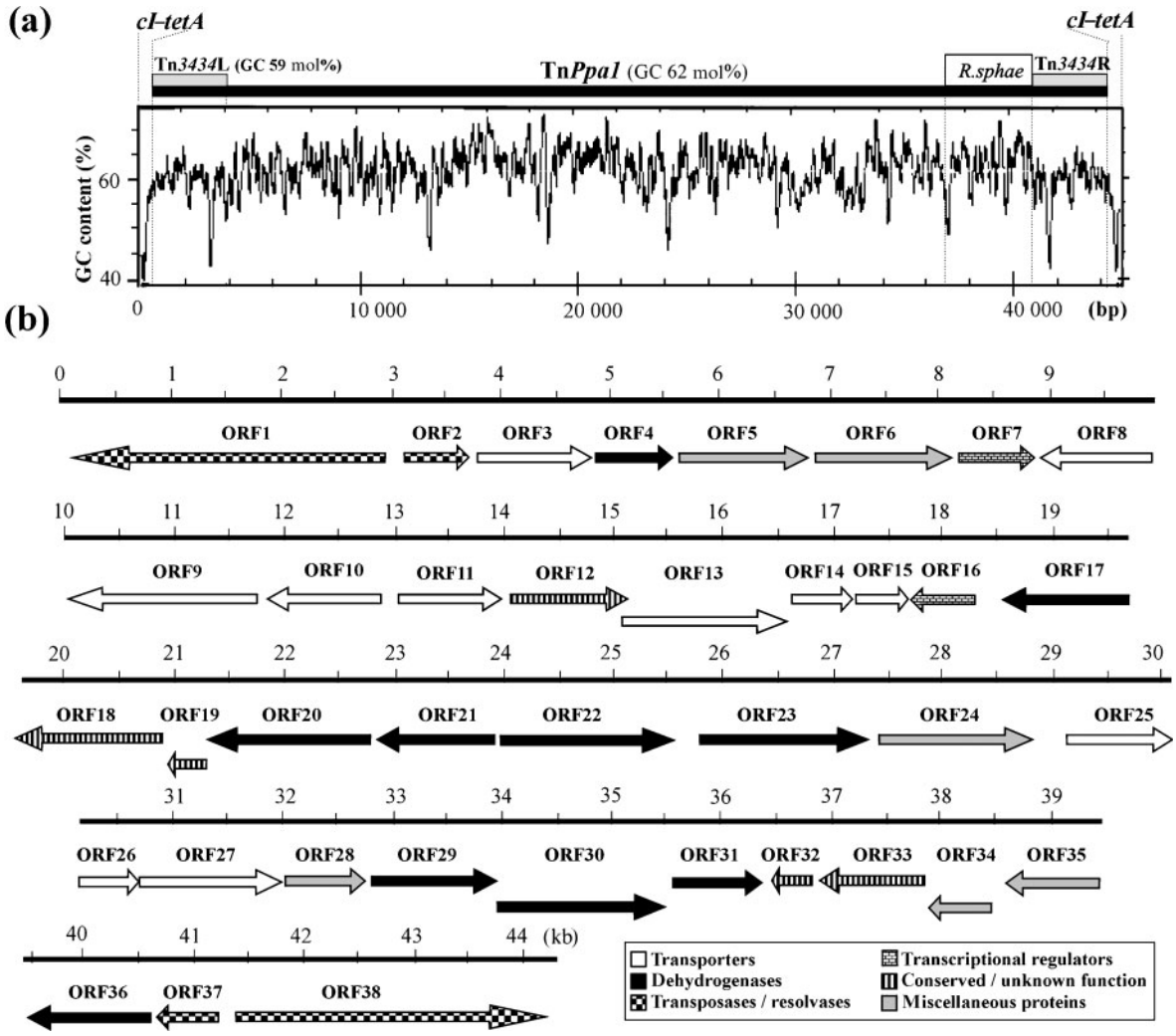

Fig. 2. Genetic organization of Tn*Ppa1*. (a) Plot of the G+C content of the Tn*Ppa1* DNA sequence. The sequence of the Tn*Ppa1* integration site, within the *cI–tetA* selective cartridge of the entrapment vector pMMB2, has been included on both sides of the Tn. The two copies of Tn*3434* are indicated by grey blocks. A DNA segment homologous in sequence and structure to a DNA region on chromosome cII of *R. sphaeroides* is denoted as *R.sphae*. (b) Genetic map of Tn*Ppa1*. Predicted coding regions are shown by arrows indicating the direction of transcription. The genes are marked according to their putative functions (see legend below map). All ORFs are numbered as in Table 3.

Inversion of Tn*Ppa1* in the DSM 11072 genome

When two *res* sites are located on the same DNA molecule in an inverted orientation (as observed in Tn*Ppa1*), recombination between them (mediated by resolvase) should result in the inversion of the flanked DNA segment (Mahillon, 1998). This mechanism may result in the rotation of a huge portion of Tn*Ppa1*. To confirm this possibility we performed PCR with total DNA of strain DSM 11072 as a template, using two sets of primers, LNUC and LINV, and LNUC and RINV, each of which should amplify DNA fragments specific for a different orientation of Tn*Ppa1*. As shown in Fig. 3(a) the former primer pair should amplify a DNA fragment of the sequenced version of Tn*Ppa1*, while with the latter pair a PCR product would be produced only following inversion of the element. With both sets of primers we observed amplicons of the expected size (Fig. 3b). DNA sequence analysis demonstrated that the internal DNA region of Tn*Ppa1* adjacent to the Tn*3434*R, may also lie in the opposite orientation, close to Tn*3434*L (data not shown), thus confirming recombinational 'flip-flop' of Tn*Ppa1* in the host genome.

Genomic localization of Tn*Ppa1* in DSM 11072 and its distribution in other strains of *P. pantotrophus*

In order to identify the location of Tn*Ppa1* in the strain DSM 11072 genome, a PCR-amplified DNA fragment of Tn*3434* (amplified with primers L3434 and R3434) was

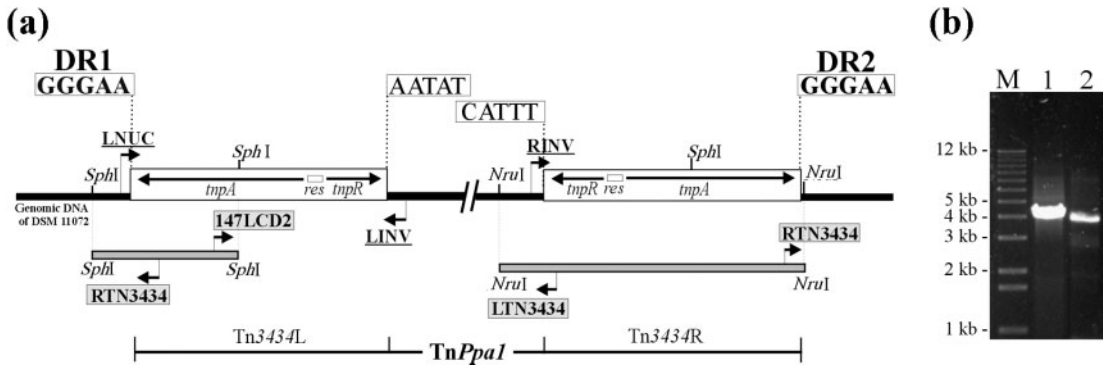

Fig. 3. (a) Determination of DNA sequences flanking Tn*Ppa1* in the genome of strain DSM 11072. The 5 bp direct repeats flanking both copies of Tn*3434* are boxed and marked as DR1 and DR2. The *Sph*I and *Nru*I restriction fragments used in the determination of the sequences flanking Tn*Ppa1* by IPCR are shown as shaded boxes (see text for details). Primers used for DNA amplification are shown in shaded bars with the direction of amplification indicated by arrows. The primers used for detection of the inverted form of Tn*Ppa1* are underlined (see text for details). (b) Identification of the inverted form of Tn*Ppa1* using PCR. Total genomic DNA extracted from strain DSM 10072 was subjected to PCR with LNUC and LINV (lane 1) or LNUC and RINV (lane 2) primers. The numbers on the left indicate the position of the molecular mass standards (lane M).

used to probe a Southern blot of the mega-sized DNA replicons of DSM 11072, visualized by in-gel cell lysis and gel electrophoresis. It has previously been shown that strain DSM 11072, besides pWKS1 (2·7 kb), also carries the megaplasmid pWKS3 (Baj *et al.*, 2000). We found, however, that this strain contained an additional high–molecular-mass replicon (designated pWKS5) of >640 kb, which was readily visible when the strain was cured of pWKS3 (strain DBB1; Fig. 4). On the Southern blot, the Tn*3434*-specific probe produced a hybridization signal with DNA of pWKS5, which revealed the location of Tn*Ppa1* (Fig. 4).

In a previous study, we showed by hybridization that two copies of Tn*3434* are present in the genome of strain DSM 11072, but that it is absent from other strains of *P. pantotrophus* (DSM 11073, DSM 65 and LMD 82.5), as well as four other paracoccal species (*P. versutus*, *P. solventivorans*, *Paracoccus methylutens*, *Paracoccus denitrificans*) (Bartosik *et al.*, 2003a). To investigate the distribution of sequences in the core region of Tn*Ppa1*, a DIG-labelled mixture of restriction fragments of Tn*Ppa1* was used to probe a Southern blot of *Eco*RI-digested total DNA from the above *P. pantotrophus* strains. A positive hybridization signal was observed exclusively with the DNA of the host strain DSM 11072, which proves that the Tn*Ppa1* sequences were strain specific (data not shown).

DISCUSSION

We have constructed a novel entrapment vector pMMB2 (containing a *repABC*-type replicon), which appears to be an appropriate tool for the capture of large TEs. The *repABC* replicons are found exclusively within megaplasmids (or chromosomes), harboured by many representatives of the Alphaproteobacteria. Their size may exceed 1 million bp, e.g. the 1·68 Mb megaplasmid pSymB of *Sinorhizobium meliloti* 1021 (Finan *et al.*, 2001). This demonstrates that *repABC* modules (in which the partitioning and replication genes are clustered in a single operon; Ramírez-Romero *et al.*, 2001) can maintain large replicon genomes and should, therefore, be suitable for the construction of large TE entrapment vectors.

Using pMMB2, we captured Tn*Ppa1*, which is the first known composite Tn of *Paracoccus* spp. and, to our knowledge, is the largest TE that has ever been identified by transposition to an entrapment vector. The results summarized in Table 2 show that it was not possible to capture Tn*Ppa1* using the previously constructed entrapment vector pMEC1 carrying the *ori* region of a small plasmid pWKS1 that naturally resides in strain DSM 11072 (Bartosik *et al.*, 2002). This observation indicates that the nature of the vector replicator region acts as a natural selection for the size of the integrated elements.

We have shown that Tn*Ppa1* resides in strain DSM 11072 within a very large replicon significantly exceeding 640 kb in size (Fig. 4). Replicons of this size have also been detected in other strains of *P. pantotrophus* (data not shown). Since many representatives of the Alphaproteobacteria contain more than one chromosome (Jumas-Bilak *et al.*, 1998), the question of whether pWKS5 is a chromosome or a mega-sized plasmid remains open.

Tn*Ppa1* has an atypical structure and possesses at each terminus a divergently oriented copy of a non-composite transposon, Tn*3434* (Tn*3* family). Tn*3434* (like ISs) carries only genetic information sufficient for its own transposition. In contrast, the closest homologue of Tn*3434*, Tn*5393*, identified in *P. pantotrophus* LMD 82.5, contains two additional streptomycin-resistance genes (*strA* and *strB*) located downstream of the *tnpR* gene (Bartosik *et al.*, 2003a).

Table 3. ORFs located within Tn*Ppa1* in *P. pantotrophus* DSM 11072

ORF no.	Coding region (bp)*	Orientation	Protein size (aa)	Possible function†	Best BLAST hit		
					Percentage identity‡ (aa)	Organism	GenBank accession no.
1	48–2 930	←	960	Transposase	100 (960/960)	*P. pantotrophus* DSM 11072	AAO85810
2	3 051–3 662	→	203	Resolvase	100 (203/203)	*P. pantotrophus* DSM 11072	AAO85811
3	3 699–4 739	→	347	*sn*-Glycerol-3-phosphate ABC transporter, ATP-binding	54 (188/346)	*Chromobacterium violaceum* ATCC 12472	AAQ61315
4	4 741–5 535	→	264	Oxidoreductase (short chain dehydrogenase/ reductase family)	54 (137/250)	*Sinorhizobium meliloti* 1021	NP 386353
5	5 600–6 748	→	382	Homoserine kinase type II	47 (162/342)	*R. sphaeroides* 2.4.1	ZP 0 0006368
6	6 755–8 080	→	441	4-Aminobutyrate aminotransaminase	60 (262/430)	*S. meliloti* 1021	NP 436219
7	8 150–8 806	→	218	Transcriptional regulator (GntR family)	42 (90/210)	*Pseudomonas aeruginosa* UCBPP-PA14	ZP 00140362
8	8 930–9 994	←	354	ABC transporter, ATP-binding	51 (167/327)	*Symbiobacterium thermophilum* IAM 14863	YP 074974
9	10 010–11 734	←	574	ABC-type Fe^{3+} transporter (permease component)	30 (164/537)	*Azotobacter vinelandii* AvOP	ZP 00415494
10	11 814–12 881	←	355	ABC-type Fe^{3+} transporter (periplasmic component)	30 (94/309)	*Thermobifida fusca*	ZP 00292778
11	13 013–13 939	→	308	PEP phosphonomutase	56 (162/285)	*Burkholderia fungorum* LB400	ZP 00282024
12	14 009–15 145	→	404	Unknown (conserved hypothetical protein)	49 (168/339)	*Mesorhizobium* sp. BNC1	EAN06321
13	15 142–16 539	→	465	Uncharacterized protein involved in propionate catabolism	46 (172/368)	*B. fungorum* LB400	ZP00284977
14	16 543–17 118	→	191	Chromate transport protein ChrA	36 (67/185)	*Burkholderia vietnamiensis* G4	ZP 00421931
15	17 134–17 664	→	176	Chromate transport protein ChrA	36 (63/171)	*Dechloromonas aromatica* RCB	ZP 00150273
16	17 682–18 275	←	197	Transcriptional regulator (GntR family)	51 (100/194)	*P. aeruginosa* PAO1	NP 249488
17	18 522–19 601	←	359	Malate/L-lactate dehydrogenase	39 (129/326)	*Mesorhizobium* sp. BNC1	EAN07781
18	19 546–20 949	←	467	Unknown (conserved hypothetical protein)	53 (248/467)	*Bord. bronchiseptica* RB50	NP 887274
19	20 946–21 248	←	100	Unknown (conserved hypothetical protein)	46 (46/99)	*Bord. bronchiseptica* RB50	NP 887273
20	21 245–22 708	←	487	Succinate dehydrogenase/fumarate reductase flavoprotein subunit	52 (252/477)	*B. fungorum* LB400	ZP 00280122
21	22 799–23 815	←	338	D-Isomer specific 2-hydroxyacid dehydrogenase	49 (154/311)	*Pseudomonas syringae* pv. tomato DC3000	NP 793070
22	23 959–25 458	→	499	Fumarate reductase/succinate dehydrogenase flavoprotein, N-terminal	54 (264/488)	*Mesorhizobium* sp. BNC1	EAN06305
23	25 730–27 247	→	505	Succinate dehydrogenase/fumarate reductase (flavoprotein subunit)	74 (366/489)	*B. fungorum* LB400	ZP 00284982
24	27 262–28 749	→	495	Uncharacterized protein involved in propionate catabolism	53 (257/479)	*B. fungorum* LB400	ZP 00284981

Table 3. cont.

ORF no.	Coding region (bp)*	Orientation	Protein size (aa)	Possible function†	Best BLAST hit		
					Percentage identity‡ (aa)	Organism	GenBank accession no.
25	29 037–30 095	→	352	TRAP-T C4-dicarboxylate transporter (DctP subunit)	27 (93/339)	*Mesorhizobium* sp. BNC1	EAN07139
26	30 092–30 688	→	198	TRAP-T transporter (DctQ subunit)	34 (33/97)	*Rhodopseudomonas palustris* CGA009	NP 949889
27	30 688–32 001	→	437	TRAP-T transporter (DctM subunit)	35 (152/434)	*Oceanobacillus iheyensis* HTE831	NP 693782
28	32 003–32 821	→	272	Zinc-dependent hydrolase	47 (126/263)	*Yersinia pseudotuberculosis* IP 32953	YP 072065
29	32 827–33 957	→	376	Zinc-containing alcohol dehydrogenase	50 (188/369)	*Rhiz. etli*	AAM54942
30	33 905–35 461	→	518	NAD-dependent aldehyde dehydrogenase	49 (240/489)	*B. fungorum* LB400	ZP 00280125
31	35 545–36 315	→	256	Short-chain dehydrogenase	36 (90/246)	*Moorella thermoacetica* ATCC 39073	ZP 00575032
32	36 388–36 717	←	109	Unknown (conserved hypothetical protein)	72 (74/102)	*R. sphaeroides* 2.4.1	ZP 0 0005343
33	36 722–37 738	←	338	Unknown, membrane protein	64 (206/319)	*R. sphaeroides* 2.4.1	ZP 0 0005342
34	37 735–38 403	←	222	Tartrate dehydratase beta subunit/fumarate hydratase (class I, C-terminal domain)	73 (144/197)	*R. sphaeroides* 2.4.1	ZP 0 0005341
35	38 400–39 356	←	318	Tartrate dehydratase alpha subunit/fumarate hydratase (class I, N-terminal domain)	85 (271/318)	*R. sphaeroides* 2.4.1	ZP 0 0005340
36	39 415–40 554	←	379	Succinate dehydrogenase/fumarate reductase	73 (278/378)	*R. sphaeroides* 2.4.1	ZP 0 0005339
37	40 625–41 236	←	203	Resolvase	100 (203/203)	*P. pantotrophus* DSM 11072	AAO85811
38	41 357–44 239	→	960	Transposase	100 (960/960)	*P. pantotrophus* DSM 11072	AAO85810

*Nucleotide position from start to stop codon in the Tn*Ppa1* sequence.

†The designated putative function is based on a protein domain search.

‡Identity in percentage and amino acids (number identical relative to total number examined) as determined with BLAST.

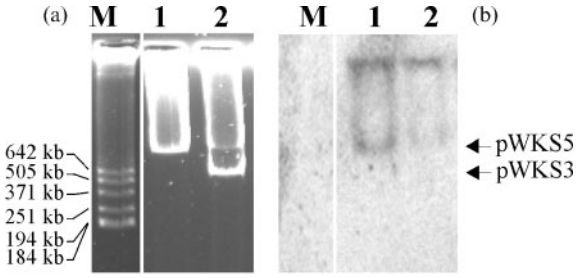

Fig. 4. Localization of Tn*Ppa1* within the *P. pantotrophus* DSM 11072 genome. (a) Mega-sized replicons of *P. pantotrophus* DSM 11072 (lane 2) and its derivative strain DBB1 (deprived of megaplasmid pWKS3) (lane 1) visualized by in-gel lysis and DNA electrophoresis. *Rhiz. etli* CE3 strain was used as a marker, carrying six megaplasmids of the sizes given on the left (lane M). (b) Southern blot of the same gel probed with a DIG-labelled Tn*3434* fragment.

Tn*Ppa1* encodes three clusters of putative transporters. (i) ORFs 14 and 15 encode putative products similar to ChrA transporters, which have been shown, in some cases, to be responsible for chromate resistance (Aguilera *et al.*, 2004). (ii) ORFs 25–27 show sequence similarities to uptake systems of the tripartite, ATP-independent, periplasmic transporter (TRAP-T) family (Kelly & Thomas, 2001). The TRAP-T systems were initially identified in *R. sphaeroides* (Jacobs *et al.*, 1996) and *Rhodococcus capsulatus* (Forward *et al.*, 1997), where they catalyse the uptake of glutamate and C4 dicarboxylates, respectively. (iii) ORFs 8–10 encode putative ABC-type transporters, with significant similarity to proteins involved in Fe^{3+} transport. Iron, which is a limiting factor in the environment, is an essential nutrient for all living organisms, being a component of key enzymes such as cytochromes, ribonucleotide reductase and other metabolically linked molecules (Köster, 2001). The presence of iron transporters within the Tn and their dissemination by lateral transfer may be highly advantageous to many bacterial hosts. The specific function of the putative transporters encoded by Tn*Ppa1* awaits experimental confirmation.

There are two possible scenarios by which Tn*Ppa1* might have arisen in strain DSM 11072. One scenario assumes the acquisition of Tn*3434* by lateral transfer and then two independent transposition events of this cryptic Tn into the host genome. In this case Tn*Ppa1* would be composed of a segment of DSM 11072 chromosome flanked by two copies of Tn*3434*. The other scenario assumes acquisition of the whole Tn*Ppa1* (e.g. on a plasmid) followed by transposition of Tn*Ppa1* into the host genome, or integration of the plasmid molecule into the genome. In this case, Tn*Ppa1*-encoded genes might not be conserved in other strains of *P. pantotrophus*. According to the first scenario, each of the integrated copies of Tn*3434* should be bordered by different repeated target sequences (DRs), while in the case of the latter, DRs should only be observed flanking both termini of Tn*Ppa1*. As seen in Fig. 3, the entire Tn*Ppa1* (but not the individual copies of Tn*3434*) is flanked by 5 bp DRs in the strain DSM 11072 genome, which favours the second scenario. Moreover, hybridization analysis revealed that, of the three tested strains of *P. pantotrophus* (DSM 65, DSM 11073 and LMD 82.5), none carried sequences homologous to Tn*Ppa1*, which unequivocally proves that Tn*Ppa1* was acquired by strain DSM 11072 through lateral transfer. Since this element is present only in one strain, it would appear to be a relatively recent acquisition that occurred after branching of the tested *P. pantotrophus* strains.

Taking into account the similar G+C content of Tn*Ppa1* and the genomic DNA of *P. pantotrophus*, and the presence of a DNA segment homologous to chromosome cII of *R. sphaeroides*, it seems that Tn*Ppa1* has been gained from a phylogenetically closely related bacterial species. The most external ORFs of the core region of Tn*Ppa1* (ORFs 3 and 36), located adjacent to both copies of Tn*3434*, are truncated, and the missing parts of the ORFs could not be identified outside the Tn. These disruptions are probably remnants of the ancient Tn*3434* transposition events which led to the formation of Tn*Ppa1* in its native host.

In strain DSM 11072, the two copies of Tn*3434* reside within the same DNA molecule despite the phenomenon of Tn immunity (where a replicon containing one copy of a Tn is highly resistant to further insertions of the same element) which is thought to apply to members of the Tn*3* family (Grindley, 2002). The divergent orientation of the Tn*3434* copies appears to ensure the stable maintenance of Tn*Ppa1* within the host genome. Tn*3434*, like other Tn*3*-like elements, encodes a resolvase module, which promotes efficient site-specific recombination between *res* sequences carried by two Tns residing within the same DNA molecule. Resolvase-mediated recombination should result in the loss of the core region of Tn*Ppa1* (when Tn*3434*L and Tn*3434*R are present in the same orientation) or its inversion (when they are arranged divergently). We confirmed that inversion does occur, resulting in rotation of the Tn within the host genome. This rearrangement does not change the local genetic environment, although more detailed studies are required to confirm that the effect of such a recombinational flip-flop of the Tn is neutral.

The identification of Tn*Ppa1* provides evidence for the transposition and lateral transfer of a large segment of DNA of chromosomal origin. Transposition of housekeeping genes into co-residing plasmids or megaplasmids may potentially have a significant impact on the structure and evolution of bacterial genomes (e.g. it may stimulate the formation of multi-chromosome genomes). Additionally, duplication of sets of genes (e.g. by replicative transposition) is considered an important mechanistic antecedent of gene innovation and, consequently, of genetic novelty (Coenye *et al.*, 2005). The duplicated genetic information may then be spread by lateral transfer among bacterial populations, producing a variety of phenotypic effects.

Although Tn*Ppa1* appears not to posses the complete genetic information for any particular metabolic pathway, it carries a number of genes (including housekeeping genes and membrane transporters) whose presence may improve the ecological fitness of the host cells. It is noteworthy that some bacterial catabolic pathways seem to have evolved by patchwork assembly, i.e. by acquisition of various genes following separate lateral transfer events. In such cases, the acquisition of even a single gene may initiate a novel metabolic activity (Copley, 2000; Springael & Top, 2004).

Taking into account all our findings, we suggest that Tn*Ppa1* may be considered a transposable GEI. GEIs are defined as strain-specific, large chromosomal regions that can be excised from the genome and transferred to other recipients. They contain mobility genes encoding integrases or transposases (required for integration and excision), as well as one or more genes that can increase the adaptability and versatility of the bacterium (Dobrindt *et al.*, 2004). The majority of the identified GEIs encode an integrase, involved in integration of GEIs close to tRNA genes. However, in the case of Tn*Ppa1*, transposition is responsible for the movement of the element. The same mechanism of translocation is also suggested for a 44·8 kb long pathogenicity island of *Bacillus anthracis* which is flanked by inverted copies of IS*1627* (Okinaka *et al.*, 1999).

Physiologically, *Paracoccus* species are highly versatile. For example, different strains are able to use a variety of organic compounds as a source of carbon. Although there is no evidence linking the presence of TEs with vital phenotypic characteristics of *P. pantotrophus*, the possibility that their physiological heterogeneity might result from the presence of various TEs cannot be ruled out. To investigate the role of Tns in genome evolution, we plan to employ the entrapment vector pMMB2 to search for such elements in other strains of *Paracoccus* and in other members of the Alphaproteobacteria.

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REFERENCES

Aguilera, S., Aguilar, M. E., Chavez, M. P., Lopez-Meza, J. E., Pedraza-Reyes, M., Campos-Garcia, J. & Cervantes, C. (2004). Essential residues in the chromate transporter ChrA of *Pseudomonas aeruginosa*. *FEMS Microbiol Lett* **232**, 107–112.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Baj, J., Piechucka, E., Bartosik, D. & Wlodarczyk, M. (2000). Plasmid occurrence and diversity in the genus *Paracoccus*. *Acta Microbiol Polon* **49**, 265–270.

Bartosik, D., Bialkowska, A., Baj, J. & Wlodarczyk, M. (1997). Construction of mobilizable cloning vectors derived from pBGS18 and their application for analysis of replicator region of a pTAV202 mini-derivative of *Paracoccus versutus* pTAV1 plasmid. *Acta Microbiol Polon* **46**, 379–383.

Bartosik, D., Baj, J. & Wlodarczyk, M. (1998). Molecular and functional analysis of pTAV320-*repABC* type replicon of a composite pTAV1 plasmid of *Paracoccus versutus*. *Microbiology* **144**, 3149–3157.

Bartosik, D., Baj, J., Sochacka, M., Piechucka, E. & Wlodarczyk, M. (2002). Molecular characterization of functional modules of plasmid pWKS1 of *Paracoccus pantotrophus* DSM 11072. *Microbiology* **148**, 2847–2856.

Bartosik, D., Sochacka, M. & Baj, J. (2003a). Identification and characterization of transposable elements of *Paracoccus pantotrophus*. *J Bacteriol* **185**, 3753–3763.

Bartosik, D., Szymanik, M. & Baj, J. (2003b). Indentification and distribution of insertion sequences of *Paracoccus solventivorans*. *Appl Environ Microbiol* **69**, 7002–7008.

Birnboim, H. C. & Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Res* **7**, 1513–1519.

Campbell, A. (2002). Eubacterial genomes. In *Mobile DNA II*, pp. 1024–1039. Edited by N. L. Craig, R. Craigie, M. Gellert & A. M. Lambowitz. Washington, DC: American Society for Microbiology.

Coenye, T., Gevers, D., Van de Peer, Y., Vandamme, P. & Swings, J. (2005). Towards a prokaryotic genomic taxonomy. *FEMS Microbiol Rev* **29**, 147–167.

Copley, S. D. (2000). Evolution of a metabolic pathway for degradation of a toxic xenobiotic: the patchwork approach. *Trends Biochem Sci* **25**, 261–265.

Devereux, J., Haeberli, P. & Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* **11**, 387–395.

Ditta, G., Stanfield, S., Corbin, D. & Helinski, D. R. (1980). Broad host-range DNA cloning system for gram-negative bacteria: construction of a gene bank of *Rhizobium meliloti*. *Proc Natl Acad Sci U S A* **77**, 7347–7351.

Dobrindt, U., Hochhut, B., Hentschel, U. & Hacker, J. (2004). Genomic island in pathogenic and environmental microorganisms. *Nature Rev Microbiol* **2**, 414–424.

Finan, T. M., Weidner, S. & 10 other authors (2001). The complete sequence of the 1,683-kb pSymB megaplasmid from the N_2-fixing endosymbiont *Sinorhizobium meliloti*. *Proc Natl Acad Sci U S A* **98**, 9889–9894.

Forward, J. A., Behrendt, M. C., Wyborn, N. R., Cross, R. & Kelly, D. P. (1997). TRAP transporters: a new family of periplasmic solute transport systems encoded by the dctPQM genes of *Rhodobacter capsulatus* and by homologs in diverse gram-negative bacteria. *J Bacteriol* **179**, 5482–5493.

Gerstenberg, C., Friedrich, B. & Schlegel, H. G. (1982). Physical evidence for plasmids in autotrophic, especially hydrogen-oxidising bacteria. *Arch Microbiol* **133**, 90–96.

Gibson, T. J. (1984). *Studies on Epstein–Barr virus genome*. PhD thesis. University of Cambridge.

Grindley, N. D. F. (2002). The movement of Tn*3*-like elements: transposition and cointegrate resolution. In *Mobile DNA II*, pp. 272–302. Edited by N. L. Craig, R. Craigie, M. Gellert & A. M. Lambowitz. Washington, DC: American Society for Microbiology.

Hanahan, D. (1983). Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* **166**, 557–580.

Jacobs, M. H., van der Heide, T., Driessen, A. J. & Konings, W. N. (1996). Glutamate transport in *Rhodobacter sphaeroides* is mediated

by a novel binding protein-dependent secondary transport system. *Proc Natl Acad Sci U S A* **93**, 12786–12790.

Jordan, S. L., McDonald, I. R., Kraczkiewicz-Dowjat, A. J., Kelly, D. P., Rainey, F. A., Murrell, J. C. & Wood, A. P. (1997). Autotrophic growth on carbon disulfide is a property of novel strains of *Paracoccus denitrificans*. *Arch Microbiol* **168**, 225–236.

Jumas-Bilak, E., Michaux-Charachon, S., Bourg, G., Ramuz, M. & Allardet-Servent, A. (1998). Unconventional genomic organization in the alpha subgroup of the Proteobacteria. *J Bacteriol* **180**, 2749–2755.

Kelly, D. J. & Thomas, G. H. (2001). The tripartite ATP-independent periplasmic (TRAP) transporters of bacteria and archaea. *FEMS Microbiol Rev* **25**, 405–424.

Köster, W. (2001). ABC transporter-mediated uptake of iron, siderophores, heme and vitamin B12. *Res Microbiol* **152**, 291–301.

Kushner, S. R. (1978). An improved method for transformation of *E. coli* with ColE1-derived plasmids. In *Genetic Engineering*, pp. 17–23. Edited by H. B. Boyer & S. Nicosia. Amsterdam: Elsevier/North-Holland.

Ludwig, W., Mittenhuber, G. & Friedrich, C. G. (1993). Transfer of *Thiosphaera pantotropha* to *Paracoccus denitrificans*. *Int J Syst Bacteriol* **43**, 363–367.

Mahillon, J. (1998). Transposons as gene haulers. *APMIS suppl* **84**, 29–36.

Noel, K. D., Sanchez, A., Fernandez, L., Leemens, J. & Cevallos, M. A. (1984). *Rhizobium phaseoli* symbiotic mutants with transposon Tn*5* insertions. *J Bacteriol* **158**, 148–155.

Okinaka, R. T., Cloud, K., Hampton, O. & 12 other authors (1999). Sequence and organization of pXO1, the large *Bacillus anthracis* plasmid harboring the anthrax toxin genes. *J Bacteriol* **181**, 6509–6515.

Ramírez-Romero, M. A., Tellez-Sosa, J., Barrios, H., Perez-Oseguera, A., Rosas, V. & Cevallos, M. A. (2001). RepA negatively autoregulates the transcription of the *repABC* operon of the *Rhizobium etli* symbiotic plasmid basic replicon. *Mol Microbiol* **42**, 195–204.

Rutherford, K., Parkhill, L., Crook, J., Horsnell, T., Rice, P., Rajandream, M.-A. & Barrell, B. (2000). Artemis: sequence visualisation and annotation. *Bioinformatics* **16**, 944–945.

Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Schneider, D., Faure, D., Noirclerc-Savoye, M., Barrière, A. C., Coursange, E. & Blot, M. (2000). A broad-host-range plasmid for isolating mobile genetic elements in gram-negative bacteria. *Plasmid* **44**, 201–207.

Solyga, A. & Bartosik, D. (2004). How to capture a functional transposable element. *Pol J Microbiol* **53**, 139–144.

Springael, D. & Top, E. M. (2004). Horizontal gene transfer and microbial adaptation to xenobiotics: new types of mobile genetic elements and lessons from ecological studies. *Trends Microbiol* **12**, 53–58.

Wheatcroft, R., McRae, G. D. & Miller, R. W. (1990). Changes in the *Rhizobium meliloti* genome and the ability to detect supercoiled plasmids during bacteroid development. *Mol Plant Microbe Interact* **3**, 9–17.

Williams, R., Macartney, D. P. & Thomas, C. M. (1998). The partitioning activity of the RK2 central control region requires only *incC*, *korB* and KorB-binding site O_B3 but other KorB-binding sites form destabilizing complexes in the absence of O_B3. *Microbiology* **144**, 3369–3378.