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Genetic Analysis of Mobile *tetQ* Elements in Oral *Prevotella* Species

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Abstract

Prevotella species are members of the bacterial oral flora and are opportunistic pathogens in polymicrobial infections of soft tissues. Antibiotic resistance to tetracyclines is common in these bacteria, and the gene encoding this resistance has been previously identified as *tetQ*. The *tetQ* gene is also found on conjugative transposons in the intestinal *Bacteroides* species; whether these related bacteria have transmitted *tetQ* to *Prevotella* is unknown. In this study, we describe our genetic analysis of mobile *tetQ* elements in oral *Prevotella* species. Our results indicate that the mobile elements encoding *tetQ* in oral species are distinct from those found in the *Bacteroides*. The intestinal bacteria may act as a reservoir for the *tetQ* gene, but *Prevotella* has incorporated this gene into an IS21-family transposon. This transposon is present in *Prevotella* species from more than one geographical location, implying that the mechanism of *tetQ* spread between oral *Prevotella* species is highly conserved.

Keywords

Periodontal disease; anaerobe; Bacteroidetes; transposon; IS21; Tn6099; Tn6100; horizontal DNA transfer

INTRODUCTION

The genus *Prevotella* is composed of obligately anaerobic bacteria associated with the human alimentary tract, as well as the bovine rumen [1]. *Prevotella intermedia* and *Prevotella nigrescens* are black-pigmented anaerobic microorganisms commonly found in human dental plaque, and are associated with the development of gingivitis and periodontal

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disease [2–5]. In addition, oral *Prevotella* species are common opportunistic pathogens in soft tissue infections of the head and neck [6–9].

Prevotella species are members of phylum *Bacteroidetes*, and thus are close taxonomic relatives with the oral pathogen *Porphyromonas gingivalis* and the intestinal *Bacteroides* species [10]. The *Bacteroides* are well characterized genetically, and they transfer DNA to other bacterial species in the intestine [11]. Studies on gene transfer in these organisms have revealed a multitude of genetic elements, primarily transposons, which can be conjugally transferred [12,13]. The primary transferable elements identified in the genera *Bacteroides* are the large (> 60 kb) conjugative transposons (CTns), which are frequently associated with tetracycline resistance, most commonly *tetQ*, a ribosomal protection gene [14].

TetQ genes similar to those identified in *Bacteroides* have been found in *Prevotella* and *Porphyromonas* species [15–17]. The *tetQ* gene cloned from one *Prevotella nigrescens* clinical isolate was nearly identical in sequence to *tetQ* from a conjugative transposon identified in *Bacteroides fragilis* [18]. Tetracycline resistant clinical isolates of *Prevotella* and *Porphyromonas* have been shown to transfer tetracycline resistance to other bacterial species by conjugation *in vitro* [19–21].

The degree to which transposable elements contribute to antibiotic resistance in the oral anaerobe community is unclear. Regional studies indicate that there is a great deal of variability in antibiotic resistance frequencies in these bacteria [22]. A study in Spain found that 4% of *P. intermedia* isolates were resistant to tetracycline [23], while other studies have shown resistance levels as high as 26% [24–27]. As with other medically relevant bacteria, it is likely that selective pressure in the form of antibiotic therapy is driving the development of more resistant organisms in the oral cavity.

Intestinal bacteria are inherently under high levels of antibiotic selective pressure. It has been proposed that these commensal organisms drive the evolution of mobile resistance elements and subsequently transmit them to other more pathogenic bacterial species [28]. While we can hypothesize that oral members of phylum *Bacteroidetes* are similar to the intestinal species in their genetic systems, no advanced genetic analysis of oral mobile elements has been attempted. In this study, we described our genetic analysis of mobile *tetQ* elements in oral *Prevotella* species. Our results indicate that the mobile elements encoding *tetQ* in oral species are distinct in size and gene content from those found in the *Bacteroides*.

MATERIALS AND METHODS

Bacterial strains and Growth Conditions

Prevotella clinical isolates (Table 1) were grown in Trypticase Soy Broth (TSB) supplemented with 7.5 µM hemin and 3 µM menadione. TSB blood agar plates were made with the addition of 5% sheep's blood and 1.5% agarose. *Prevotella* strains were grown anaerobically at 37° C in a Coy anaerobic chamber under 86% nitrogen: 10% carbon dioxide: 4% hydrogen. Selection for antibiotic resistant *Prevotella* was with 10 µg/ml erythromycin, or 5 µg/ml tetracycline. Dual resistance was selected on 10 µg/ml erythromycin and 1 µg/ml tetracycline. Select *Prevotella* strains were made rifampin resistant (MIC > 30 µg/ml) by serial passage on increasing concentrations of the antibiotic. *E. coli* strains DH5α, S17-1, and STBL-4 (Invitrogen) were grown in Luria-Bertani (LB) media supplemented as needed with ampicillin (100 µg/ml).

Construction of a *tetQ* Mobile Element Capture Plasmid

A transposon capture strategy for *Prevotella* was designed utilizing plasmid pFD665, a *Bacteroides-E. coli* suicide vector containing an *E. coli* pSC101 plasmid origin of

replication and RK2 origin of transfer, and an *ermF* selectable marker for expression in members of the Bacteroidetes [29]. pFD665 replicates as a low-copy number plasmid in *E. coli*, and must integrate via homologous recombination into the chromosome to be maintained in a Bacteroidetes recipient. For homologous recombination into *Prevotella* recipients containing the *tetQ* gene, PCR primers 5'-GGGAAGGCGGTACCTCTCCTTAACGTACG-3' and 5'-GGGAAGGCGGATCCAATGCTTCTATCTCC-3' were used to amplify a 2.6 kb fragment containing the *tetQ* gene. The primers contain *KpnI* and *BamHI* restriction sites at the 5' ends, and the resulting PCR fragment was digested and cloned into the corresponding sites in pFD665, resulting in plasmid pFD665(*tetQ*) (Figure 1A). pFD665(*tetQ*) was subsequently mobilized from *E. coli* strain S17-1 into a *Prevotella tetQ*+ recipient, and stable integration of the suicide vector into the recipient chromosome (via *tetQ* homology) was selected for by erythromycin resistance (Figure 1B). The resulting plasmid-transposon chimera was recovered in *E. coli*, using the *Prevotella* Tc^rEm^r strain as a donor in DNA conjugation mixes with *E. coli* recipient strain STBL4. Recipients containing the pFD665(*Strain*) plasmid were selected on LB media with 100 µg/ml ampicillin. The transposon capture strategy was utilized to recover mobile elements containing *tetQ* from clinical isolates *P. nigrescens* PDRC11 and PDRC22B (Table 1).

***Prevotella* DNA conjugation**

Plasmid pFD665(*tetQ*) was conjugated from *E. coli* S17-1 donors to *Prevotella* recipients by mixing log phase cultures at ratios of 1 donor to 100 recipients, pelleting the mixed cultures and resuspending the cells in 50 µl TSB, and incubating the bacterial pellets overnight on pre-reduced blood agar plates in a candle jar at 37°C. *Prevotella* recipients were selected by incubating 7 to 10 days anaerobically on TSB blood agar containing 100 µg/ml gentamicin, and 10 µg/ml erythromycin. Plasmid conjugal transfers from *Prevotella* donors to *E. coli* recipients were similar to *E. coli* to *Prevotella* transfers, except the mating pellet was incubated on pre-reduced TSB blood agar plates anaerobically overnight, and *E. coli* recipients were selected aerobically on LB media with 100 µg/ml ampicillin. *Prevotella* to *Prevotella* matings were performed with equal ratios of bacterial strains, and mating mixtures were incubated 24 hours anaerobically at 37°C. Conjugation efficiencies were calculated by dividing the number of transconjugants obtained by the number of input donor cells. Controls for DNA exchange by transformation were *Prevotella* heat killed donors mixed with viable *Prevotella* recipients incubated anaerobically overnight. Controls were negative for all donor strains tested.

Molecular biology

DNA cloning, sequencing, PCR amplification, Southern blotting, *E. coli* plasmid purification, and other common molecular biology techniques were carried out by standard procedures [30]. *E. coli* plasmid purification for DNA sequencing was done using the QiaPrep Spin Miniprep kit (Qiagen). DNA sequencing was performed by the University of Florida Interdisciplinary Center for Biotechnology Research Sequencing Core (Gainesville, FL), and by SeqWright DNA Technology Services (Houston, TX). Sequence assembly, DNA and protein analysis was done with CLC Combined Workbench, v3 (CLC bio). Total DNA was purified from *Prevotella* using the Promega Wizard Genomic DNA purification kit, with further purification by phenol/chloroform extraction if necessary. Southern hybridizations employed the Amersham AlkPhos Direct Labelling and Detection System (GE Healthcare).

RESULTS

TetQ transposon capture

Prevotella nigrescens strains PDRC11 and PDRC22B were originally isolated from diseased sites in patients with refractory periodontitis; the PDRC11 strain is resistant to penicillins and tetracyclines, and can transfer these resistances to other bacteria in conjugation assays (LaCroix & Walker, 1996). The gene conferring tetracycline resistance in PDRC11 was cloned and determined by DNA sequence analysis to be *tetQ*, and was found to be chromosomally-encoded [18]. Using the sequence data available for PDRC11 *tetQ*, we designed a transposon capture strategy utilizing plasmid pFD665, a *Bacteroides-E. coli* shuttle vector originally used to clone and sequence a conjugative transposon (cTn341) from *B. fragilis* strain 341 [29]. The strategy used to capture the *tetQ* mobile element is described in the Materials and Methods, and illustrated in Figure 1B.

Our original approach was to mobilize pFD665(*tetQ*) directly into PDRC11, however we were unable to obtain any transconjugants from repeated attempts at this mating. Introduction of foreign DNA into *Prevotella* species is notoriously difficult and some *Prevotella* are known to produce extracellular endonucleases and restriction/modification systems [31,32]. *P. nigrescens* VPI-8944 has been shown to reliably accept plasmid DNA from *E. coli* [33], thus we modified our strategy to utilize this genetically tractable strain. VPI-8944 was made rifampicin resistant (Rf^r; MIC > 30 µg/ml) by serial passage. The VPI-8944 Rf^r strain was then utilized as a recipient in a mating with PDRC11 as the donor, and VPI-8944 Tc^rRf^r transconjugants were obtained at a frequency of 8.12×10^{-5} . The presence of the *tetQ* gene in the VPI-8944 Tc^rRf^r transconjugants was confirmed by PCR.

The VPI-8944 Tc^rRf^r strain was then used as the recipient for the pFD665(*tetQ*) plasmid, and Em^r transconjugants were obtained at a frequency of 2.2×10^{-8} . Insertion of the pFD665(*tetQ*) plasmid into the *tetQ* gene of the VPI-8944 Tc^rRf^rEm^r transconjugants was confirmed by Southern blot. The VPI-8944 Tc^rRf^rEm^r strain was then used as a donor in a mating with *E. coli* STBL4 as the recipient, to recover the *tetQ* mobile element in plasmid form. The conjugation frequency for this transfer was 3.3×10^{-8} . After successful capture of the *P. nigrescens* PDRC11 mobile element, we utilized the same strategy to capture the element from *P. nigrescens* PDRC22B.

Genetic Analysis of the Captured Transposon

Plasmid DNA from an *E. coli* Ap^r transconjugant was purified and analyzed by restriction digest and agarose gel electrophoresis (Figure 1C). Comparison of the supercoiled pFD665(*tetQ*) vector to the vector recovered from the VPI-8944 Tc^rRf^rEm^r strain, pFD665(PDRC11), illustrates a significant increase in size in the recovered plasmid, as would result from capture of a mobile *Prevotella* DNA element. The original and recovered plasmids were further compared by restriction digest with *EcoRI*, *HindIII*, and *PstI*. There are no *PstI* sites in pFD665(*tetQ*), and one each of *EcoRI* and *HindIII* (Figure 1A). The recovered plasmid, pFD665(PDRC11), contained one *PstI* site, and two each for *EcoRI* and *HindIII*. Comparison of restriction fragment sizes from the original and recovered plasmids indicated a captured PDRC11 DNA fragment of approximately 7.5 kb. Similar analysis of the recovered pFD665(PDRC22B) plasmid indicated capture of a slightly smaller DNA fragment (approximately 6 kb; data not shown.)

DNA Sequence Analysis of the Captured Transposon

The pFD665(PDRC11) recovered plasmid contains a duplication of the *tetQ* gene (Figure 1B), making it difficult to sequence the captured element directly in this vector. We sub-cloned the *EcoRI* fragment representing the captured fragment into pUC18, and used M13

forward and reverse primers to initiate first and second strand sequencing of the mobile element by primer walking. Once a draft of the captured sequence was complete, we designed PCR primers to amplify the mobile element directly from the original host strain, *P. nigrescens* PDRC11. These PCR fragments were sequenced directly, to eliminate inclusion of potential DNA mutations or deletions acquired during transfer of the element into *P. nigrescens* VPI-8894 and *E. coli* STBL4. The final mobile element sequence results from a minimum of three-fold coverage. The PDRC22B captured DNA fragment was sequenced using the same strategy. A Tn number was assigned for each sequenced element using the Tn number registry at the Eastman Dental Institute <http://www.ucl.ac.uk/eastman/tn/index.php>. The final sequence data for these elements is archived under the GenBank Accession numbers HM561907 for the PDRC11 element (Tn6099) and HM561908 for PDRC22B (Tn6100).

The final length of the PDRC11 mobile element Tn6099 is 7095 bp, and the element encodes five putative proteins of greater than 50 amino acids (Figure 2). The DNA sequence encompassing the first two open reading frames has the most extensive homology (94% over 3590 bp) to the *tetQ-rteAB* operon of the conjugative transposons found in the intestinal *Bacteroides*. Alignment of the PDRC11 sequence with the closest homolog, from cTn341 [29], reveals that the *rteA* open reading frame in PDRC11 is truncated, and the *rteB* gene is absent (Figure 2). In Tn6099, the 3' region of *rteA* is replaced with two open reading frames encoding proteins with homology to the IS21 transposase IstA and accessory protein IstB [34]. The putative IstA protein contains an DDE motif, commonly found in transposase enzymes, and the IstB protein contains a DEAD/DEAH box helicase motif [35]. Although the IstB protein from Tn6099 is truncated relative to IstB of Tn6100, the DEAD helicase motif is intact in both proteins. Downstream of *istB* is a fifth open reading frame, which has no homology at the DNA or protein level to sequences found in GenBank. This open reading frame is designated *orfA*, and is found on the reverse strand relative to the other four genes. Secondary structure analysis of Tn6099 reveals a large multiloop hairpin structure encompassing a 1 kb region; this hairpin is downstream of *istB*, with a $\Delta G = -270.1$ kcal/mol. The sequenced mobile element Tn6100 is identical in sequence to Tn6099 over the first 5.7 kb, but the 1 kb region of secondary structure found in Tn6099 is absent from Tn6100, and instead a 308 bp region containing a 3' continuation of the *istB* open reading frame is present.

Detection of *tetQ-istA-istB* in *Prevotella* clinical isolates

Clinical strains *P. nigrescens* PDRC11 and *P. nigrescens* PDRC22B are from the collection of the Periodontal Disease Research Center at the University of Florida. We wished to determine if the association of *tetQ* with *istAB* transposition genes exists in isolates of *Prevotella* from independent sources. We screened a collection of *Prevotella* isolates originating from Osaka, Japan for tetracycline resistance by growth on media with 1 µg/ml Tc, and identified five strains of *P. nigrescens* and one strain of *P. denticola* that were tetracycline resistant and contained the *tetQ* gene (Figure 3)[36]. We also acquired a *tetQ* positive *P. intermedia* strain from Brazil (strain MRS1). Using PCR, we tested genomic DNA from each strain for the presence of *istA* and *istB*, and for linkage between the *tetQ-rteA* genes and the *istA-istB* genes. All five isolates from the Osaka collection were positive for *istAB* and for *rteA-istA*, and the MRS1 isolate was weakly positive for both bands.

Horizontal DNA transfer of *tetQ* from *Prevotella* clinical isolates

In our *tetQ* mobile element capture strategy, we successfully utilized *P. nigrescens* VPI-8944 as a recipient strain in matings with *P. nigrescens* PDRC11 and 22B donors. We wished to determine if the *Prevotella* isolates from Osaka and Brazil are also able to mobilize *tetQ* by horizontal DNA transfer. Conjugation assays revealed that all *istAB*

positive strains can mediate *tetQ* transfer to *P. nigrescens* VPI-8944; the *P. intermedia* MRS1 isolate, containing a very weak *istAB* band, was not (Table 2). Notably, the highest rates of transfer were from the *P. denticola* Osaka donor and *P. nigrescens* PDRC22B, which were not statistically different. (P value = 0.68).

Genetic Analysis of *P. nigrescens* VPI-8944 *tetQ*+ Transconjugants

Genomic DNA from *P. nigrescens* PDRC11 and a *P. nigrescens* VPI-8944 transconjugant from a PDRC11 mating were analyzed by Southern blot, using the entire Tn6099 element as the probe (Figure 4A). Genomic DNA was digested with *AvaI* (one site), *XbaI* (5 sites), or *SphI* (0 sites); the relative locations of the restriction sites are shown in Figure 4B. The *AvaI* digest results in a strong band just under 8 kb in size, and one weak band over 10 kb. We expect two bands from a linear, integrated mobile element; one band has a much stronger signal than the other indicating that the *AvaI* site is very close to one end of the integrated element. In the *XbaI* digest, we expect six bands from the integrated element; the 0.4 kb, 0.9 kb, 1.0 kb, and 1.8 kb bands are present as anticipated. The 2.8 kb band is missing, and replaced with a strong band at approximately 5 kb, and a weak band at approximately 3.5 kb. This indicates that the 2.8 kb *XbaI* fragment contains the integration site or “ends” of the transposable element, which is consistent with the *AvaI* results. This places the putative transposon ends near the 3' end of the *istB* gene and the stem-loop structure; this region is represented in the Figure 4B restriction map as a dot. To confirm if this site represents the integration region for Tn6099, we used PCR to test genomic DNA from *P. nigrescens* PDRC11 for the presence of an intact region of DNA between *istB* and *tetQ* (Figure 4C). The region between *istB* and *tetQ* is not intact in genomic DNA, confirming that this is the region containing the integrating ends of the transposable element.

P. nigrescens VPI-8944 transconjugants from matings with five different donors were analyzed to confirm the presence of *tetQ* in the chromosome, and to determine if the mobile elements from different strains utilized the same insertion site. Genomic DNA from select transconjugants was digested with *BamHI*, which has no sites in Tn6099 or Tn6100, and post-electrophoresis was analyzed by Southern blot, using the *tetQ* gene as the probe (Figure 4D). Transconjugants from all 5 donors produced single bands between 12 and 13 kb; the *P. nigrescens* 8944 recipient control had no band. Thus the *tetQ* mobile element in each of the donor strains also lack *BamHI* sites, consistent with the sequenced elements. Bands from *P. nigrescens* strains 28, 29, and 32 are the same size, indicating that these three *tetQ* mobile elements insert into the same site. The hybridizing fragments from the *P. denticola* and *P. nigrescens* 22B donors are slightly larger; these elements either insert in a different site, or these elements may be slightly larger than those found in *P. nigrescens* strains 28, 29, and 32.

DISCUSSION

Plaque biofilms offer an excellent opportunity to study gene transfer in a disease associated bacterial population. Interspecies genetic exchange has the potential to transfer antibiotic resistance genes, which may result in infections resistant to therapy. Additionally, members of the oral flora may acquire or transfer antibiotic resistance to other bacteria that reside elsewhere in the human flora. Many *Prevotella* strains contain the *tetQ* tetracycline resistance gene, which is highly similar to the *tetQ* gene found conjugative transposons in intestinal species of *Bacteroides*.

Our capture and genetic analysis of two *tetQ* mobile elements from *P. nigrescens* reveals these oral transposable elements to be distinct from the *tetQ* carrying *Bacteroides* elements in size, and in gene composition. The *Bacteroides* conjugative transposons are large (40–60 kb); encode multiple genes required for formation of a conjugal pore and DNA mobilization

(*tra* A-Q; *mob*); and mediate integration into the bacterial chromosome using integrase-family enzymes and a site-specific recombination mechanism [37]. The elements identified in *Prevotella* are relatively small (6–7 kb), do not encode a conjugal transfer apparatus, and appear to utilize an IS21-family transposase enzyme. The lack of an obvious origin of transfer or mobilization protein leaves the mechanism of horizontal transfer for the *Prevotella* elements in question. The genome sequence of *P. intermedia* strain 17 (TIGR; Rockville, MD, USA) contains a locus with high homology to the transfer genes found in the *Bacteroides* conjugative transposons; the *Prevotella tetQ* elements may thus be more similar in mechanism to the mobilizable transposons of the *Bacteroides* and utilize a DNA transfer apparatus provided by the host cell or a co-resident mobile element [12,13]. In *Porphyromonas gingivalis*, a member of the oral Bacteroidetes, chromosomal DNA undergoes horizontal transfer [38]; it is possible that the *Prevotella tetQ* transposons are horizontally transferred as part of genomic DNA and subsequently transpose into the recipient genome, or are incorporated as part of the transferred genomic DNA by homologous recombination. Regardless of the detailed mechanism of horizontal transfer, it is clear that the *Prevotella* mobile *tetQ* elements are genetically and functionally distinct from those found in the *Bacteroides*.

The clear DNA homology breakpoints in the Tn6099 sequence at genes *rteA* (compared to cTn341) and *istB* (compared to Tn6100) imply a modular or cassette assembly of the mobile element. In the *Bacteroides*, *RteA* and *RteB* are two component signal transduction molecules that regulate DNA transfer in response to tetracycline; these genes are located downstream and in an operon with *tetQ* [39]. In the *Prevotella* elements, *rteA* is not intact and *rteB* is absent, implying that the *tetQ* gene was acquired as the only functional gene from a *tetQ-rteA-rteB* operon. The *istB* gene is truncated in Tn6099 compared to Tn6100. The *istB* predicted function is as a transposition accessory protein, and as such may not be required for transposition; however, the DEAD box motif found in the protein is intact. The Tn6099 *istB* 3' end is replaced with a region of strong secondary structure; in Tn6099 this region also contains the ends of the transposon. GC content analysis of the *Prevotella tetQ* elements indicates that the overall GC content is 45%, similar to the expected 43% GC content of *Prevotella* genome sequences.

Prevotella clinical isolates from other geographical locations also contained *tetQ*, and five out of six contained the *istAB* genes in the same distance and orientation relative to *tetQ* as the PDRC mobile elements. The *istAB* positive strains were capable of mediating *tetQ* transfer to *P. nigrescens* VPI-8944, the *P. intermedia* MRS1 isolate was not; this implies that *tetQ* association with the *istAB* genes is important for transfer. *P. intermedia* MRS1 genomic DNA was weakly amplified by the *istAB* and *tetQ-istA* primers; this strain may have a diverged and/or non-functional version of the *Prevotella tetQ* transposon. Horizontal DNA transfer normally occurs at the highest frequencies between bacteria of the same species, however in this study *P. denticola* donated *tetQ* to a *P. nigrescens* recipient at conjugation frequencies comparable to (or higher than) *P. nigrescens* donors. Thus the ability of the donor to mobilize these *Prevotella* elements, along with inter-species restriction/modification compatibility, may be a determining factor in successful *tetQ* transfer to a recipient.

These studies indicate that the mechanism of *tetQ* spread between oral *Prevotella* species is conserved, and these bacteria use a transposon with a DNA sequence unique to the *Prevotella*. *TetQ* genes were found in *Prevotella* clinical isolates from diverse geographical locations, and the mobile elements all appear to be associated with an IS21-like transposon. The intestinal *Bacteroides* may act as a reservoir for the *tetQ* gene, but the oral *Prevotella* have evidently incorporated this gene into their own mobilome, based on an IS21-family

transposon. The horizontal DNA transfer apparatus that mediates the spread of these *tetQ* transposons has yet to be identified.

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Figure 1. Transposon Capture Method. 1A

E. coli-Prevotella suicide vector pFD665, with 2.3 kb *tetQ* gene. Genes functional in *Prevotella* are shown in light gray, those functional in *E. coli* are shown in dark gray. The origin of transfer is shown in black. **1B.** Transposon capture is mediated by homologous recombination of pFD665(*tetQ*) (light gray) into the integrated *tetQ* transposon (black). **1C.** Restriction digest of captured *tetQ* transposon in pFD665.

Figure 2. DNA sequence Alignment of *Prevotella* Captured Elements with a *Bacteroides tetQ* operon

Homologous DNA is represented in grey, non-homologous DNA in white. The PDRC11 transposon sequence Tn6099 is the reference. The region of strong secondary structure in Tn6099 is represented by a hairpin.

Figure 3. Detection of *tetQ* elements in diverse isolates of *Prevotella*

3A. Location and expected size of PCR products based on the PDRC11 *tetQ* element sequence. **3B.** PCR products from *Prevotella* strains isolated in Japan (Pi17, Pn28, Pn29, Pn32, Pn34, Pd-Osaka), Brazil (MRS1), and Florida (PDRC11, PDRC22B).

Figure 4. Analysis of *Prevotella* mobile element insertion

4A Southern blot of genomic DNA from *P. nigrescens* PDRC11 and its transconjugant offspring *P. nigrescens* VPI-8894. Genomic DNA was digested with *AvaI* (A), *XbaI* (X), and *SphI* (S). **4B.** *XbaI* and *AvaI* restriction map of PDRC11 *tetQ* element Tn6099, and PCR fragments amplified from genomic DNA. **4C.** PCR results from genomic amplification of strain PDRC11. **4D.** Southern blot of *tetQ* in *BamHI* restricted genomic DNA from transconjugants of *P. nigrescens* VPI-8894. Transconjugant donors: lane 1, *P. nigrescens* strain 28; lane 2, *P. nigrescens* strain 29; lane 3, *P. nigrescens* strain 32; lane 4, *P. denticola* Osaka; lane 5, *P. nigrescens* strain 22B; Controls: lane 6, *P. nigrescens* 22B; lane 7, *P. nigrescens* VPI-8944.

Table 1**Bacterial Strains**

Bacterial species	Strain	Country of Origin	Antibiotic resistance	Reference
<i>Prevotella nigrescens</i>	PDRC-11	Florida, United States	T _c ^R	[18]
“	PDRC-22B	Florida, United States	T _c ^R	[18]
“	VPI-8944 (ATCC 33563)	Virginia, United States	none	[40]
“	Pn28, Pn29, Pn32, Pn34	Osaka, Japan	T _c ^R	[36]
<i>P. denticola</i>	Osaka	Osaka, Japan	T _c ^R	[36]
<i>P. intermedia</i>	Pi17	Osaka, Japan	none	[36]
“	MRS1	Brazil	T _c ^R	This study

Table 2

tetQ Conjugation Efficiencies from *Prevotella* clinical isolates into recipient *P. nigrescens* VPI-8944.

Donor Strain	Conjugation Efficiency	Standard Deviation
<i>P. nigrescens</i> 28	4.34E-07	1.11E-07
<i>P. nigrescens</i> 29	1.63E-06	1.58E-06
<i>P. nigrescens</i> 32	6.65E-08	9.40E-08
<i>P. denticola</i> Osaka	1.18E-04	1.47E-04
<i>P. intermedia</i> MRS1	<10 ⁻⁹	ND
<i>P. nigrescens</i> PDRC-22B	3.56E-04	5.01E-04
<i>P. nigrescens</i> PDCR-11	8.12E-05	1.11E-05