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Genetic analysis of mobile *tetQ* elements in oral *Prevotella* species

Gena D. Tribble [a,*], John J. Garza [a], Victor L. Yeung [a], Todd W. Rigney [a], Doan-Hieu V. Dao [a], Paulo H. Rodrigues [b,1], Clay B. Walker [b], Charles J. Smith [c]

[a] Department of Periodontics, Dental Branch, University of Texas Health Science Center at Houston, Houston, TX 77030, USA
[b] Department of Oral Biology and Center for Molecular Microbiology, College of Dentistry, University of Florida, Gainesville, FL 32610-0424, USA
[c] Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, Greenville, NC 27834, USA

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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.

1. 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 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 *P. 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.

* Corresponding author. 6516 M.D. Anderson Blvd, Room 319, University of Texas Dental Branch, Houston, TX 77030. Tel.: +1 713 486 4483; fax: +1 713 500 4393.
E-mail address: gena.d.tribble@uth.tmc.edu (G.D. Tribble).
[1] Current address: Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP 05587-050, Brazil.

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Table 1
Bacterial Strains.

Bacterial species	Strain	Country of Origin	Antibiotic resistance	Reference
Prevotella nigrescens	PDRC11	Florida, United States	Tc^R	[18]
Prevotella nigrescens	PDRC22B	Florida, United States	Tc^R	[18]
Prevotella nigrescens	VPI-8944 (ATCC 33563)	Virginia, United States	none	[40]
Prevotella nigrescens	Pn28, Pn29, Pn32, Pn34	Osaka, Japan	Tc^R	[36]
Prevotella denticola	Osaka	Osaka, Japan	Tc^R	[36]
Prevotella intermedia	Pi17	Osaka, Japan	none	[36]
Prevotella intermedia	MRS1	Brazil	Tc^R	This study

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*.

2. Materials and methods

2.1. 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. Selected *Prevotella* strains were made rifampin resistant (MIC> 30 μg/ml) by serial passage on increasing concentrations of the antibiotic. *Escherichia coli* strains DH5α, S17-1, and STBL4 (Invitrogen) were grown in Luria-Bertani (LB) media supplemented as needed with ampicillin (100 μg/ml).

2.2. 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*) (Fig. 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 (Fig. 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

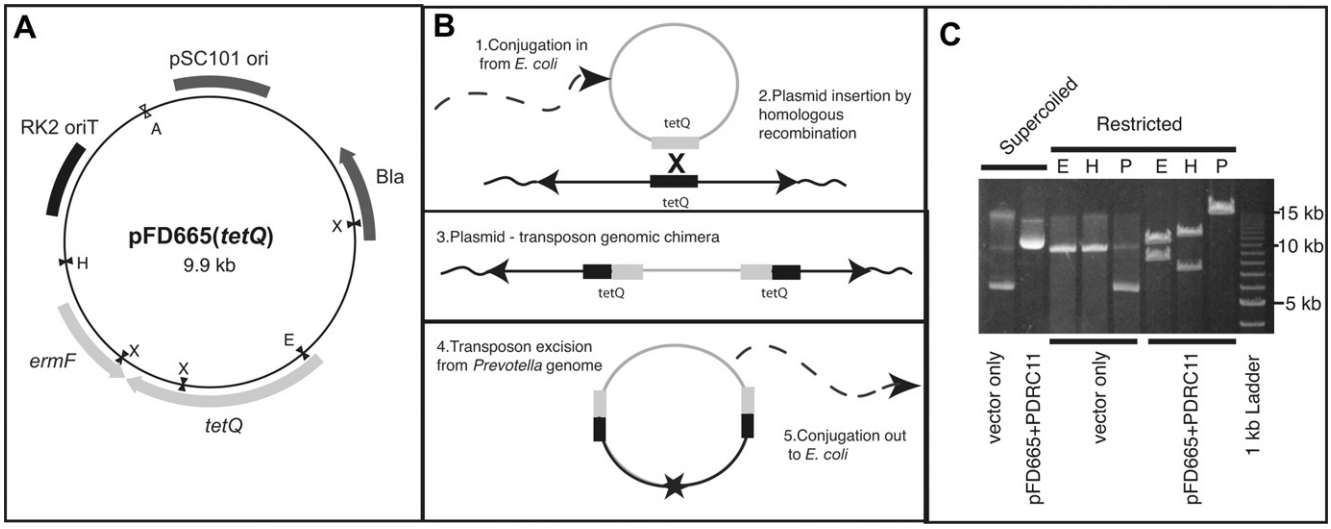

Fig. 1. Transposon Capture Method. 1A. *E. coli–Prevotella* suicide vector pFD665, with 2.3 kb *tetQ* gene. Genes functional in *Prevotella* are shown in light gray, those functional in *E. coli* are shown in dark gray. The origin of transfer is shown in black. 1B. Transposon capture is mediated by homologous recombination of pFD665(*tetQ*) (light gray) into the integrated *tetQ* transposon (black). 1C. Restriction digest of captured *tetQ* transposon in pFD665. In panels A and C, restriction enzymes are represented by the following abbreviations: E - Eco RI, H - Hind III, P - Pst I, X - Xba I, A - Ava I.