Identification of Tet45, a tetracycline efflux pump, from a poultry-litter-exposed soil isolate and persistence of tet(45) in the soil

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Objectives: To characterize a tetracycline resistance (TcR) determinant, closely related to the TetL efflux pump, in a Bhargavaea cecembensis strain previously isolated from a poultry-litter-impacted soil.

Methods: Genomic DNA of B. cecembensis DMV42A was shotgun cloned and expressed in Escherichia coli. Antimicrobial susceptibility testing and a [3H]tetracycline uptake assay were used to confirm the function of the target gene. Transferability of the gene was examined using filter matings and confirmed by PCR and sequencing. Real-time quantitative PCR was performed on soil metagenomic DNA to evaluate the prevalence of the gene in the soil from which B. cecembensis DMV42A was isolated and in more pristine local soils.

Results: The TcR determinant from B. cecembensis DMV42A, designated Tet45, was identified as a tetracycline efflux pump sharing 78% amino acid identity with certain TetL proteins. In B. cecembensis DMV42A, tet(45) was adjacent to truncated and non-functional arsenic resistance genes with high sequence similarities to genes from staphylococcal plasmids. After filter matings, the tet(45) gene could be found in E. coli transconjugants, although the transfer mechanism was unknown. Tet45 homologues are also present in the genomes of several Bacillus cereus strains and a Bacillus thuringiensis strain. tet(45) was detected in the poultry-litter-impacted soil, and persisted at a similar level 2 years after removal of the chicken waste, although it was not detected in several more pristine soils.

Conclusions: Tet45 is a tetracycline efflux pump closely related to TetL. Horizontal gene transfer may have contributed to the dissemination and persistence of tet(45) in a poultry-litter-impacted soil.

Keywords: tetracycline resistance, efflux pump, Tn5397-like element, poultry farm soil

Introduction

Bacterial resistance to tetracyclines occurs primarily through three mechanisms: actively pumping the antibiotics out of the cell, producing ribosomal protection proteins, or enzymatic degradation of the antibiotics.1 The first two are the predominant mechanisms found in clinical settings.2 So far 44 classes of tetracycline resistance (TcR) determinants have been identified, of which 28 are efflux pumps (http://faculty.washington.edu/marilynr/; last updated October 2012).1–3 Twenty-five of the efflux pumps belong to the major facilitator superfamily and utilize proton motive force (PMF).1–3 Tetracycline efflux genes exist in both Gram-negative and Gram-positive bacteria. Those found in the former are normally carried by large conjugative plasmids, whereas those in the latter are often on small transmissible plasmids that are occasionally found integrated into chromosomes.1

The newly characterized Bhargavaea cecembensis strains are Gram-positive bacteria in the order Bacillales, and were previously shown to be susceptible to tetracycline.4 Several TcR B. cecembensis strains were isolated from a soil adjacent to a chicken farm waste storage shed, and all were shown to contain tet(L) genes based on a PCR assay.5 However, subsequent sequencing of those PCR-amplified gene fragments revealed that the gene in one strain, DMV42A, was potentially distinct from the tet(L) genes in the other strains, as well as all known tet(L) genes. In this study, to characterize that gene, we cloned B. cecembensis DMV42A genomic DNA, which led to the identification of a new tetracycline efflux pump, designated Tet45 by the nomenclature centre.6 Additionally, a Tn5397-like transposon carrying tet(M) was identified in strain DMV42A after cloning. Real-time quantitative PCR (qPCR) found tet(45) and tet(M) genes in the poultry-litter-impacted soil at 10^4 – 10^5 copies/g of wet soil when the farm was in operation, and at 10^4 copies/g of wet soil 2 years after closure of the farm and removal of the waste. In contrast, neither of the tested tet genes was detected at a much less contaminated site on the same farm, or at several sites in a local state forest that had not been in agricultural use for decades.
Materials and methods

Bacterial strains and plasmids

Four *B. cecembensis* strains, previously shown to contain tet(L) genes, were used in this study (Table 1). These strains were isolated from a soil located adjacent to a waste storage shed on a chicken farm as described in You et al. The farm had been operated for over 20 years, but closed down shortly after we had collected the soil samples in 2008. In 2010, a second set of soil samples were collected from the same location on the farm (also described in You et al.). Additional bacterial strains and plasmids used in this study are also listed in Table 1.

DNA manipulation and PCR

Soil metagenomic DNA, genomic DNA and plasmid DNA were extracted using the PowerMax Soil DNA Isolation Kit or PowerSoil DNA Isolation Kit (MoBio Labs), the DNeasy Blood & Tissue Kit (Qiagen) and the PowerMax Soil DNA Isolation Kit or PowerSoil DNA Isolation Kit (MoBio Labs), the DNeasy Blood & Tissue Kit (Qiagen) and the QiAprep Spin MinPrep Kit (Qiagen), respectively. Extraction yield and DNA quality were evaluated using a NanoDrop spectrophotometer. PCR was performed using proofreading ExTaq DNA polymerase (Takara) and the primers listed in Table 2. A typical PCR consisted of an initial denaturation at 94°C for 5 min followed by 30–35 cycles of 94°C for 45 s, the annealing temperature (Table 2) for 1 min and 72°C for 1 min, followed by a final extension at 72°C for 10 min. Positive and negative controls were included in each run. For colony PCR, half of a colony was added to 50 μL of sterilized Milli-Q water or ultrapure water (Quality Biological, Inc.) and dispersed by thorough vortexing, after which the cells were lysed by heating at 100°C for 5 min. After centrifugation at 3000 g for 5 min, 2 μL of the supernatant was used as template.

Cloning of TcR determinants

*B. cecembensis* DMV42A genomic DNA was digested with EcoRI (Promega), ligated (using T4 DNA ligase or the Quick Ligation Kit, NEB) into EcoRI-digested and dephosphorylated (using antarctic phosphatase, NEB) pUC18 and transformed into *E. coli* TOP10 cells (Invitrogen) according to the manufacturer's protocol. Prior to ligation, the DNAs were purified using the QIAquick PCR Purification Kit (Qiagen). Transformants were selected on LB agar containing 50 mg/L of ampicillin (to select for the vector) and 8 mg/L of tetracycline (to select for TcR-encoding inserts).

DNA sequencing and sequence analysis

PCR products and cloned inserts were sequenced on both strands using a 3730xl DNA Analyzer (Applied Biosystems) at the Johns Hopkins University School of Medicine Sequencing Facility. PCR products were sequenced using PCR primers. The inserts in pUC18 were initially sequenced using universal M13/pUC18 forward and reverse primers, and subsequently by primer walking. SeqMan Pro (DNAStar) was used for contig assembly. Open reading frames (ORFs) were identified using the NCBI ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and Glimmer program, followed by BLASTP and conserved domain searches to identify potential coding sequences. Transmembrane helices were predicted using TMHMM 2.0. The reliability of the resulting tree was assessed by bootstrap analysis of 1000 replicates. Genome sequences involved in comparisons included *NZ_ABD40000000, NC_0000715*, *NZ_JH72281*, *NZ_JH79208*, *NZ_JH791969*, *NZ_CM000717*, *NC_0000756*, *NC_0000741*, *NC_014829*, *NC_006270*, *NC_003997*, *NC_007052*, *NC_AB00000000*, *NZ_AA01000000*, *NZ_ADJ80000000*, *NC_010382*, *NC_006510*, *NC_009328*, *NC_012793*, *NC_AEP00000000* and *NC_009089*. A new tetracycline efflux gene tet(45)

Antimicrobial susceptibility testing

The MICs of tetracycline, sodium arsenite, sodium arsenate and 3-nitro-4-hydroxyphenylarsonic acid (roxarsone) were determined by a 2-fold broth microdilution method to test the functionality of cloned genes. Exponentially growing cultures with an OD 600 reading of 0.2 were 500-fold diluted with antimicrobial-free media. The diluted culture (100 μL) was inoculated into an equal volume of the same medium containing individual antimicrobials in a microtitre plate. *B. cecembensis* cultures were incubated at 30°C for 48 h, and *E. coli* cultures at 37°C for 24 h. Ampicillin (50 mg/L) was added to *E. coli* cultures for plasmid maintenance. Assays were performed in duplicate.
Table 2. Primers used for conventional PCR, qPCR and DNA sequencing

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>Target</th>
<th>Sequence (5′→3′)</th>
<th>Annealing temperature (°C)</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
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</thead>
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<tr>
<td>TetL45-Fb</td>
<td>tet(L) and tet(45)</td>
<td>TTTTCCTCTTGAGCGTTTATGC</td>
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<td>171</td>
<td>this study</td>
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<tr>
<td>TetM-F</td>
<td>tet(M)</td>
<td>TGGCGTGTCTATGATGTTCAC</td>
<td>55</td>
<td>145</td>
<td>this study</td>
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<td>TetM-R</td>
<td>tet(M)</td>
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<td>this study</td>
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<td>TetL45-R</td>
<td>tet(45)</td>
<td>TTTTCCTCTTGAGCGTTTATGC</td>
<td>63</td>
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<tr>
<td>Tet45-R</td>
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<td>this study</td>
</tr>
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<td>Tn916-F</td>
<td>orf13 genes of Tn916-family elements</td>
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<td>63</td>
<td>145</td>
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<td>this study</td>
</tr>
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<td>16S-F</td>
<td>16S rRNA genes</td>
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<td>29</td>
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<tr>
<td>16S-R</td>
<td>16S rRNA genes</td>
<td>GGTGTTAAGGTGCGTGGGTTGA</td>
<td>60</td>
<td>142</td>
<td>29</td>
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</tbody>
</table>

*aSome annealing temperatures varied from references.  
*bThese primers amplified a similar internal region of both tet(L) and tet(45) genes.  
*cThese primers did not target Tn5397-type tet(M).

[^H]tetracycline uptake assay

The tetracycline efflux function of Tet45 was tested for as previously described.\(^1\) E. coli TOP10 cells containing p4T22 or pUC18 were grown to exponential phase in LB broth supplemented with ampicillin and tetracycline or ampicillin alone, respectively. The cells were washed twice with PBS and then resuspended in PBS. The suspensions was immediately measured using a Beckman LS 3801 liquid scintillation counter with Opti-Fluor liquid scintillation cocktail (Perkin Elmer), and the OD\(_{530}\) was recorded. 2,4-Dinitrophenol (1 mM, Sigma-Aldrich) was added to certain reactions at 25 min to de-energize the cells by collapsing their PMF. All assays were performed in duplicate.

Transformation and conjugation assays

To identify Tc\(^6\) encoding plasmids in B. cecembensis DMV42A, undigested genomic DNA from this strain was transformed into E. coli TOP10, and Tc\(^6\) transformants were selected for on LB agar containing tetracycline (4 or 8 mg/L).

Filterings similar to those reported previously\(^12\) were performed to test the transferability of the Tc\(^6\) determinants identified in B. cecembensis DMV42A. An ampicillin-resistant (Amp\(^R\)) green fluorescent protein (gfp)-expressing E. coli strain DH5\(_x\) pGFPmut3 (Table 1; tetracycline MIC 2 mg/L) was used as a convenient recipient to determine whether the tet genes in the Gram-positive donor could transfer to other bacteria, including distantly related Gram-negative species. The recipient’s Amp\(^R\) and fluorescent green colour helped to distinguish between recipients/transconjugants and breakthrough growth of donors on the selection plates.

Donor and recipient cultures were grown to exponential phase in LB broth supplemented with ampicillin and tetracycline (4 mg/L). Transfer of tet(45) was confirmed in certain transconjugants by colony PCR (using primers TetL45-F/ TetL45-R or Tet45-F/Tet45-R listed in Table 2) followed by DNA sequencing. Control experiments involving the strain alone were performed similarly.

qPCR assays

qPCR was performed on metagenomic DNA using a Bio-Rad iCycler to determine the abundance of the Tc\(^6\) determinants in soil samples. Eubacterial 16S rRNA genes, tet(45), tet(M) and the orf13 genes of Tn5397 and Tn916-like elements (Tn916, Tn2010, Tn5251, Tn5397, Tn6002 and Tn6003) were targeted using primers listed in Table 2. Typical reaction mixtures contained 2 \(\mu\)L of the DNA template, 0.48 \(\mu\)M of each primer and IQ SYBR Green Supermix (Bio-Rad). The thermal programme consisted of an initial denaturation at 95°C for 3 min followed by 40 cycles of 10 s at 95°C, 20 s at the annealing temperature (Table 2) and 30 s at 72°C. Standard curves (4–6 log range in duplicate or triplicate, \(R^2>0.99\)) were generated using plasmids listed in Table 1, with amplification efficiencies of 90%–110%. For each reaction, the detection limits were <85 copies for 16S rRNA genes, <51 copies for tet(45), <121 copies for tet(M), <23 copies for Tn5397-orf13 and <138 copies for Tn916-orf13. Three to six independent serial dilutions (up to 10\(^{-3}\)) of each soil DNA were subjected to qPCR, and reaction specificity was verified by a melt curve analysis as well as qPCR product sequencing. The copy number of each gene was normalized to 16S rRNA gene abundance to account for variations between soil samples in DNA extraction efficiencies and bacterial numbers.
Results

Cloning and sequencing of TcR determinants from B. cecembensis DMV42A

The TcR B. cecembensis strain DMV42A (tetracycline MIC 32 mg/L) was originally suggested to contain tet(L)5 by PCR analysis involving tet(L)-specific primers used in previous studies.13 However, sequencing of the PCR-generated gene fragment showed that this gene shared only \( \approx \) 80% nucleotide identity with known tet(L) genes. To obtain the complete sequence of this gene, fragments of DMV42A genomic DNA were cloned in E. coli TOP10, which yielded TcR E. coli TOP10 transformants. Two distinct plasmids were identified in these transformants, suggesting that at least two tet genes might be present in DMV42A. One plasmid, p4T22, contained a 6 kb insert, while the other, pT11RSC, contained an 18 kb insert. Sequencing was performed to identify the tet genes cloned in p4T22 and pT11RSC, which conferred tetracycline MICs of 64 and 32 mg/L on E. coli TOP10, respectively.

Identification of a new tetracycline efflux pump Tet45

Sequencing of the 6 kb insert in p4T22 (JF837331) identified an ORF of 1377 bp, whose translated 458-amino-acid sequence showed 78% identity to TetL efflux pumps encoded by several small mobilizable plasmids (e.g. pSU1 reported in You et al.5). The gene did not have the characteristic pattern of mosaic genes, such as those encoding ribosomal protection proteins;2

**Figure 1.** Phylogeny of tetracycline efflux pumps. Protein sequences were aligned by ClustalX 2.0 using the BLOSUM series,9 and the neighbour-joining tree was generated by MEGA4 using the JTT model.10 The bootstrap consensus tree was inferred from 1000 replicates, and rooted using Otr(C) not within the major facilitator superfamily. Branches with bootstrap values < 50 were collapsed. The bar represents 0.5 amino acid substitutions per site. Grey shading highlights group 2 tetracycline efflux pumps.
in that no section had significantly higher identity to tet(L) than others (also see Figure S1, available as Supplementary data at JAC Online). This determinant was assigned the name Tet45 by the nomenclature centre.6 Tet45 belonged to the group 2 tetracycline efflux pumps (Figure 1),1,2 which have 14 transmembrane α-helices. An ORF encoding a putative leader peptide was identified immediately upstream of tet(45).

The functionality of Tet45 was tested by comparing [3H]tetracycline accumulation in E. coli TOP10 p4T22 with that in E. coli TOP10 pUC18. Over the entire period of the experiment, cells bearing tet(45) contained lower levels of [3H]tetracycline than those bearing the cloning vector alone (Figure 2). The addition of 2,4-dinitrophenol, a protonophore that collapses the PMF, resulted in increased tetracycline accumulation in E. coli TOP10 p4T22, but decreased accumulation in E. coli TOP10 pUC18. These results were similar to the reported behaviour of various tetracycline efflux pumps in E. coli.11,14 thus indicating that Tet45 also conferred resistance by actively pumping tetracycline out of the cell. This function was not investigated in B. cecembensis, due to the unavailability of a tet(45) deletion mutant.

**tet(45) homologues are present in Bacillus spp. strains, although the flanking regions differ**

PCR using tet(45)-specific primers (Tet45-F/Tet45-R in Table 2) confirmed that the other three B. cecembensis strains (listed in Table 1), isolated from the same poultry-litter-impacted soil as DMV42A,5 did not contain tet(45). Nonetheless, a BLASTP search against the microbial genome database identified Tet45 (AE6M2953) homologues (>90% amino acid identities) in genome-sequenced Bacillus cereus strains (ZP_04320974 in ATCC 10876 and ZP_03233850 in AH1134). However, their flanking regions differed from the tet(45)-flanking regions in B. cecembensis DMV42A (Figure 3). In the B. cereus genomes, several metabolic genes are located downstream of the tet(45) homologues, while in p4T22 two divergently transcribed genes, maf and radC, were identified downstream of tet(45). maf and radC are normally associated with the mrrBCD cluster in the order Bacilales.15 which was not observed in B. cecembensis DMV42A. In p4T22 two truncated acr3 genes, encoding putative arsB-like efflux pumps, were identified upstream of tet(45), with an arsR gene located further upstream, encoding a putative transcriptional regulator. Arsenic resistance genes were not found adjacent to the B. cereus tet(45) homologues.

MICs of arsenite, arsenate and roxarsone were the same for E. coli TOP10 p4T22 and E. coli TOP10 pUC18, indicating that the truncated acr3 genes did not confer arsenite resistance on E. coli. Moreover, B. cecembensis DMV42A showed no significant difference in arsenic resistance levels when compared with the other B. cecembensis strains included in this study (data not shown), although whether these strains contained arsenic resistance genes was unknown.

Including one of the acr3 genes, the cloned B. cecembensis DMV42A genomic fragment contained several putative genes whose products showed significant sequence similarities to proteins encoded by plasmids, including p18813-P04 from an epidemic Staphylococcus aureus isolate16 and SAP008A from a poultry litter staphylococcal strain17 (Table S1, available as Supplementary data at JAC Online). A disparity of G+C content was also observed within the cloned B. cecembensis DMV42A genomic fragment (Figure 3). While tet(45) and the region upstream showed a G+C content of 33.3%, the region downstream of tet(45) showed a G+C content of 51.1%, much closer to the 59.5% G+C content of the B. cecembensis type strain DSE10.14 A recent search of the microbial genome database identified further Tet45 homologues (>90% amino acid identities) in other B. cereus strains (VD022, VD045, BAG3X2-2 and 172560W) and in a Bacillus thuringiensis strain, BGS-4BD1. While the flanking regions of these homologues varied from one genome to another, none contained arsenic resistance genes as observed for the tet(45)-flanking region in B. cecembensis DMV42A.

**Identification of a Tn5397-like element carrying tet(M) in pT11RSC**

Sequencing of the 20 kb insert in pT11RSC (JF915701) identified a tet(M)-carrying element (Figure S2, available as Supplementary data at JAC Online). This element was 100% identical to the conjugative transposon Tn3 (a variant of Tn5397) in Clostridium difficile 630,18 excluding a 2647 bp deletion (containing the group II intron) within orf14 and a tandem 8 bp direct repeat within orf6. As in C. difficile 630, the Tn5397-like element integrated into the fic region in B. cecembensis DMV42A, likely reflecting target-site selection.19 Sequences further upstream of the element were predicted to encode a UV damage endonuclease, a copper transport repressor and a copper transport ATPase (Table S1, available as Supplementary data at JAC Online). While the Tn5397-like element showed a G+C content of 38.9%, the genomic region upstream of the element showed a G+C content of 54.9%, which was closer to the 59.5% G+C content of the B. cecembensis type strain DSE1017 (Figure S2, available as Supplementary data at JAC Online).8

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You et al.
Transferability of the B. cecembensis DMV42A tet(45) gene

Matings between B. cecembensis DMV42A and the TcR AmpR gfp-expressing E. coli recipient, performed under the 4 mg/L tetracycline condition, successfully generated Tc R AmpR gfp-expressing E. coli colonies. Screening of 21 of these colonies by PCR confirmed the presence of tet(45) in eight E. coli transconjugants. Sequencing of a PCR-amplified tet(45) fragment (GU584222) in one such transconjugant confirmed that it was identical to the tet(45) gene of the donor. Thus, tet(45) appeared transferable between B. cecembensis DMV42A and the E. coli strain, although the mechanism of transfer was obscure. Previously, an alkaline lysis method yielded no plasmids from B. cecembensis DMV42A, and in this study transforming undigested genomic DNA from B. cecembensis DMV42A into E. coli TOP10 did not yield TcR transformants, suggesting that plasmids might not play a role in the transfer process. The presence of maf and radC, two genes normally located on bacterial chromosomes, immediately downstream of tet(45) in B. cecembensis DMV42A (Figure 3) also suggested that this region was not plasmid borne. The possibility that tet(45) could be located on a large, low copy number plasmid has, however, not yet been refuted.

Persistence of TcR determinants in the poultry-litter-impacted soil

As shown in Table 3, tet(45), tet(M), Tn5397 (reflected by its orf13 gene) and various Tn916-like elements (reflected by their orf13 genes) were detected in the poultry-litter-impacted soil, from which B. cecembensis DMV42A was isolated, in both 2008 (before the farm closed down) and 2010 (2 years after shutdown and removal of all the waste). They were not detected in a more pristine soil collected ≈10 m away from the chicken waste shed or in soils collected from a local state forest. While only a subset of tet(M) genes (non-Tn5397 type) were targeted by the primers used here, more copies of tet(M) than tet(45) (P < 0.001, Welch test) were identified in the poultry-litter-impacted soil, in both 2008 and 2010. Over the 2 year period, the copy number of all tested genes declined, although only
although this strain showed a high spontaneous TcR mutation.

terias was generated using an E. coli genes, several putative genes in the acr3 staphylococcal plasmids, including one plasmid from poultry region showed high sequence similarity to genes encoded by tet genomes containing arsenic resistance genes, a feature not seen in other bacterial Tn tet genes identified in B. cecembensis. Experimental evidence that strain DMV42A could transfer tet genes to other bacteria was generated using an E. coli strain as the recipient, although this strain showed a high spontaneous Tc\(^8\) mutation rate (data not shown). Therefore, other strains including Gram-positive bacteria should be tested as recipients in future, and additional studies should be performed with various donor–recipient ratios. Meanwhile, since the tet(45)-containing region in B. cecembensis DMV42A showed no sign of a conjugal element, the transfer of tet(45) to E. coli could have been mediated by integrative and conjugal elements co-existing in the donor\(^{23,24}\) or by a mechanism other than conjugation. To understand the transfer mechanism requires a further investigation.

Concomitant with the identification of tet(45), we captured a mobile (data not shown) Tn5393-like element carrying tet(M) from the B. cecembensis strain. The Tn5397-tet(M) showed considerable sequence variation from other Tn916-tet(M) genes, and could not be detected\(^5\) by two commonly used primers.\(^20\) So far, Tn5397 and its derivatives have only been found in C. difficile and Enterococcus faecium,\(^{18,25,26}\) but these elements can transfer between C. difficile strains, between C. difficile and Bacillus subtilis, and between C. difficile and Enterococcus faecalis.\(^{25,27}\)

In conclusion, we have identified a novel tetracycline resistance determinant, tet(45), in a poultry-litter-exposed soil strain of B. cecembensis. Tet65 is closely related to TetL and both are group 2 tetracycline efflux pumps belonging to the major facilitator superfamily, utilizing PMF for function. Genes highly similar to tet(45) are present in the genomes of several B. cereus and B. thuringiensis strains.

Table 3. Persistence of two tet genes and the orf13 genes of Tn5397 and various Tn916-family elements in a poultry-litter-impacted soil

<table>
<thead>
<tr>
<th>Year</th>
<th>16S rRNA gene(^a)</th>
<th>tet(45)</th>
<th>tet(M)</th>
<th>Tn5397-orf13</th>
<th>Tn916-family orf13</th>
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<tr>
<td>2008</td>
<td>(5.03±1.20)x10(^{10})</td>
<td>(4.54±1.87)x10(^4)</td>
<td>(2.77±1.04)x10(^5)</td>
<td>(1.12±0.17)x10(^5)</td>
<td>(7.06±1.99)x10(^5)</td>
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<tr>
<td>2010</td>
<td>(6.56±1.62)x10(^9)</td>
<td>(1.74±0.79)x10(^4)</td>
<td>(5.01±2.17)x10(^6)</td>
<td>(6.69±0.55)x10(^4)</td>
<td>(1.29±0.42)x10(^5)</td>
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\(^a\)Previously reported data.\(^5\)

\(^b\)Data are mean±SD of replicate measurements from independent qPCR runs.

Discussion

Among the 44 tetracycline (tet) and oxytetracycline (otr) resistance genes characterized so far, 28 encode efflux pumps, most of which belong to the major facilitator superfamily, and use PMF to actively extrude the compounds. In this study, we report the discovery of a new tetracycline efflux gene, tet(45), in a B. cecembensis strain isolated from a poultry-litter-impacted soil. In B. cecembensis DMV42A, tet(45) was flanked by truncated arsenic resistance genes, a feature not seen in other bacterial genomes containing tet(45) homologues. Including one of the acr3 genes, several putative genes in the tet(45)-flanking region showed high sequence similarity to genes encoded by staphylococcal plasmids, including one plasmid from poultry litter,\(^17\) thereby indicating the potential source of the tet(45)-flanking region in B. cecembensis DMV42A. However, no plasmids were identified in this strain, although the presence of a large, low-copy-number plasmid could not be ruled out. Further studies are required to address this possibility and to validate poultry litter as a potential reservoir of the tet(45) and acr3 genes identified in B. cecembensis DMV42A. Experimental evidence that strain DMV42A could transfer tet(45) to other bacteria was generated using an E. coli strain as the recipient, although this strain showed a high spontaneous Tc\(^8\) mutation rate (data not shown). Therefore, other strains including Gram-positive bacteria should be tested as recipients in future, and additional studies should be performed with various donor–recipient ratios. Meanwhile, since the tet(45)-containing region in B. cecembensis DMV42A showed no sign of a conjugal element, the transfer of tet(45) to E. coli could have been mediated by integrative and conjugal elements co-existing in the donor\(^{23,24}\) or by a mechanism other than conjugation. To understand the transfer mechanism requires a further investigation.

Concomitant with the identification of tet(45), we captured a mobile (data not shown) Tn5393-like element carrying tet(M) from the B. cecembensis strain. The Tn5397-tet(M) showed considerable sequence variation from other Tn916-tet(M) genes, and could not be detected\(^5\) by two commonly used primers.\(^20\) So far, Tn5397 and its derivatives have only been found in C. difficile and Enterococcus faecium,\(^{18,25,26}\) but these elements can transfer between C. difficile strains, between C. difficile and Bacillus subtilis, and between C. difficile and Enterococcus faecalis.\(^{25,27}\)

In conclusion, we have identified a novel tetracycline resistance determinant, tet(45), in a poultry-litter-exposed soil strain of B. cecembensis. Tet65 is closely related to TetL and both are group 2 tetracycline efflux pumps belonging to the major facilitator superfamily, utilizing PMF for function. Genes highly similar to tet(45) are present in the genomes of several B. cereus and B. thuringiensis strains.

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Transparency declarations

None to declare.
Supplementary data

Figures S1 and S2, and Table S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


