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Genetic characterization and potential molecular dissemination mechanism of *tet*(31) gene in *Aeromonas caviae* from an oxytetracycline wastewater treatment system

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ABSTRACT

Recently, the rarely reported *tet*(31) tetracycline resistance determinant was commonly found in *Aeromonas salmonicida*, *Gallibacterium anatis*, and *Oblitimonas alkaliphila* isolated from farming animals and related environment. However, its distribution in other bacteria and potential molecular dissemination mechanism in environment are still unknown. The purpose of this study was to investigate the potential mechanism underlying dissemination of *tet*(31) by analysing the *tet*(31)-carrying fragments in *A. caviae* strains isolated from an aerobic biofilm reactor treating oxytetracycline bearing wastewater. Twenty-three *A. caviae* strains were screened for the *tet*(31) gene by polymerase chain reaction (PCR). Three strains (two harbouring *tet*(31), one not) were subjected to whole genome sequencing using the PacBio RSII platform. Seventeen *A. caviae* strains carried the *tet*(31) gene and exhibited high resistance levels to oxytetracycline with minimum inhibitory concentrations (MICs) ranging from 256 to 512 mg/L. *tet*(31) was comprised of the transposon Tn*6432* on the chromosome of *A. caviae*, and Tn*6432* was also found in 15 additional *tet*(31)-positive *A. caviae* isolates by PCR. More important, Tn*6432* was located on an integrative conjugative element (ICE)-like element, which could mediate the dissemination of the *tet*(31)-carrying transposon Tn*6432* between bacteria. Comparative analysis demonstrated that Tn*6432* homologs with the structure ISCR2-Δ*phzF*-*tetR*(31)-*tet*(31)-Δ*glmM*-*sul2* were also carried by *A. salmonicida*, *G. anatis*, and *O. alkaliphila*, suggesting that this transposon can be transferred between species and even genera. This work provides the first report on the identification of the *tet*(31) gene in *A. caviae*, and will be helpful in exploring the dissemination mechanisms of *tet*(31) in water environment.

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Introduction

Tetracycline resistance has been widely detected in different environmental niches (Andersen and Sandaa, 1994; Huang et al., 2016; Jiang et al., 2017; Li et al., 2010; Rhodes et al., 2000), and tetracycline-resistant bacteria have been used as potential indicators of antimicrobial resistance in water environments (Harnisz et al., 2011). Until now, more than thirty-eight tetracycline resistance (*tet*) genes were identified (Roberts, 2005), which *tet*(A), *tet*(G), *tet*(X), and *tet*(M) have been frequently detected in various environments (Ling et al., 2013; Liu et al., 2012; Ma et al., 2016; Shi et al., 2013; Zhang and Zhang, 2011) and in phylogenetically diverse bacterial genera (Chopra and Roberts, 2001; Roberts, 2005). Among these *tet* genes, *tet*(31) was firstly reported in *Aeromonas salmonicida* isolated from diseased fish (L'Abée-Lund and Sørum, 2000) in Norway. Until 2011, the rarely reported *tet*(31) gene was found commonly in *Gallibacterium anatis* isolated from chicken during the period 1965–2000 in Demark and Czech Republic. Recently, Leclercq et al. (2016) identified the *tet*(31) gene in a tetracycline resistant mobilome from a Chinese pig manure sample. Therefore, *tet*(31) was mainly found in farming animals and the related environmental samples containing antibiotics, which might pose a potential risk to the receiving water environment.

It has been reported that the dissemination of widely detected *tet*(A), *tet*(G), *tet*(X), and *tet*(M) genes has been associated with transposons (Coyne et al., 2011; Ghosh et al., 2009; Jasni et al., 2010; Lau et al., 2008); for example, members of the Tn*916* family, which belongs to the integrative conjugative element (ICE), have been reported to mediate the dissemination of the *tet*(M) gene among many commensal and pathogenic bacteria (Roberts and Mullany, 2009). Different from above *tet* genes, *tet*(31) was only found to be associated with an IS*CR2* (insertion sequence common region) (previously reported transposase_32) element in the chromosome of *G. anatis* (Bojesen et al., 2011). Since ISCR elements could only move from one genetic location to another in the same cell (Bennett, 2008), the molecular mechanism underlying the dissemination of the *tet*(31) gene between bacteria in environment is still unknown. At the same time, it is important to expand the host range of *tet*(31) in water environment.

In this study, we isolated 17 *A. caviae* strains carrying *tet*(31) from a biofilm reactor treating synthetic oxytetracycline bearing wastewater. Whole genome sequencing and subsequent bioinformatics analyses revealed that the *tet*(31) gene was carried by the novel transposon Tn*6432*, and is possibly disseminated in *A. caviae* isolates as part of a novel ICE-like element. This study will be helpful in exploring the potential molecular mechanisms of the dissemination of *tet*(31) between bacteria in water environment.

1. Materials and methods

1.1. Bacterial isolation and identification

Sludge samples were collected from an aerobic biofilm reactor treating synthetic oxytetracycline bearing wastewater (influent concentration of oxytetracycline: 25 mg/L) (Shi et al., 2018). Serial 10-fold dilutions of sludge samples were prepared in physiological saline, and 100-μL aliquots were added in triplicate to tryptic soy agar (TSA) and R2 agar (R2A) non-selective plates, respectively. These plates were incubated at 30°C for 12–48 hr. After incubation, all bacterial colonies with different morphologies were recovered from each plate and purified.

Genomic DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega Madison, USA) according to the manufacturer's instructions. All isolates were initially identified by 16S rRNA gene polymerase chain reaction (PCR) using bacterial universal primers 27F and 1492R (Appendix A Table S1) (Lane, 1991), according to a previously described procedure (Li et al., 2010). Identification at the species level of isolates belonging to *Aeromonas* was based on the sequencing of the housekeeping gene *gyrB* (encoding the β-subunit of DNA gyrase) using primers 3F and 14R (Table S1) (Yanez et al., 2003). To analyse the evolutionary process of *A. caviae*, a phylogenetic tree based on the *gyrB* gene was constructed by the neighbour-joining method using MEGA 5.0 software using the maximum-composite-likelihood distances (Tamura et al., 2011) and 1000 bootstrap replicates.

1.2. Antibiotic susceptibility testing

Susceptibility to oxytetracycline was determined by microdilution (96-well plates) (Andrews, 2001) using Mueller–Hinton (MH) broth, according to the standard guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2009), with oxytetracycline concentrations ranging from 0.25 to 1024 mg/L. After 18 hr of incubation at 37°C (Nawaz et al., 2006), minimum inhibitory concentrations (MICs) were determined when there was no visible bacterial growth (CLSI, 2009). The oxytetracycline breakpoint was resistant ≥16 mg/L (Penders and Stobberingh, 2008). The *Escherichia coli* strain ATCC 25922 was used for quality control.

1.3. Detection of tetracycline resistance genes

All *A. caviae* isolates were screened by PCR for the presence of *tet* genes, namely *tet*(A), *tet*(C), *tet*(G), *tet*(L), *tet*(31), *tet*(Q), and *tet*(X) (Appendix A Table S1). Bands of interest were further confirmed by direct sequencing, as mentioned above. The nearest matches were identified in GenBank using the BLASTN program. Bacterial strains carrying *tet* genes, as verified by sequencing, were used as positive controls, and standard PCR mixtures without DNA template were used as negative controls. To analyse the evolutionary process of the *tet*(31)-positive isolates in the present study and deposited in the GenBank database, a phylogenetic tree based on the 16S rRNA gene was constructed using the method mentioned above.

1.4. Genome sequencing and analysis

Genomic DNA of three *A. caviae* strains, two of which harboured the *tet*(31) gene and one not, were extracted using the Wizard® Genomic DNA Purification Kit (Promega Madison, USA), as described above. The SMRTbell™ library preparation

was performed according to the manufacturer's instructions (Pacific Biosciences, Menlo Park, CA, USA) following the Procedure & Checklist — 10 kb Template Preparation using BluePippin™ Size-Selection System. The SMRT sequencing was carried out at the Beijing Institute of Genomics (China) on a PacBio RSII platform (Pacific Biosciences) using P6-C4 chemistry with average median depth of coverage 100× (Khong et al., 2016). De novo assembly of the three genomes was performed using the hierarchical genome assembly process (HGAP3, SMRTAnalysis 2.3.0) workflow with standard parameters. Full chromosome sequences were further polished using Quiver to obtain high consensus accuracy (Chin et al., 2013). Genome annotation was primarily conducted using the RAST (Rapid Annotation using Subsystem Technology) tool (Aziz et al., 2008), then checked and eventually corrected manually using BLASTX in the NCBI-NR database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Annotations of the antibiotic resistance genes (ARGs), insertion sequences (ISs), integrons, and transposons were carried out using online databases, including the Comprehensive Antibiotic Resistance Database (CARD) (http://arpcard.mcmaster.ca) (McArthur et al., 2013), ISfinder database (https://www-is.biotoul.fr/) (Siguier et al., 2006), INTEGRALL database (http://integrall.bio.ua.pt/) (Moura et al., 2009), and Tn Number Registry (Roberts et al., 2008), respectively. IS and transposon terminal inverted repeats (IRs) and other features were identified by comparison with the reference sequences in the ISfinder database and known published elements. Sequence comparisons and alignments were performed using Easyfig software (version 2.2.2) (Sullivan et al., 2011) and BLASTN. Gene organization diagrams were drawn in the tool Inkscape (version 0.92.2) (www.inkscape.org). Furthermore, genomic islands (GIs) were predicted in the three *A. caviae* genomes using IslandViewer 4 (Bertelli et al., 2017).

1.5. PCR analysis of the genetic environment of tet(31)

Transposon Tn*6432* was tested in 15 additional *A. caviae* isolates through PCR, with the forward primer targeting the ISCR2 transposase gene and reverse primer targeting the *tet*(31) gene (Appendix A Table S1). Three of the PCR products were then sequenced.

1.6. Nucleotide sequence accession number

Complete genome sequences of *A. caviae* strains R25-2, R25-6, and T25-39 were deposited in the NCBI database with accession numbers CP025777, CP025705, CP025706. Other sequences of *A. caviae* were also deposited in the NCBI database with accession numbers ranging from MG737558 to MG737580 for 16S rRNA, MG751854 to MG751876 for the *gyrB* gene, MG737581 to MG737583 for the *tet*(31) gene, and MG737584 to MG737586 for Tn*6432*.

2. Results and discussion

2.1. Taxonomic, oxytetracycline resistance phenotypes and genotypes of A. caviae

A total of 23 *A. caviae* strains were recovered from the sludge samples collected from a biofilm reactor exposed to 25 mg/L of oxytetracycline for three months. Phylogenetic analysis of the *gyrB* gene showed that the *A. caviae* isolates contained two clades (Appendix A Fig. S1). Clade 1 included 17 isolates and was 100% identical at the *gyrB* locus to the *A. caviae* clone DK-A.caviae-14 (GenBank no. KJ747119.1). Clade 2 contained six isolates and showed 99% identity with the *A. caviae* clone DK-A.caviae-15 (GenBank no. KJ747120.1). The two clades showed 98% identity to each other.

As shown in Table 1, all *A. caviae* isolates exhibited high resistance levels to oxytetracycline, with MICs ranging from 256 mg/L to >1024 mg/L. Seven *tet* genes were further screened in the *A. caviae* isolates by PCR. As shown in Table 1, *tet*(31) was detected in 73.9% (17/23) of isolates; however, the other *tet* genes, including *tet*(A), *tet*(C), *tet*(G), *tet*(L), *tet*(Q), and *tet*(X), were not detected. To our knowledge, this is the first report on the *tet*(31) gene in *A. caviae* isolated from an oxytetracycline wastewater treatment system.

tet(31) was firstly found in *A. salmonicida* isolated from diseased fish in 2000. As a matter of fact, this gene appeared in chicken pathogenic *G. anatis* in 1965, and was a common determinant in *G. anatis* isolated from chickens from very different production systems and localities (Czech Republic and Denmark) (Bojesen et al., 2011). However, few studies have reported about this gene. Until now, the host range of *tet*(31) is very limited (Fig. 1). Only *A. salmonicida*, *G. anatis*, and *O. alkaliphila* were found to harbour the *tet*(31) gene (Bojesen et al., 2011; L'Abée-Lund and Sørum, 2000; Lauer et al., 2015). As shown in Fig. 1, all the *tet*(31)-positive bacteria were phylogenetically distant from each other, except that *A. salmonicida* and *A. caviae* belongs to the same genus.

Table 1 – Antimicrobial susceptibility of *A. caviae*, determined by broth microdilution, and the presence of the *tet*(31) gene and transposon Tn*6432* by PCR.

Strain	MIC (mg/L)	*tet*(31)	Tn*6432*
R25-5	>1024	–	–
R25-6	>1024	–	–
R25-41	>1024	–	–
R25-42	>1024	–	–
R25-43	>1024	–	–
T25-40	>1024	–	–
R25-2	256	+	+
R25-7	256	+	+
R25-39	256	+	+
T25-19	256	+	+
T25-24	256	+	+
T25-26	256	+	+
T25-30	256	+	+
T25-36	256	+	+
T25-38	256	+	+
T25-43	256	+	+
T25-44	256	+	+
T25-18	512	+	+
T25-35	512	+	+
T25-37	512	+	+
T25-39	512	+	+
T25-41	512	+	+
T25-58	512	+	+

–: *tet*(31) negative strains; +: *tet*(31) positive strains.

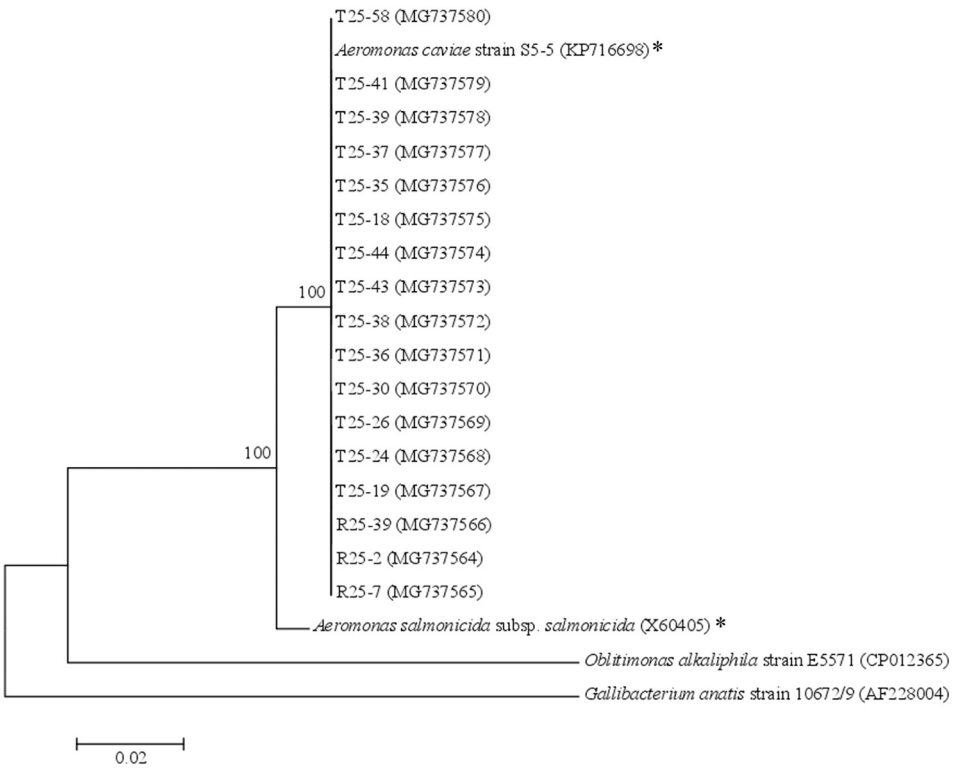

Fig. 1 – Phylogenetic distribution of *tet*(31)-carrying bacteria based on the nucleotide sequence of the 16S rRNA. The phylogenetic tree was constructed by the neighbour-joining method using MEGA 5.0 software *via* the maximum-composite-likelihood distances. *Aeromonas caviae strain S5–5 and Aeromonas salmonicida subspp. salmonicida were phylogenetic control without the *tet*(31) gene.

2.2. Genetic background of the tet(31) gene in A. caviae by whole genome sequencing

To further investigate the genetic environment of the *tet*(31) gene in the *A. caviae* isolates obtained in this study, PacBio RSII sequencing was used to determine the genome sequences of *A. caviae* strains R25-2 and T25-39 (carrying *tet*(31)) and R25-6 (not carrying *tet*(31)) (Table 1). We found that *tet*(31) (with 99.8% similarity with those reported in *A. salmonicida* and *G. anatis*) was located in the chromosomes of R25-2 and T25-39 (Fig. 2a), and was comprised of the IS*CR2*-*ISAhy2*-Δ*phzF*-*tetR*(31)-*tet*(31)-*ISAeca5*-Δ*glmM*-*sul2*-*IS256* unit, named transposon Tn*6432* in this study. Tn*6432* as a transposon was supported by the fact that it was flanked by 119-bp (upstream of IS*CR2* *tnpA*) and 302-bp (downstream of *sul2* gene) conserved regions, and showed more than 99% nucleotide sequence identity to all IS*CR2*-*sul2* putative transposons identified so far (Leclercq et al., 2016). Using PCR with specifically designed primers, Tn*6432* was also demonstrated to exist in the 15 additional *tet*(31)-positive *A. caviae* isolates (Table 1), indicating the prevalence of the Tn*6432* in *A. caviae* strains.

The *tet*(31) gene was first found on the plasmid pRAS2 of an *A. salmonicida* strain isolated from a diseased fish in Norway (L'Abée-Lund and Sørum, 2000), and then identified in the chromosome of chicken pathogen *G. anatis* in Denmark (Bojesen et al., 2011). Interestingly, the *tet*(31) gene was located on the same genetic unit, IS*CR2*-Δ*phzF*-*tetR*(31)-*tet*(31)-Δ*glmM*-*sul2*, in the two host genomes (Fig. 2a). The same *tet*(31)-carrying unit was also found in the chromosome of *O. alkaliphila* (GenBank accession no. CP012365) from a historical collection in the USA (Lauer et al., 2015) (Fig. 2a). Recently, Leclercq et al. (2016) detected the IS*CR2*-Δ*phzF*-*tetR*(31)-*tet*(31)-Δ*glmM*-*sul2* structure (Tn*6300*) from a tetracycline mobilome within a Chinese pig manure sample, with an additional *IS10R* insertion between the *tetR*(31) and *tet*(31) genes (Fig. 2a). In our study, *tet*(31)-carrying Tn*6432* was similar to Tn*6300* in structure except for various *IS* element insertions. The simultaneous presence of the *tet*(31)-containing genetic unit in different bacterial genera suggests the possibility of the horizontal transfer of this genetic unit (Bojesen et al., 2011; L'Abée-Lund and Sørum, 2000; Lauer et al., 2015).

Interestingly, the region surrounding Tn*6432* seems to be a hotspot for transposon integration. First, a complete Tn*floR* transposon (Doublet et al., 2005) composed of the florfenicol resistance gene (*floR*), *virD2*, Δ*lysR*, and full-length IS*CR2* transposase *tnpA* (Figs. 2a and 3a) was present. This transposon forms a circular intermediate that inserts at a specific TATACGT site (Doublet et al., 2005), which was clearly identifiable in our sequence data. Second, upstream of Tn*floR*, two streptomycin resistance genes, *strA* and *strB*, which are part of another known transposon, Tn*5393c* (L'Abée-Lund and Sørum, 2000), showed clear 5-bp direct repeats indicative of recent insertion into the current structure (Figs. 2a and 3a).

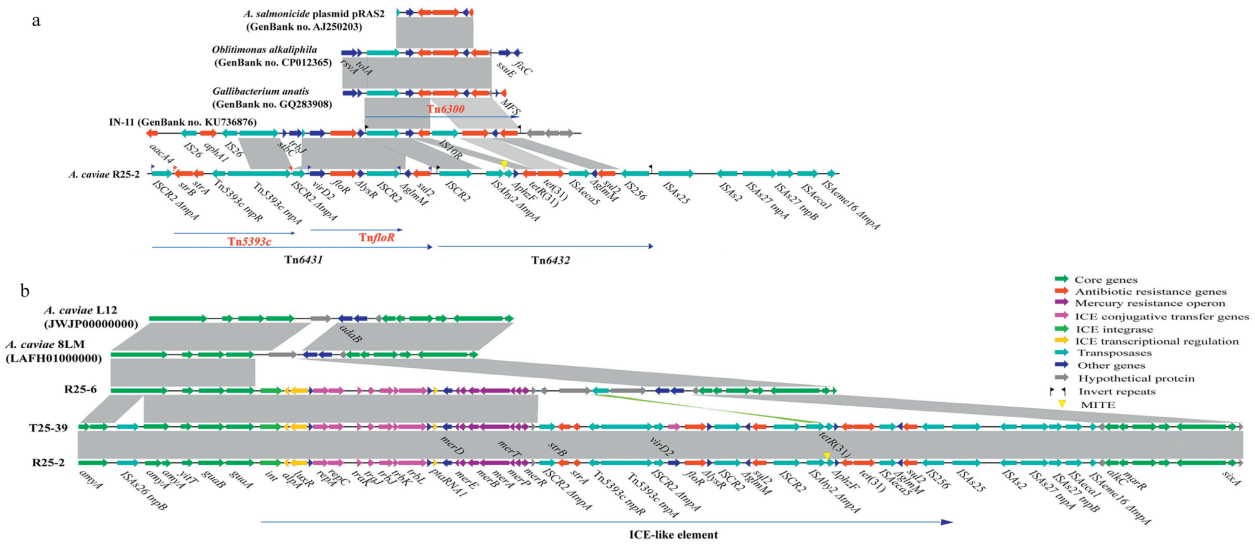

Fig. 2 – Genetic context of the *tet*(31) gene and comparison with related sequences in GenBank. (a) Comparison of the *tet*(31)-carrying fragment in *A. caviae* with related sequences in GenBank. (b) Comparison of the sequences harbouring the *tet*(31)-carrying ICE-like element with related sequences in *A. caviae* strains R25-6, L12, and 8LM in GenBank. *A. caviae* strain L12 was isolated from a fresh water lake in Malaysia, and strain 8LM was isolated from a patient in southern Brazil. Vertical blocks between sequences indicate regions of shared similarity at the nucleotide level, shaded according to BLASTN (grey for matches in the same direction and green for inverted matches). Gene names are given when known. ICE: integrative conjugative element; MITE: miniature inverted-repeated transposable element.

When Tn*floR* and Tn*5393c* were removed from the sequence, a new structure appeared, consisting of a full-length ISCR2 transposase *tnpA*, Δ*glmM*, and sulphonamide resistance gene *sul2* (Fig. 3a). The ISCR2…Δ*glmM*…*sul2* structure, including the inserted Tn*floR* and Tn*5393c*, might be another ISCR2-*sul2* transposon, which has not been reported previously and was

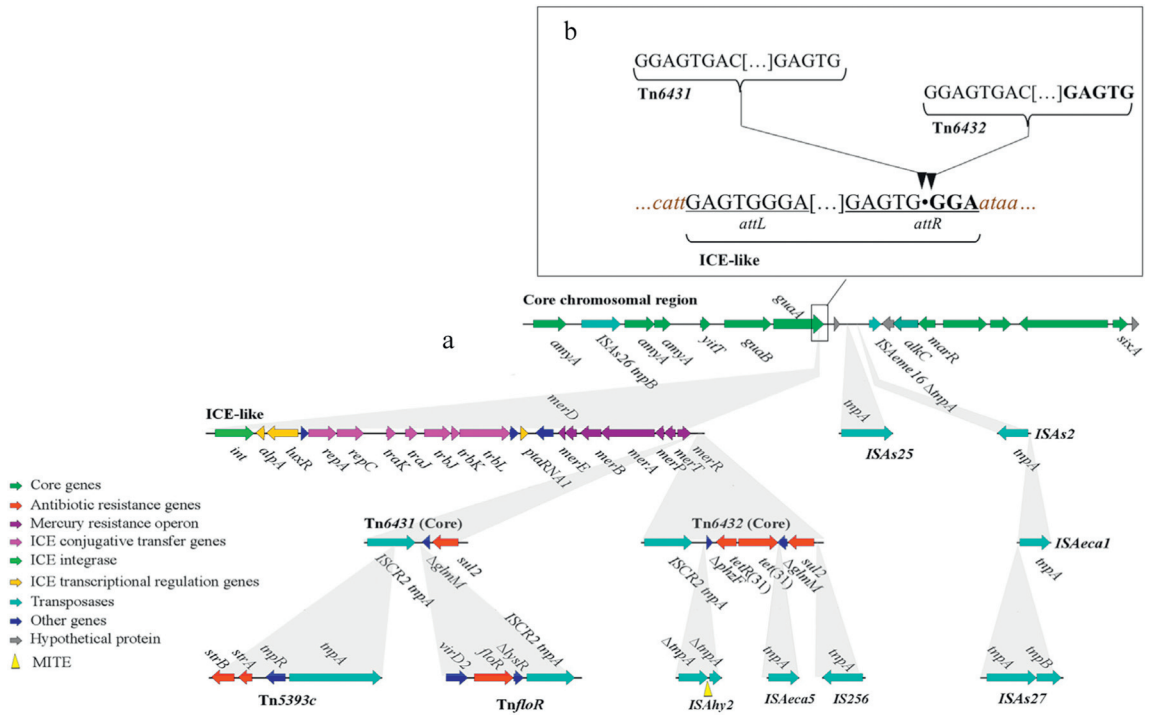

Fig. 3 – Decomposition of the *tet*(31)-carrying chromosomal region of *A. caviae* R25–2. (a) Each genetic unit is displayed independently, with all ORFs represented on scale. Gene names are given when known. Element insertion sites are represented by grey shading. (b) Sequence view of the ICE-like insertion site. *attR* reconstructed after Tn*6432* insertion is displayed in bold (5′-GAGTGGGA-3′).

named Tn*6431* in this study. Similar to Tn*6432*, Tn*6431* was also flanked by 119-bp (upstream of IS*CR2 tnpA*) and 302-bp (downstream of *sul2* gene) conserved regions, in agreement with a transposon unit (Leclercq et al., 2016).

2.3. Potential molecular mechanism underlying the dissemination of the tet(31) gene between bacteria

To further reveal how Tn*6431* and Tn*6432* were integrated into the chromosome of *A. caviae* strains R25-2 and T25-39, the genomic regions containing these transposons were compared to the sequences of three other *A. caviae* strains, including L12 isolated from a fresh water lake in Malaysia (GenBank no. JWJP00000000) (Chan et al., 2015), 8LM from a patient in southern Brazil (GenBank no. LAFH01000000) (Moriel et al., 2015), and the *tet*(31)-free sequenced strain R25-6 in the present study. Sequence analysis revealed that the two transposons were inserted at the end of a 38,598-bp ICE-like structure located downstream of the GMP synthase *guaA* in the R25-2 and T25-39 genomes (Fig. 2b).

ICEs are large mobile genetic elements, which can excise from the chromosome in a donor cell, transfer between cells *via* conjugation, and then integrate into the chromosome in a recipient cell (Wozniak and Waldor, 2010). The basic ICE structure is usually composed of four essential modules, including the integration/excision module that ensures intracellular mobility functions, replication/DNA processing module, DNA secretion module, and DNA regulation module (Ghinet et al., 2011; Guglielmini et al., 2011). It is thought that accessory genes were usually acquired by ICEs, which could temporarily decrease the fitness cost of the ICEs (Gaillard et al., 2008). Many studies have revealed that ICEs carried diverse ARGs, and contributed to the increase of antibiotic resistance (Michael et al., 2012; Roberts and Mullany, 2011; Whittle et al., 2001). The ICE-like structure identified in this study harboured core genes of an ICE, including an *int* integrase gene, *pta*RNA1, *alp*A, and *lux*R genes of the transcriptional regulation module, and *repA*, *repC*, *traK*, *traJ*, *trbJ*, *trbK*, and *trbL* genes homologous of the conjugative transfer system (Figs. 2b and 3a). The structure also harboured a mercury resistance operon, and transposons Tn*6431* and Tn*6432* (Fig. 2b).

The position of the ICE-like structure downstream of the *guaA* gene prompted our search for the 5′-GAGTGGGA-3′ pattern, which is a core motif of the integration site (*attB*) of almost all *guaA* family genomic islands (Song et al., 2012). In R25-6, where Tn*6431* and Tn*6432* were absent, an 8-bp conserved sequence was detected upstream of the *int* integrase and downstream of the mercury resistance operon, providing the boundaries (*attL* and *attR*) of the ICE-like element. Comparison of R25-6 and R25-2 sequences revealed that both Tn*6431* and Tn*6432* were inserted in the *attR* of the ICE-like element, downstream of the mercury operon (Fig. 3b). Finally, by removing the ICE-like element from the structure, the *ISAs25*, Δ*ISAeme16*, and nested *ISAs2-ISAs27-ISAeca1* elements were found to be inserted into the chromosome adjacent to the *guaA* gene and were not part of the ICE-like structure (Fig. 3a).

A BLASTN search in GenBank revealed that no additional copy of the *tet*(31)-carrying ICE-like element in the database, suggesting that it belonged to a novel ICE family. Further study should be carried out to confirm whether the ICE-like element mediated horizontal gene transfer of *tet*(31). To understand the molecular transfer mechanism of *tet*(31), the *tet*(31)-carrying chromosomal region of *A. caviae* strain R25-2 was decomposed as shown in Fig. 3. One possible acquisition mechanism was that the ICE-like element carrying Tn*6432* was integrated and transferred into the chromosome of *A. caviae* strain R25-2 through conjugation and site-specific recombination at the 8-bp (5′-GAGTGGGA-3′) conserved *attB* site (Fig. 3b) downstream of the *guaA* gene (Song et al., 2012). It should be noted that Tn*6432* was integrated into the *attR* of the ICE-like element, which should therefore preclude the element's mobility. However, the 3′ end of the transposon actually regenerated the pattern (5′-GAGTGGGA-3′) (Fig. 3b), suggesting that the ICE-like element may still be mobile while carrying Tn*6432*. In comparison, the fact that *A. caviae* strain R25-6, also isolated from the same sludge sample, harboured the ICE-like element devoid of Tn*6432*, suggesting that *tet*(31)-carrying Tn*6432* may have been acquired through independent and subsequent integration into the *attR* of the ICE-like element already present in the *A. caviae* strain. The ICE-like element identified in this study carried a total of six ARGs conferring resistance to four classes of antibiotics, and further increased the risk of horizontal transfer of these ARGs.

In this study, the rarely reported *tet*(31) tetracycline resistance determinant has been commonly found in some stains isolated from the farming animals including fish, chicken and pig, and the related environment, in which antibiotics are widely used for disease prevention and growth promotion (Casewell et al., 2003). In the present study, we found *tet*(31) and the other 5 ARGs co-occurred in the multidrug resistant ICE-like elements widely distributed in *A. caviae* strains isolated from wastewater treatment system under high concentration of oxytetracycline. Thus, antibiotic selection pressure in environment might be an important factor shaping the antibiotic resistance of environmental bacteria. The removal of antibiotic pressure in water environment will be helpful for blocking the dissemination of ARGs in environment.

It should be noted that the dissemination mechanism of *tet*(31) revealed in this study was based on bioinformatics analysis. To further validate the dissemination mechanism of the *tet*(31) gene, additional research is required to transfer *tet*(31) to a recipient bacteria by conjugation (Jasni et al., 2010). Furthermore, the donor strain labelled a single-copy fluorescent reporter gene fusion to the *tet*(31)-carrying ICE-like element (Reinhard et al., 2013) will be seeded in a controlled bioreactor, and the dissemination of *tet*(31)-carrying ICE-like element will be monitored by fluorescence microscopy.

3. Conclusions

Herein, the distribution of the *tet*(31) gene in sludge bacteria and its potential molecular dissemination mechanism in water environment under high concentration of oxytetracycline were investigated by using culture-based approaches and whole genome sequencing. To the best of our knowledge, this is the first report on the *tet*(31) gene in *A. caviae* isolated from an oxytetracycline wastewater treatment system, and on a novel transposon, termed Tn*6432*. Sequence comparison

analysis demonstrated that Tn*6432* homologs with the structure ISCR2-Δ*phzF*-*tetR*(31)-*tet*(31)-Δ*glmM*-*sul2* were carried by *A. salmonicida*, *G. anatis*, *O. alkaliphila*, and *A. caviae*, suggesting that this transposon can be transferred between species and even genera. Sequence analysis also identified that the *tet*(31)-carrying transposon Tn*6432* was syntenic with another novel transposon Tn*6431*, and located on an ICE-like element, which highlights the strong dissemination potential of the *tet*(31) gene. The results revealed the potential molecular dissemination mechanism of the *tet*(31) gene in water environmental bacteria under high concentration of antibiotic, which could provide useful information for understanding the antibiotic resistance development in the water environments with antibiotic pollution.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.05.008.

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