

# Supplementary Information

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**Table S1.** Matched high-throughput sequencing reads of antibiotic resistance genes (ARGs) in sludge fed with 0 mg/L tetracycline of Day 6 against antibiotic resistance database (ARDB).

| No. | ARDB Accession Number | Identity (%) n | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name       | Function                                       | Related Antibiotics                     |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|----------------|--|---|
| 1   | CAE53425              | 90.62          | 25                | $7.0 \times 10^{-8}$  | 1628            | <i>sul2</i>    | Sulfonamide-resistant dihydropteroate synthase | sulfonamide                             |
| 2   | YP_001969930          | 90             | 28                | $6.0 \times 10^{-9}$  | 182             | <i>sul2</i>    | Sulfonamide-resistant dihydropteroate synthase | sulfonamide                             |
| 3   | YP_002112964          | 100            | 27                | $1.0 \times 10^{-11}$ | 29              | <i>aph33ib</i> | Aminoglycoside O-phosphotransferase            | streptomycin                            |
| 4   | YP_001571041          | 90             | 28                | $5.0 \times 10^{-9}$  | 21              | <i>macB</i>    | Macrolide-specific efflux system               | macrolide                               |
| 5   | YP_002029849          | 90.32          | 27                | $2.0 \times 10^{-8}$  | 21              | <i>smeE</i>    | Multidrug resistance efflux pump               | fluoroquinolone                         |
| 6   | YP_002890644          | 90.32          | 31                | $3.0 \times 10^{-10}$ | 21              | <i>bacA</i>    | Undecaprenyl pyrophosphate phosphatase         | bacitracin                              |
| 7   | CAM88415              | 100            | 32                | $2.0 \times 10^{-12}$ | 13              | <i>aph6id</i>  | Aminoglycoside O-phosphotransferase            | streptomycin                            |
| 8   | ABG36700              | 90.62          | 32                | $2.0 \times 10^{-12}$ | 12              | <i>sul1</i>    | Sulfonamide-resistant dihydropteroate synthase | sulfonamide                             |
| 9   | YP_001836040          | 93.94          | 29                | $9.0 \times 10^{-11}$ | 12              | <i>ermB</i>    | rRNA adenine N-6-methyltransferase             | lincosamide, macrolide, streptogramin_b |
| 10  | CAC86407              | 100            | 32                | $3.0 \times 10^{-13}$ | 11              | <i>aph7id</i>  | Aminoglycoside O-phosphotransferase            | streptomycin                            |
| 11  | YP_002894485          | 93.94          | 26                | $4.0 \times 10^{-10}$ | 11              | <i>ant3ia</i>  | Aminoglycoside O-nucleotidylyltransferase      | spectinomycin, streptomycin             |

Table S1. Cont.

| No. | ARDB Accession Number | Identity (%) n | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name      | Function                                       | Related Antibiotics  |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|---------------|--|--|
| 12  | AAQ92181              | 90.91          | 25                | $2.0 \times 10^{-7}$  | 6               | <i>mexB</i>   | Multidrug resistance efflux pump               | aminoglycoside,<br>beta_lactam,<br>fluoroquinolone,<br>tetracycline, tigecycline |
| 13  | ACB20261              | 100            | 33                | $7.0 \times 10^{-13}$ | 6               | <i>aph6id</i> | Aminoglycoside O-phosphotransferase            | streptomycin   |
| 14  | BAF36555              | 100            | 32                | $1.0 \times 10^{-11}$ | 6               | <i>sul1</i>   | Sulfonamide-resistant dihydropteroate synthase | sulfonamide  |
| 15  | CAG34265              | 90.62          | 32                | $4.0 \times 10^{-10}$ | 6               | <i>smeE</i>   | Multidrug resistance efflux pump               | fluoroquinolone  |
| 16  | ZP_04577926           | 90             | 30                | $3.0 \times 10^{-9}$  | 6               | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase         | bacitracin   |
| 17  | YP_523088             | 90.32          | 27                | $2.0 \times 10^{-9}$  | 5               | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase         | bacitracin   |
| 18  | ABM94007              | 90.32          | 31                | $3.0 \times 10^{-10}$ | 4               | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase         | bacitracin   |
| 19  | EEB58431              | 93.75          | 32                | $1.0 \times 10^{-10}$ | 4               | <i>mexF</i>   | Multidrug resistance efflux pump               | chloramphenicol,<br>fluoroquinolone  |
| 20  | EEQ96502              | 90             | 30                | $8.0 \times 10^{-10}$ | 4               | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase         | bacitracin   |
| 21  | YP_002006347          | 90.62          | 32                | $5.0 \times 10^{-11}$ | 4               | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase         | aminoglycoside,<br>bacitracin  |
| 22  | YP_586061             | 90.91          | 32                | $4.0 \times 10^{-11}$ | 4               | <i>mexB</i>   | Multidrug resistance efflux pump               | tetracycline, tigecycline  |
| 23  | ZP_03790349           | 90.91          | 33                | $2.0 \times 10^{-7}$  | 4               | <i>ceoB</i>   | Multidrug resistance efflux pump               | chloramphenicol  |
| 24  | AAY92510              | 90.32          | 31                | $1.0 \times 10^{-9}$  | 3               | <i>mexF</i>   | Multidrug resistance efflux pump               | chloramphenicol,<br>fluoroquinolone  |
| 25  | ABQ96629              | 100            | 32                | $5.0 \times 10^{-12}$ | 3               | <i>sul1</i>   | Sulfonamide-resistant dihydropteroate synthase | sulfonamide  |

Table S1. Cont.

| No. | ARDB Accession Number | Identity (%) n | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name    | Function                                       | Related Antibiotics                              |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|-------------|--|--|
| 26  | BAH18720              | 100            | 33                | $2.0 \times 10^{-13}$ | 3               | <i>ermB</i> | rRNA adenine N-6-methyltransferase             | lincosamide,<br>macrolide,<br>streptogramin_b    |
| 27  | CAE50480              | 100            | 32                | $2.0 \times 10^{-13}$ | 3               | <i>sul1</i> | Sulfonamide-resistant dihydropteroate synthase | sulfonamide<br>acriflavin,<br>aminoglycoside,    |
| 28  | CAG74079              | 90.32          | 31                | $2.0 \times 10^{-10}$ | 3               | <i>acrB</i> | Multidrug resistance efflux pump               | beta_lactam,<br>glycylcycline,<br>macrolide      |
| 29  | Q82Y49                | 90.91          | 30                | $1.0 \times 10^{-10}$ | 3               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase         | bacitracin                                       |
| 30  | YP_001187672          | 90             | 30                | $2.0 \times 10^{-9}$  | 3               | <i>mexF</i> | Multidrug resistance efflux pump               | chloramphenicol,<br>fluoroquinolone              |
| 31  | YP_001477377          | 90.32          | 31                | $4.0 \times 10^{-10}$ | 3               | <i>rosB</i> | Potassium antiporter                           | fosmidomycin<br>acriflavin,<br>aminoglycoside,   |
| 32  | YP_001908403          | 90.62          | 32                | $6.0 \times 10^{-10}$ | 3               | <i>acrB</i> | Multidrug resistance efflux pump               | beta_lactam,<br>glycylcycline,<br>macrolide      |
| 33  | YP_002800512          | 90.62          | 32                | $3.0 \times 10^{-10}$ | 3               | <i>mexF</i> | Multidrug resistance efflux pump               | chloramphenicol,<br>fluoroquinolone              |
| 34  | YP_348389             | 90.32          | 31                | $1.0 \times 10^{-10}$ | 3               | <i>mexF</i> | Multidrug resistance efflux pump               | chloramphenicol,<br>fluoroquinolone              |
| 35  | YP_985461             | 92.31          | 26                | $2.0 \times 10^{-7}$  | 3               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase         | bacitracin<br>aminoglycoside,<br>beta_lactam,    |
| 36  | ZP_01363330           | 90.91          | 26                | $5.0 \times 10^{-8}$  | 3               | <i>mexB</i> | Multidrug resistance efflux pump               | fluoroquinolone,<br>tetracycline,<br>tigecycline |

Table S1. Cont.

| No. | ARDB Accession Number | Identity (%) n | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name         | Function   | Related Antibiotics   |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|------------------|--|---|
| 37  | ABZ01843              | 100            | 31                | $4.0 \times 10^{-12}$ | 2               | <i>tetG</i>      | Major facilitator superfamily transporter, tetracycline efflux pump    | tetracycline  |
| 38  | ACC96132              | 100            | 32                | $6.0 \times 10^{-13}$ | 2               | <i>aadD</i>      | Aminoglycoside O-nucleotidyltransferase                                | kanamycin, tobramycin   |
| 39  | ACN73425              | 96.97          | 33                | $3.0 \times 10^{-12}$ | 2               | <i>sul1</i>      | Sulfonamide-resistant dihydropteroate synthase                         | sulfonamide   |
| 40  | ACO92337              | 92.31          | 26                | $4.0 \times 10^{-8}$  | 2               | <i>ant2ia</i>    | Aminoglycoside O-nucleotidyltransferase                                | dibekacin, gentamicin, kanamycin, sisomicin, tobramycin acriflavin, aminoglycoside, |
| 41  | BAC77251              | 93.55          | 31                | $4.0 \times 10^{-11}$ | 2               | <i>acrB</i>      | Multidrug resistance efflux pump                                       | beta_lactam, glycylcycline, macrolide lincosamide, macrolide, streptogramin_b       |
| 42  | CAA70206              | 100            | 33                | $9.0 \times 10^{-14}$ | 2               | <i>ermC</i>      | rRNA adenine N-6-methyltransferase                                     | carbenicillin, penicillin   |
| 43  | CAI46978              | 100            | 32                | $6.0 \times 10^{-13}$ | 2               | <i>bl2c_pse3</i> | Class A beta-lactamase   | lincomycin  |
| 44  | CAJ75670              | 100            | 32                | $7.0 \times 10^{-14}$ | 2               | <i>lnuA</i>      | Lincosamide nucleotidyltransferase                                     | tetracycline  |
| 45  | CAM12478              | 96.97          | 33                | $6.0 \times 10^{-13}$ | 2               | <i>tet32</i>     | Ribosomal protection protein   | chloramphenicol   |
| 46  | CAM88409              | 100            | 32                | $7.0 \times 10^{-13}$ | 2               | <i>cml_e3</i>    | Major facilitator superfamily transporter, chloramphenicol efflux pump | mexW  |
| 47  | CAY51926              | 93.75          | 28                | $4.0 \times 10^{-8}$  | 2               | <i>mexW</i>      | Multidrug resistance efflux pump                                       | <i>tetC</i>   |
| 48  | NP_511233             | 100            | 32                | $9.0 \times 10^{-14}$ | 2               | <i>tetC</i>      | Major facilitator superfamily transporter, tetracycline efflux pump    | tetracycline  |

Table S1. Cont.

| No. | ARDB Accession Number | Identity (%) n | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name       | Function   | Related Antibiotics   |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|----------------|--|---|
| 49  | NP_792892             | 90.62          | 32                | $3.0 \times 10^{-11}$ | 2               | <i>mexF</i>    | Multidrug resistance efflux pump                                       | chloramphenicol,<br>fluoroquinolone<br>lincosamide,<br>macrolide,<br>streptogramin_b  |
| 50  | NP_862543             | 100            | 32                | $7.0 \times 10^{-14}$ | 2               | <i>ermT</i>    | rRNA adenine N-6-methyltransferase                                     | aminoglycoside,<br>glycylcycline  |
| 51  | YP_001346277          | 90.91          | 25                | $1.0 \times 10^{-7}$  | 2               | <i>mexI</i>    | Multidrug resistance efflux pump                                       | acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide<br>erythromycin,<br>fluoroquinolone,<br>glycylcycline,<br>roxithromycin |
| 52  | YP_001348630          | 90.32          | 31                | $5.0 \times 10^{-11}$ | 2               | <i>mexY</i>    | Multidrug resistance efflux pump                                       | bacitracin<br>tetracycline  |
| 53  | YP_001350280          | 90.91          | 32                | $1.0 \times 10^{-10}$ | 2               | <i>mexW</i>    | Multidrug resistance efflux pump                                       |   |
| 54  | YP_002240037          | 93.94          | 33                | $1.0 \times 10^{-11}$ | 2               | <i>acrB</i>    | Multidrug resistance efflux pump                                       |   |
| 55  | YP_608682             | 90.62          | 32                | $9.0 \times 10^{-11}$ | 2               | <i>mexD</i>    | Multidrug resistance efflux pump                                       |   |
| 56  | YP_970399             | 100            | 33                | $1.0 \times 10^{-12}$ | 2               | <i>bacA</i>    | Undecaprenyl pyrophosphate phosphatase                                 | bacitracin  |
| 57  | ZP_02632674           | 100            | 32                | $7.0 \times 10^{-13}$ | 2               | <i>tetP(B)</i> | Ribosomal protection protein   | tetracycline  |
| 58  | ZP_03552050           | 90.91          | 31                | $2.0 \times 10^{-11}$ | 2               | <i>bacA</i>    | Undecaprenyl pyrophosphate phosphatase                                 | bacitracin  |
| 59  | ZP_03752765           | 96.97          | 33                | $8.0 \times 10^{-15}$ | 2               | <i>ant6ia</i>  | Aminoglycoside O-nucleotidylyltransferase                              | streptomycin  |
| 60  | ZP_04405450           | 100            | 33                | $9.0 \times 10^{-14}$ | 2               | <i>aph6id</i>  | Aminoglycoside O-phosphotransferase                                    | streptomycin  |
| 61  | AAB84282              | 93.94          | 33                | $3.0 \times 10^{-12}$ | 1               | <i>tetV</i>    | Major facilitator superfamily transporter,<br>tetracycline efflux pump | tetracycline  |

Table S1. Cont.

| No. | ARDB Accession Number | Identity (%) n | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name         | Function   | Related Antibiotics  |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|------------------|--|--|
| 62  | AAC36915              | 100            | 32                | $3.0 \times 10^{-14}$ | 1               | <i>ermQ</i>      | rRNA adenine N-6-methyltransferase   | lincosamide, macrolide, streptogramin_b erythromycin               |
| 63  | AAK69613              | 100            | 32                | $6.0 \times 10^{-13}$ | 1               | <i>ereA</i>      | Erythromycin esterase  |  |
| 64  | AAM08183              | 93.94          | 33                | $3.0 \times 10^{-12}$ | 1               | <i>bl2D_oxa5</i> | Class D beta-lactamase   | cloxacillin, penicillin  |
| 65  | AAN06707              | 100            | 33                | $4.0 \times 10^{-14}$ | 1               | <i>tetA</i>      | Major facilitator superfamily transporter, tetracycline efflux pump                          | tetracycline   |
| 66  | AAO38916              | 100            | 33                | $1.0 \times 10^{-13}$ | 1               | <i>tetO</i>      | Ribosomal protection protein   | tetracycline acriflavin, aminoglycoside,                           |
| 67  | AAQ21216              | 90.91          | 33                | $9.0 \times 10^{-11}$ | 1               | <i>acrB</i>      | Multidrug resistance efflux pump   | beta_lactam, glycylcycline, macrolide                              |
| 68  | AAR21617              | 100            | 32                | $4.0 \times 10^{-13}$ | 1               | <i>sul1</i>      | Sulfonamide-resistant dihydropteroate synthase   | sulfonamide  |
| 69  | AAR29485              | 100            | 33                | $1.0 \times 10^{-14}$ | 1               | <i>tetO</i>      | Ribosomal protection protein   | tetracycline   |
| 70  | AAS20534              | 90.91          | 33                | $1.0 \times 10^{-11}$ | 1               | <i>bl2D_oxa2</i> | Class D beta-lactamase   | cloxacillin, penicillin  |
| 71  | AAU89126              | 100            | 33                | $1.0 \times 10^{-14}$ | 1               | <i>aac6ib</i>    | Aminoglycoside N-acetyltransferase   | amikacin, dibekacin, isepamicin, netilmicin, sisomicin, tobramycin |
| 72  | AYY68306              | 100            | 33                | $2.0 \times 10^{-13}$ | 1               | <i>mefA</i>      | Major facilitator superfamily transporter, Macrolide-Lincosamide-Streptogramin B efflux pump | macrolide  |

Table S1. Cont.

| No. | ARDB Accession Number | Identity (%) n | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name       | Function                                  | Related Antibiotics  |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|----------------|---|--|
| 73  | ABG77966              | 93.94          | 33                | $1.0 \times 10^{-11}$ | 1               | <i>acrB</i>    | Multidrug resistance efflux pump          | acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide<br>dibekacin,<br>gentamicin,<br>netilmicin, sisomicin,<br>tobramycin                             |
| 74  | ABL95928              | 96.88          | 32                | $2.0 \times 10^{-15}$ | 1               | <i>aac6iia</i> | Aminoglycoside N-acetyltransferase        | cephalosporin,<br>penicillin<br>dibekacin,<br>gentamicin,<br>kanamycin,<br>sisomicin,<br>tobramycin  |
| 75  | ABN80430              | 100            | 32                | $4.0 \times 10^{-13}$ | 1               | <i>bl2_veb</i> | Class A beta-lactamase                    | streptomycin<br>acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide<br>acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide |
| 76  | ABO42050              | 90.62          | 32                | $1.0 \times 10^{-12}$ | 1               | <i>ant2ia</i>  | Aminoglycoside O-nucleotidylyltransferase | acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide<br>acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide                 |
| 77  | ABP57330              | 100            | 32                | $5.0 \times 10^{-15}$ | 1               | <i>ant6ia</i>  | Aminoglycoside O-nucleotidylyltransferase | acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide<br>acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide                 |
| 78  | ABR13271              | 90.91          | 33                | $6.0 \times 10^{-12}$ | 1               | <i>acrB</i>    | Multidrug resistance efflux pump          | acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide<br>acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide                 |
| 79  | ABW72062              | 96.97          | 33                | $2.0 \times 10^{-11}$ | 1               | <i>acrB</i>    | Multidrug resistance efflux pump          | acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide  |

Table S1. Cont.

| No. | ARDB Accession Number | Identity (%) n | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name       | Function  | Related Antibiotics  |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|----------------|---|--|
| 80  | ACK77685              | 90.91          | 33                | $2.0 \times 10^{-11}$ | 1               | <i>acrB</i>    | Multidrug resistance efflux pump  | acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide<br>sulfonamide |
| 81  | ACR66841              | 96.15          | 26                | $2.0 \times 10^{-10}$ | 1               | <i>sul1</i>    | Sulfonamide-resistant dihydropteroate synthase<br>Bifunctional enzyme that catalyzes the oxidative decarboxylation of UDP-glucuronic acid (UDP-GlcUA) to UDP-4-keto-arabinose (UDP-Ara4O) and the addition of a formyl group to UDP-4-amino-4-deoxy-L-arabinose (UDP-L-Ara4N) to form UDP-L-4-formamido-arabinose (UDP-L-Ara4FN). The modified arabinose is attached to lipid A and is required for resistance to polymyxin and cationic antimicrobial peptides |  |
| 82  | B7LM76                | 93.94          | 33                | $1.0 \times 10^{-14}$ | 1               | <i>arnA</i>    |   | polymyxin  |
| 83  | BAB71966              | 100            | 33                | $6.0 \times 10^{-13}$ | 1               | <i>tetP(A)</i> | Major facilitator superfamily transporter, tetracycline efflux pump   | tetracycline   |
| 84  | CAA70208              | 100            | 32                | $3.0 \times 10^{-14}$ | 1               | <i>ermC</i>    | rRNA adenine N-6-methyltransferase  | lincosamide,<br>macrolide,<br>streptogramin_b  |
| 85  | CAJ45367              | 100            | 33                | $3.0 \times 10^{-13}$ | 1               | <i>mefA</i>    | Major facilitator superfamily transporter, Macrolide-Lincosamide-Streptogramin B efflux pump  | macrolide  |
| 86  | CAL44999              | 100            | 33                | $2.0 \times 10^{-15}$ | 1               | <i>InuA</i>    | Lincosamide nucleotidyltransferase  | lincomycin   |

Table S1. Cont.

| No. | ARDB Accession Number | Identity (%) n | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name         | Function  | Related Antibiotics  |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|------------------|---|--|
| 87  | NP_348075             | 93.94          | 33                | $6.0 \times 10^{-13}$ | 1               | <i>tetP(A)</i>   | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline   |
| 88  | NP_387454             | 100            | 33                | $5.0 \times 10^{-14}$ | 1               | <i>tetC</i>      | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline   |
| 89  | Q51429                | 100            | 27                | $4.0 \times 10^{-9}$  | 1               | <i>bl2D_oxa2</i> | Class D beta-lactamase  | cloxacillin,penicillin   |
| 90  | XP_002333050          | 96.97          | 33                | $6.0 \times 10^{-12}$ | 1               | <i>tetC</i>      | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline   |
| 91  | YP_001155044          | 92.31          | 26                | $4.0 \times 10^{-9}$  | 1               | <i>bacA</i>      | Undecaprenyl pyrophosphate phosphatase                              | bacitracin<br>erythromycin,<br>fluoroquinolone,<br>glycylcycline,<br>roxithromycin |
| 92  | YP_001350571          | 90.91          | 33                | $7.0 \times 10^{-11}$ | 1               | <i>mexD</i>      | Multidrug resistance efflux pump                                    | fosmidomycin<br>lincosamide,<br>macrolide,<br>streptogramin_b                      |
| 93  | YP_001477378          | 90.62          | 32                | $6.0 \times 10^{-12}$ | 1               | <i>rosA</i>      | Major facilitator superfamily transporter                           | chloramphenicol,<br>fluoroquinolone  |
| 94  | YP_001716198          | 100            | 33                | $1.0 \times 10^{-13}$ | 1               | <i>ermT</i>      | rRNA adenine N-6-methyltransferase                                  | bacitracin<br>fluoroquinolone  |
| 95  | YP_001749316          | 90.32          | 31                | $2.0 \times 10^{-10}$ | 1               | <i>mexF</i>      | Multidrug resistance efflux pump                                    | chloramphenicol,<br>fluoroquinolone  |
| 96  | YP_001797193          | 93.55          | 31                | $3.0 \times 10^{-11}$ | 1               | <i>bacA</i>      | Undecaprenyl pyrophosphate phosphatase                              | bacitracin   |
| 97  | YP_002030219          | 93.75          | 32                | $8.0 \times 10^{-12}$ | 1               | <i>smeB</i>      | Multidrug resistance efflux pump                                    | fluoroquinolone  |
| 98  | YP_002081505          | 90.62          | 32                | $4.0 \times 10^{-11}$ | 1               | <i>mexF</i>      | Multidrug resistance efflux pump                                    | chloramphenicol,<br>fluoroquinolone  |
| 99  | YP_002084975          | 90.62          | 32                | $3.0 \times 10^{-11}$ | 1               | <i>mexW</i>      | Multidrug resistance efflux pump                                    |  |
| 100 | YP_002260505          | 90.91          | 33                | $4.0 \times 10^{-12}$ | 1               | <i>bacA</i>      | Undecaprenyl pyrophosphate phosphatase                              | bacitracin   |
| 101 | YP_002332890          | 100            | 33                | $2.0 \times 10^{-12}$ | 1               | <i>tetA</i>      | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline   |

Table S1. Cont.

| No. | ARDB Accession Number | Identity (%) n | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name    | Function  | Related Antibiotics                 |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|-------------|---|-------------------------------------|
| 102 | YP_002847505          | 93.75          | 32                | $2.0 \times 10^{-10}$ | 1               | <i>mdtF</i> | Multidrug resistance efflux pump  | doxorubicin,<br>erythromycin        |
| 103 | YP_236040             | 93.75          | 32                | $9.0 \times 10^{-11}$ | 1               | <i>mexF</i> | Multidrug resistance efflux pump  | chloramphenicol,<br>fluoroquinolone |
| 104 | YP_236080             | 90.62          | 32                | $1.0 \times 10^{-11}$ | 1               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase  | bacitracin                          |
| 105 | YP_274481             | 90.62          | 32                | $3.0 \times 10^{-11}$ | 1               | <i>mexF</i> | Multidrug resistance efflux pump  | chloramphenicol,<br>fluoroquinolone |
| 106 | YP_316450             | 90.62          | 32                | $9.0 \times 10^{-11}$ | 1               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase  | bacitracin                          |
| 107 | YP_550152             | 96.97          | 33                | $7.0 \times 10^{-13}$ | 1               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase  | bacitracin                          |
| 108 | YP_746446             | 90.91          | 33                | $6.0 \times 10^{-12}$ | 1               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase  | bacitracin                          |
| 109 | YP_932216             | 90.91          | 33                | $9.0 \times 10^{-11}$ | 1               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase  | bacitracin                          |
| 110 | YP_981592             | 96.97          | 33                | $1.0 \times 10^{-12}$ | 1               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase  | bacitracin                          |
| 111 | ZP_02901439           | 93.33          | 30                | $2.0 \times 10^{-09}$ | 1               | <i>mdtF</i> | Multidrug resistance efflux pump  | doxorubicin,<br>erythromycin        |
| 112 | ZP_02904980           | 96.97          | 33                | $7.0 \times 10^{-14}$ | 1               | <i>ceoB</i> | Multidrug resistance efflux pump<br><br>Bifunctional enzyme that catalyzes the oxidative decarboxylation of UDP-glucuronic acid (UDP-GlcUA) to UDP-4-keto-arabinose (UDP-Ara4O) and the addition of a formyl group to UDP-4-amino-4-deoxy-L-arabinose (UDP-L-Ara4N) to form UDP-L-4-formamido-arabinose (UDP-L-Ara4FN). The modified arabinose is attached to lipid A and is required for resistance to polymyxin and cationic antimicrobial peptides | chloramphenicol                     |
| 113 | ZP_03075977           | 90.32          | 31                | $9.0 \times 10^{-11}$ | 1               | <i>arnA</i> |   | polymyxin                           |

Table S1. Cont.

| No. | ARDB Accession Number | Identity (%) n | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name    | Function                               | Related Antibiotics   |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|-------------|--|---|
| 114 | ZP_03222019           | 96.15          | 26                | $1.0 \times 10^{-7}$  | 1               | <i>acrB</i> | Multidrug resistance efflux pump       | acriflavin,<br>aminoglycoside,<br>beta_lactam,<br>glycylcycline,<br>macrolide |
| 115 | ZP_03541894           | 90.91          | 33                | $8.0 \times 10^{-12}$ | 1               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase | bacitracin  |
| 116 | ZP_03583212           | 93.75          | 32                | $3.0 \times 10^{-10}$ | 1               | <i>ceoB</i> | Multidrug resistance efflux pump       | chloramphenicol<br>acriflavin,<br>aminoglycoside,                             |
| 117 | ZP_03832747           | 90.91          | 33                | $2.0 \times 10^{-11}$ | 1               | <i>acrB</i> | Multidrug resistance efflux pump       | beta_lactam,<br>glycylcycline,<br>macrolide<br>acriflavin,<br>aminoglycoside, |
| 118 | ZP_04612532           | 90.91          | 33                | $6.0 \times 10^{-12}$ | 1               | <i>acrB</i> | Multidrug resistance efflux pump       | beta_lactam,<br>glycylcycline,<br>macrolide                                   |
| 119 | ZP_04612557           | 90.32          | 31                | $4.0 \times 10^{-10}$ | 1               | <i>rosB</i> | Potassium antiporter                   | fosmidomycin  |
| 120 | ZP_04630103           | 90.62          | 32                | $1.0 \times 10^{-10}$ | 1               | <i>rosB</i> | Potassium antiporter                   | fosmidomycin  |
| 121 | ZP_04631861           | 90.91          | 33                | $7.0 \times 10^{-11}$ | 1               | <i>rosB</i> | Potassium antiporter                   | fosmidomycin  |

**Table S2.** Matched high-throughput sequencing reads of antibiotic resistance genes (ARGs) in sludge fed with 20 mg/L tetracycline of Day 6 against antibiotic resistance database (ARDB).

| No. | ARDB Accession Number | Identity (%) ≥ | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name      | Function                                       | Related Antibiotics   |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|---------------|--|---|
| 1   | CAE53425              | 96.97          | 25                | $8.0 \times 10^{-10}$ | 72              | <i>sul2</i>   | Sulfonamide-resistant dihydropteroate synthase | sulfonamide   |
| 2   | YP_002029849          | 90.32          | 25                | $1.0 \times 10^{-8}$  | 43              | <i>smeE</i>   | Multidrug resistance efflux pump               | fluoroquinolone   |
| 3   | ABG36700              | 92.59          | 27                | $2.0 \times 10^{-9}$  | 28              | <i>sul1</i>   | Sulfonamide-resistant dihydropteroate synthase | sulfonamide   |
| 4   | YP_002894485          | 90.62          | 26                | $4.0 \times 10^{-10}$ | 22              | <i>ant3ia</i> | Aminoglycoside O-nucleotidyltransferase        | spectinomycin,<br>streptomycin                                |
| 5   | YP_002890644          | 90.62          | 32                | $1.0 \times 10^{-11}$ | 20              | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase         | bacitracin  |
| 6   | YP_001571041          | 90.32          | 31                | $1.0 \times 10^{-9}$  | 14              | <i>macB</i>   | Macrolide-specific efflux system               | macrolide   |
| 7   | BAF36555              | 96.88          | 32                | $8.0 \times 10^{-12}$ | 13              | <i>sul1</i>   | Sulfonamide-resistant dihydropteroate synthase | sulfonamide   |
| 8   | EEB58431              | 90.62          | 32                | $4.0 \times 10^{-10}$ | 13              | <i>mexF</i>   | Multidrug resistance efflux pump               | chloramphenicol,<br>fluoroquinolone                           |
| 9   | AAR21617              | 100            | 32                | $4.0 \times 10^{-13}$ | 11              | <i>sul1</i>   | Sulfonamide-resistant dihydropteroate synthase | sulfonamide   |
| 10  | CAG34265              | 90.62          | 32                | $2.0 \times 10^{-10}$ | 11              | <i>smeE</i>   | Multidrug resistance efflux pump               | fluoroquinolone<br>aminoglycoside,                            |
| 11  | YP_586061             | 90.91          | 32                | $2.0 \times 10^{-11}$ | 10              | <i>mexB</i>   | Multidrug resistance efflux pump               | beta_lactam,<br>fluoroquinolone,<br>tetracycline, tigecycline |
| 12  | ABQ96629              | 100            | 26                | $4.0 \times 10^{-9}$  | 9               | <i>sul1</i>   | Sulfonamide-resistant dihydropteroate synthase | sulfonamide   |
| 13  | YP_001749316          | 90.91          | 32                | $2.0 \times 10^{-11}$ | 9               | <i>mexF</i>   | Multidrug resistance efflux pump               | chloramphenicol,<br>fluoroquinolone                           |
| 14  | YP_001797193          | 90.62          | 32                | $5.0 \times 10^{-11}$ | 8               | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase         | bacitracin  |
| 15  | ZP_04577926           | 90.91          | 31                | $5.0 \times 10^{-11}$ | 8               | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase         | bacitracin  |

Table S2. Cont.

| No. | ARDB Accession Number | Identity (%) ≥ | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name          | Function   | Related Antibiotics  |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|-------------------|--|--|
| 16  | ABO42050              | 100            | 32                | $2.0 \times 10^{-12}$ | 7               | <i>ant2ia</i>     | Aminoglycoside O-nucleotidylyltransferase                              | dibekacin, gentamicin, kanamycin, sisomicin, tobramycin          |
| 17  | CAC41338              | 100            | 32                | $2.0 \times 10^{-12}$ | 7               | <i>tetA</i>       | Major facilitator superfamily transporter, tetracycline efflux pump    | tetracycline   |
| 18  | YP_002081505          | 90.62          | 32                | $8.0 \times 10^{-10}$ | 7               | <i>mexF</i>       | Multidrug resistance efflux pump                                       | chloramphenicol, fluoroquinolone                                 |
| 19  | CAE50480              | 100            | 32                | $3.0 \times 10^{-13}$ | 6               | <i>sul1</i>       | Sulfonamide-resistant dihydropteroate synthase                         | sulfonamide  |
| 20  | CAM88409              | 100            | 32                | $5.0 \times 10^{-12}$ | 6               | <i>cml_e3</i>     | Major facilitator superfamily transporter, chloramphenicol efflux pump | chloramphenicol  |
| 21  | Q82Y49                | 90.91          | 29                | $1.0 \times 10^{-9}$  | 6               | <i>bacA</i>       | Undecaprenyl pyrophosphate phosphatase                                 | bacitracin   |
| 22  | AAS92348              | 93.94          | 32                | $1.0 \times 10^{-12}$ | 5               | <i>bl2D_oxa10</i> | Class D beta-lactamase   | cloxacillin, penicillin  |
| 23  | ABS19074              | 96.97          | 32                | $7.0 \times 10^{-13}$ | 5               | <i>tetA</i>       | Major facilitator superfamily transporter, tetracycline efflux pump    | tetracycline   |
| 24  | YP_001350280          | 90             | 30                | $6.0 \times 10^{-10}$ | 5               | <i>mexW</i>       | Multidrug resistance efflux pump                                       | acriflavin, aminoglycoside, beta_lactam, glycycycline, macrolide |
| 25  | YP_001908403          | 90.91          | 27                | $5.0 \times 10^{-9}$  | 5               | <i>acrB</i>       | Multidrug resistance efflux pump                                       | bacitracin   |
| 26  | YP_002006347          | 90.62          | 32                | $2.0 \times 10^{-11}$ | 5               | <i>bacA</i>       | Undecaprenyl pyrophosphate phosphatase                                 | cloxacillin, penicillin  |
| 27  | AAF72942              | 100            | 32                | $3.0 \times 10^{-13}$ | 4               | <i>bl2D_oxa10</i> | Class D beta-lactamase   | dibekacin, gentamicin, netilmicin, sisomicin, tobramycin         |
| 28  | ABL95928              | 100            | 32                | $3.0 \times 10^{-13}$ | 4               | <i>aac6iia</i>    | Aminoglycoside N-acetyltransferase                                     | streptomycin   |
| 29  | ACB20261              | 100            | 32                | $1.0 \times 10^{-13}$ | 4               | <i>aph6id</i>     | Aminoglycoside O-phosphotransferase                                    |  |

Table S2. Cont.

| No. | ARDB Accession Number | Identity (%) ≥ | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name         | Function  | Related Antibiotics   |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|------------------|---|---|
| 30  | CAC86407              | 100            | 32                | $1.0 \times 10^{-13}$ | 4               | <i>aph6id</i>    | Aminoglycoside O-phosphotransferase                                   | streptomycin  |
| 31  | CAI46978              | 100            | 32                | $2.0 \times 10^{-12}$ | 4               | <i>bl2c_pse3</i> | Class A beta-lactamase  | carbenicillin, penicillin   |
| 32  | NP_511233             | 96.97          | 32                | $7.0 \times 10^{-13}$ | 4               | <i>tetC</i>      | Major facilitator superfamily transporter, tetracycline efflux pump   | tetracycline  |
| 33  | NP_598114             | 90.62          | 31                | $4.0 \times 10^{-11}$ | 4               | <i>mexD</i>      | Multidrug resistance efflux pump                                      | erythromycin, fluoroquinolone, glycylcycline, roxithromycin                           |
| 34  | YP_001187667          | 90.91          | 32                | $2.0 \times 10^{-11}$ | 4               | <i>bacA</i>      | Undecaprenyl pyrophosphate phosphatase                                | bacitracin  |
| 35  | YP_001969930          | 100            | 30                | $2.0 \times 10^{-11}$ | 4               | <i>sul2</i>      | Sulfonamide-resistant dihydropteroate synthase                        | sulfonamide   |
| 36  | YP_002112964          | 96.97          | 32                | $3.0 \times 10^{-13}$ | 4               | <i>aph33ib</i>   | Aminoglycoside O-phosphotransferase                                   | streptomycin, aminoglycoside, beta_lactam, fluoroquinolone, tetracycline, tigecycline |
| 37  | ZP_01363330           | 90.62          | 32                | $6.0 \times 10^{-10}$ | 4               | <i>mexB</i>      | Multidrug resistance efflux pump                                      | bacitracin  |
| 38  | AAG05347              | 90.62          | 32                | $4.0 \times 10^{-11}$ | 3               | <i>bacA</i>      | Undecaprenyl pyrophosphate phosphatase                                | tetracycline  |
| 39  | ABZ01843              | 100            | 33                | $6.0 \times 10^{-13}$ | 3               | <i>tetG</i>      | Major facilitator superfamily transporter, tetracycline efflux pump   | acriflavin, aminoglycoside, beta_lactam, glycylcycline, macrolide                     |
| 40  | BAC77251              | 90.91          | 32                | $9.0 \times 10^{-11}$ | 3               | <i>acrB</i>      | Multidrug resistance efflux pump                                      | tetracycline  |
| 41  | CAC47932              | 100            | 32                | $9.0 \times 10^{-14}$ | 3               | <i>tetX</i>      | NADP-requiring oxidoreductase, an enzyme that can modify tetracycline |   |

Table S2. Cont.

| No. | ARDB Accession Number | Identity (%) ≥ | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name         | Function  | Related Antibiotics   |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|------------------|---|---|
| 42  | CAG74079              | 90.62          | 32                | $3.0 \times 10^{-10}$ | 3               | <i>acrB</i>      | Multidrug resistance efflux pump                                    | acriflavin, aminoglycoside, beta_lactam, glycylcycline, macrolide                                   |
| 43  | P51563                | 96.3           | 27                | $2.0 \times 10^{-8}$  | 3               | <i>tetG</i>      | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline  |
| 44  | Q51429                | 100            | 30                | $1.0 \times 10^{-11}$ | 3               | <i>bl2D_oxa2</i> | Class D beta-lactamase  | cloxacillin, penicillin   |
| 45  | YP_001346277          | 90.32          | 31                | $6.0 \times 10^{-10}$ | 3               | <i>mexI</i>      | Multidrug resistance efflux pump                                    |   |
| 46  | YP_001477377          | 90.62          | 31                | $9.0 \times 10^{-11}$ | 3               | <i>rosB</i>      | Potassium antiporter  | fosmidomycin<br>erythromycin,<br>fluoroquinolone,<br>glycylcycline,<br>roxithromycin                |
| 47  | YP_608682             | 90.62          | 32                | $3.0 \times 10^{-10}$ | 3               | <i>mexD</i>      | Multidrug resistance efflux pump                                    |   |
| 48  | ZP_01974653           | 92.59          | 26                | $3.0 \times 10^{-8}$  | 3               | <i>ant3ia</i>    | Aminoglycoside O-nucleotidylyltransferase                           | spectinomycin,<br>streptomycin  |
| 49  | AAK69613              | 100            | 32                | $3.0 \times 10^{-12}$ | 2               | <i>ereA</i>      | Erythromycin esterase   | erythromycin  |
| 50  | AAN06707              | 100            | 33                | $1.0 \times 10^{-13}$ | 2               | <i>tetA</i>      | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline  |
| 51  | AAR29485              | 100            | 32                | $5.0 \times 10^{-12}$ | 2               | <i>tetO</i>      | Ribosomal protection protein  | tetracycline  |
| 52  | AAS20534              | 90.32          | 31                | $2.0 \times 10^{-10}$ | 2               | <i>bl2D_oxa2</i> | Class D beta-lactamase  | cloxacillin, penicillin<br>amikacin, dibekacin,<br>isepamicin, netilmicin,<br>sisomicin, tobramycin |
| 53  | AAU89126              | 100            | 32                | $1.0 \times 10^{-12}$ | 2               | <i>aac6ib</i>    | Aminoglycoside N-acetyltransferase                                  |   |
| 54  | AAZ91706              | 96.97          | 32                | $1.0 \times 10^{-11}$ | 2               | <i>ereA</i>      | Erythromycin esterase   | erythromycin  |
| 55  | ABM94007              | 90.32          | 31                | $7.0 \times 10^{-11}$ | 2               | <i>bacA</i>      | Undecaprenyl pyrophosphate phosphatase                              | bacitracin  |
| 56  | CAY49157              | 90.62          | 32                | $4.0 \times 10^{-11}$ | 2               | <i>mexF</i>      | Multidrug resistance efflux pump                                    | chloramphenicol,<br>fluoroquinolone   |

Table S2. Cont.

| No. | ARDB Accession Number | Identity (%) ≥ | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name      | Function  | Related Antibiotics  |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|---------------|---|--|
| 57  | YP_001186705          | 90.32          | 31                | $2.0 \times 10^{-10}$ | 2               | <i>mexW</i>   | Multidrug resistance efflux pump  |  |
| 58  | YP_002030219          | 90.91          | 32                | $7.0 \times 10^{-11}$ | 2               | <i>smeB</i>   | Multidrug resistance efflux pump  | fluoroquinolone  |
| 59  | YP_002260505          | 93.75          | 32                | $6.0 \times 10^{-12}$ | 2               | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase                                    | bacitracin   |
| 60  | YP_523088             | 90.62          | 32                | $2.0 \times 10^{-11}$ | 2               | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase                                    | bacitracin   |
| 61  | YP_746446             | 90.91          | 32                | $3.0 \times 10^{-12}$ | 2               | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase                                    | bacitracin   |
| 62  | ZP_02901439           | 93.75          | 30                | $4.0 \times 10^{-10}$ | 2               | <i>mdtF</i>   | Multidrug resistance efflux pump  | doxorubicin, erythromycin<br>acriflavin, aminoglycoside,<br>beta_lactam, glycylcycline,<br>macrolide |
| 63  | ZP_03222019           | 96.97          | 33                | $1.0 \times 10^{-11}$ | 2               | <i>acrB</i>   | Multidrug resistance efflux pump  |  |
| 64  | ZP_03552050           | 90.91          | 33                | $2.0 \times 10^{-11}$ | 2               | <i>bacA</i>   | Undecaprenyl pyrophosphate phosphatase                                    | bacitracin   |
| 65  | AAL51021              | 96.97          | 33                | $2.0 \times 10^{-11}$ | 1               | <i>aac6ib</i> | Aminoglycoside N-acetyltransferase  | amikacin, dibekacin,<br>isepamicin, netilmicin,<br>sisomicin, tobramycin                             |
| 66  | AAP22012              | 100            | 32                | $3.0 \times 10^{-12}$ | 1               | <i>cml_e8</i> | Major facilitator superfamily transporter,<br>chloramphenicol efflux pump | chloramphenicol  |
| 67  | AAQ21216              | 93.94          | 33                | $1.0 \times 10^{-11}$ | 1               | <i>acrB</i>   | Multidrug resistance efflux pump  | acriflavin, aminoglycoside,<br>beta_lactam, glycylcycline,<br>macrolide<br>aminoglycoside,           |
| 68  | AAQ92181              | 93.75          | 32                | $5.0 \times 10^{-11}$ | 1               | <i>mexB</i>   | Multidrug resistance efflux pump  | beta_lactam,<br>fluoroquinolone,<br>tetracycline, tigecycline  |
| 69  | ABB92626              | 100            | 33                | $9.0 \times 10^{-16}$ | 1               | <i>catB3</i>  | Group B chloramphenicol acetyltransferase                                 | chloramphenicol  |
| 70  | ABW72062              | 93.75          | 32                | $3.0 \times 10^{-11}$ | 1               | <i>acrB</i>   | Multidrug resistance efflux pump  | acriflavin, aminoglycoside,<br>beta_lactam, glycylcycline,<br>macrolide                              |

Table S2. Cont.

| No. | ARDB Accession Number | Identity (%) ≥ | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name          | Function  | Related Antibiotics  |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|-------------------|---|--|
| 71  | ABY60438              | 100            | 33                | $4.0 \times 10^{-13}$ | 1               | <i>bl2D_oxa10</i> | Class D beta-lactamase  | cloxacillin, penicillin  |
| 72  | ACI02041              | 100            | 26                | $8.0 \times 10^{-10}$ | 1               | <i>tetW</i>       | Ribosomal protection protein  | tetracycline   |
| 73  | ACK77685              | 90.91          | 33                | $2.0 \times 10^{-11}$ | 1               | <i>acrB</i>       | Multidrug resistance efflux pump                                    | acriflavin, aminoglycoside, beta_lactam, glycycycline, macrolide |
| 74  | ACN65403              | 100            | 30                | $8.0 \times 10^{-12}$ | 1               | <i>sul1</i>       | Sulfonamide-resistant dihydropteroate synthase                      | sulfonamide  |
| 75  | ACR66841              | 100            | 32                | $2.0 \times 10^{-12}$ | 1               | <i>sul1</i>       | Sulfonamide-resistant dihydropteroate synthase                      | sulfonamide  |
| 76  | CAA24909              | 100            | 33                | $5.0 \times 10^{-16}$ | 1               | <i>tetA</i>       | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline   |
| 77  | CAA33123              | 100            | 33                | $3.0 \times 10^{-13}$ | 1               | <i>sul1</i>       | Sulfonamide-resistant dihydropteroate synthase                      | sulfonamide  |
| 78  | CAE81273              | 100            | 33                | $2.0 \times 10^{-14}$ | 1               | <i>catB3</i>      | Group B chloramphenicol acetyltransferase                           | chloramphenicol  |
| 79  | CAI57693              | 96.97          | 33                | $6.0 \times 10^{-15}$ | 1               | <i>dfrA7</i>      | Group A drug-insensitive dihydrofolate reductase                    | trimethoprim   |
| 80  | CAP69660              | 100            | 33                | $4.0 \times 10^{-13}$ | 1               | <i>bl2D_oxa5</i>  | Class D beta-lactamase  | cloxacillin, penicillin  |
| 81  | CAX33142              | 100            | 33                | $9.0 \times 10^{-14}$ | 1               | <i>bl2D_oxa10</i> | Class D beta-lactamase  | cloxacillin, penicillin  |
| 82  | CAY51926              | 93.94          | 33                | $2.0 \times 10^{-11}$ | 1               | <i>mexW</i>       | Multidrug resistance efflux pump                                    |  |
| 83  | EEQ96502              | 90.91          | 33                | $1.0 \times 10^{-11}$ | 1               | <i>bacA</i>       | Undecaprenyl pyrophosphate phosphatase                              | bacitracin   |
| 84  | NP_348075             | 96.3           | 27                | $1.0 \times 10^{-8}$  | 1               | <i>tetP(A)</i>    | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline   |
| 85  | NP_940742             | 100            | 33                | $5.0 \times 10^{-14}$ | 1               | <i>tet33</i>      | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline   |
| 86  | XP_002333050          | 100            | 33                | $3.0 \times 10^{-13}$ | 1               | <i>tetC</i>       | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline   |

Table S2. Cont.

| No. | ARDB Accession Number | Identity (%) ≥ | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name     | Function  | Related Antibiotics   |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|--------------|---|---|
| 87  | YP_001096238          | 96.97          | 33                | $5.0 \times 10^{-11}$ | 1               | <i>tet33</i> | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline  |
| 88  | YP_001348630          | 90.91          | 33                | $5.0 \times 10^{-12}$ | 1               | <i>mexY</i>  | Multidrug resistance efflux pump                                    | aminoglycoside, glycylcycline erythromycin,                       |
| 89  | YP_001350571          | 90.91          | 33                | $1.0 \times 10^{-10}$ | 1               | <i>mexD</i>  | Multidrug resistance efflux pump                                    | fluoroquinolone, glycylcycline, roxithromycin                     |
| 90  | YP_001416709          | 90.62          | 32                | $1.0 \times 10^{-11}$ | 1               | <i>bacA</i>  | Undecaprenyl pyrophosphate phosphatase                              | bacitracin  |
| 91  | YP_001438541          | 90.62          | 32                | $4.0 \times 10^{-11}$ | 1               | <i>macB</i>  | Macrolide-specific efflux system                                    | macrolide   |
| 92  | YP_001477378          | 96.88          | 32                | $6.0 \times 10^{-12}$ | 1               | <i>rosA</i>  | Major facilitator superfamily transporter                           | fosmidomycin  |
| 93  | YP_001747848          | 90.62          | 32                | $6.0 \times 10^{-10}$ | 1               | <i>mexW</i>  | Multidrug resistance efflux pump                                    |   |
| 94  | YP_002240037          | 93.75          | 32                | $5.0 \times 10^{-11}$ | 1               | <i>acrB</i>  | Multidrug resistance efflux pump                                    | acriflavin, aminoglycoside, beta_lactam, glycylcycline, macrolide |
| 95  | YP_002332890          | 100            | 32                | $2.0 \times 10^{-11}$ | 1               | <i>tetA</i>  | Major facilitator superfamily transporter, tetracycline efflux pump | tetracycline  |
| 96  | YP_002382193          | 90.91          | 33                | $8.0 \times 10^{-12}$ | 1               | <i>macB</i>  | Macrolide-specific efflux system                                    | macrolide   |
| 97  | YP_002800512          | 96.97          | 33                | $2.0 \times 10^{-12}$ | 1               | <i>mexF</i>  | Multidrug resistance efflux pump                                    | chloramphenicol, fluoroquinolone                                  |
| 98  | YP_002847505          | 93.94          | 33                | $5.0 \times 10^{-11}$ | 1               | <i>mdtF</i>  | Multidrug resistance efflux pump                                    | doxorubicin, erythromycin   |
| 99  | YP_151445             | 96.88          | 32                | $8.0 \times 10^{-12}$ | 1               | <i>acrB</i>  | Multidrug resistance efflux pump                                    | acriflavin, aminoglycoside, beta_lactam, glycylcycline, macrolide |

Table S2. Cont.

| No. | ARDB Accession Number | Identity (%) ≥ | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name    | Function   | Related Antibiotics  |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|-------------|--|--|
| 100 | YP_236040             | 90.62          | 32                | $1.0 \times 10^{-10}$ | 1               | <i>mexF</i> | Multidrug resistance efflux pump   | chloramphenicol,<br>fluoroquinolone                                    |
| 101 | YP_274481             | 90.32          | 31                | $1.0 \times 10^{-10}$ | 1               | <i>mexF</i> | Multidrug resistance efflux pump   | chloramphenicol,<br>fluoroquinolone                                    |
| 102 | YP_348389             | 90.62          | 32                | $2.0 \times 10^{-11}$ | 1               | <i>mexF</i> | Multidrug resistance efflux pump   | chloramphenicol,<br>fluoroquinolone                                    |
| 103 | YP_454364             | 96.43          | 28                | $4.0 \times 10^{-9}$  | 1               | <i>acrB</i> | Multidrug resistance efflux pump   | acriflavin, aminoglycoside,<br>beta_lactam, glycycycline,<br>macrolide |
| 104 | YP_550152             | 96.97          | 33                | $2.0 \times 10^{-12}$ | 1               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase   | bacitracin   |
| 105 | YP_606823             | 90.32          | 31                | $6.0 \times 10^{-10}$ | 1               | <i>mexW</i> | Multidrug resistance efflux pump   |  |
| 106 | YP_970399             | 93.94          | 33                | $6.0 \times 10^{-12}$ | 1               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase   | bacitracin   |
| 107 | YP_981592             | 90             | 30                | $1.0 \times 10^{-09}$ | 1               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase   | bacitracin   |
| 108 | YP_997055             | 96.97          | 33                | $7.0 \times 10^{-13}$ | 1               | <i>bacA</i> | Undecaprenyl pyrophosphate phosphatase<br><br>Bifunctional enzyme that catalyzes the<br>oxidative decarboxylation of<br>UDP-glucuronic acid (UDP-GlcUA) to<br>UDP-4-keto-arabinose (UDP-Ara4O) and<br>the addition of a formyl group to<br>UDP-4-amino-4-deoxy-L-arabinose<br>(UDP-L-Ara4N) to form<br>UDP-L-4-formamido-arabinose<br>(UDP-L-Ara4FN). The modified arabinose<br>is attached to lipid A and is required for<br>resistance to polymyxin and cationic<br>antimicrobial peptides | bacitracin   |
| 109 | ZP_02901868           | 90.91          | 33                | $1.0 \times 10^{-12}$ | 1               | <i>arnA</i> | UDP-4-amino-4-deoxy-L-arabinose<br>(UDP-L-Ara4N) to form<br>UDP-L-4-formamido-arabinose<br>(UDP-L-Ara4FN). The modified arabinose<br>is attached to lipid A and is required for<br>resistance to polymyxin and cationic<br>antimicrobial peptides  | polymyxin  |

Table S2. Cont.

| No. | ARDB Accession Number | Identity (%) ≥ | Hit Length (AA) u | E Value t             | Number of Reads | ARG Name      | Function  | Related Antibiotics   |
|-----|-----------------------|----------------|-------------------|-----------------------|-----------------|---------------|---|---|
| 110 | ZP_03075977           | 96.97          | 33                | $4.0 \times 10^{-13}$ | 1               | <i>arnA</i>   | Bifunctional enzyme that catalyzes the oxidative decarboxylation of UDP-glucuronic acid (UDP-GlcUA) to UDP-4-keto-arabinose (UDP-Ara4O) and the addition of a formyl group to UDP-4-amino-4-deoxy-L-arabinose (UDP-L-Ara4N) to form UDP-L-4-formamido-arabinose (UDP-L-Ara4FN). The modified arabinose is attached to lipid A and is required for resistance to polymyxin and cationic antimicrobial peptides | polymyxin   |
| 111 | ZP_03583212           | 100            | 33                | $7.0 \times 10^{-13}$ | 1               | <i>ceoB</i>   | Multidrug resistance efflux pump  | chloramphenicol   |
| 112 | ZP_03790349           | 93.75          | 32                | $4.0 \times 10^{-11}$ | 1               | <i>ceoB</i>   | Multidrug resistance efflux pump  | chloramphenicol   |
| 113 | ZP_04405450           | 96             | 25                | $2.0 \times 10^{-10}$ | 1               | <i>aph6id</i> | Aminoglycoside O-phosphotransferase   | streptomycin  |
| 114 | ZP_04612532           | 90.62          | 32                | $2.0 \times 10^{-11}$ | 1               | <i>acrB</i>   | Multidrug resistance efflux pump  | acriflavin, aminoglycoside, beta_lactam, glycylcycline, macrolide |
| 115 | ZP_04615639           | 90.32          | 31                | $9.0 \times 10^{-11}$ | 1               | <i>rosB</i>   | Potassium antiporter  | fosmidomycin  |

**Table S3.** Matched high-throughput sequencing reads of plasmids in sludge cultured with 0mg/L tetracycline of Day 6 against NCBI Plasmid Genome Database.

| Accession Number | Bacterial Host                              | Plasmid Name    | Identity (%) t | Hit Length (bp) | E Value t             | Number of Reads |
|------------------|---|-----------------|----------------|-----------------|-----------------------|-----------------|
| NC_006994.1      | <i>Pasteurella multocida</i>                | pCCK381         | 95             | 90              | $6.0 \times 10^{-36}$ | 4274            |
| NC_011092.1      | <i>Salmonella enterica</i>                  | pCVM19633_110   | 95             | 93              | $5.0 \times 10^{-37}$ | 3920            |
| NC_013365.1      | <i>Escherichia coli</i>                     | pO111_1         | 95             | 90              | $6.0 \times 10^{-36}$ | 2409            |
| NC_011602.1      | <i>Escherichia coli</i>                     | pE2348-2        | 95             | 90              | $6.0 \times 10^{-36}$ | 1003            |
| NC_014641.1      | <i>Achromobacter xylosoxidans</i>           | pA81            | 95             | 90              | $2.0 \times 10^{-35}$ | 509             |
| NC_003296.1      | <i>Ralstonia solanacearum</i>               | pGMI1000MP      | 95             | 90              | $1.0 \times 10^{-33}$ | 439             |
| NC_013193.1      | <i>Candidatus Accumulibacter phosphatis</i> | pAph01          | 95             | 92              | $2.0 \times 10^{-35}$ | 331             |
| NC_013509.1      | <i>Edwardsiella tarda</i>                   | pEIB202         | 96.04          | 92              | $7.0 \times 10^{-40}$ | 245             |
| NC_002524.1      | <i>Uncultured eubacterium</i>               | pIE1115         | 98             | 96              | $3.0 \times 10^{-43}$ | 236             |
| NC_010394.1      | <i>Mycobacterium abscessus</i>              | unnamed plasmid | 95.83          | 90              | $5.0 \times 10^{-37}$ | 172             |
| NC_010935.1      | <i>Comamonas testosteroni</i>               | pCNB            | 95             | 90              | $2.0 \times 10^{-36}$ | 166             |
| NC_014911.1      | <i>Alicyphilus denitrificans</i>            | pALIDE02        | 95             | 90              | $8.0 \times 10^{-35}$ | 162             |
| NC_014211.1      | <i>Nocardiopsis dassonvillei</i>            | pNDAS01         | 95             | 90              | $3.0 \times 10^{-34}$ | 141             |
| NC_010912.1      | <i>Avibacterium paragallinarum</i>          | pYMH5           | 98             | 97              | $3.0 \times 10^{-43}$ | 137             |
| NC_010510.1      | <i>Methylobacterium radiotolerans</i>       | pMRAD01         | 95             | 90              | $3.0 \times 10^{-34}$ | 135             |
| NC_007974.2      | <i>Cupriavidus metallidurans</i>            | megaplasmid     | 95             | 90              | $1.0 \times 10^{-33}$ | 123             |
| NC_014167.1      | <i>Corynebacterium resistens</i>            | pJA144188       | 98             | 93              | $1.0 \times 10^{-42}$ | 120             |
| NC_006823.1      | <i>Azoarcus</i> sp.                         | plasmid 1       | 95             | 90              | $3.0 \times 10^{-34}$ | 110             |
| NC_003350.1      | <i>Pseudomonas putida</i>                   | pWW0            | 95             | 90              | $2.0 \times 10^{-35}$ | 110             |
| NC_014208.1      | <i>Klebsiella oxytoca</i>                   | pKOX105         | 95             | 91              | $3.0 \times 10^{-38}$ | 106             |
| NC_009140.1      | <i>Salmonella enterica</i>                  | pSN254          | 95             | 92              | $6.0 \times 10^{-36}$ | 85              |
| NC_004973.1      | <i>Uncultured eubacterium</i>               | pIE1130         | 95.05          | 100             | $3.0 \times 10^{-38}$ | 84              |
| NC_011667.1      | <i>Thauera</i> sp.                          | pTha01          | 95             | 92              | $2.0 \times 10^{-36}$ | 82              |
| NC_001740.1      |   | RSF1010         | 98             | 96              | $4.0 \times 10^{-42}$ | 80              |
| NC_014621.1      | <i>Ketogulonicigenium vulgare</i>           | pYP1            | 95             | 90              | $2.0 \times 10^{-36}$ | 79              |
| NC_004604.2      | <i>Bacillus megaterium</i>                  | pBM400          | 95             | 90              | $2.0 \times 10^{-35}$ | 75              |

Table S3. Cont.

| Accession Number | Bacterial Host                      | Plasmid Name | Identity (%) t | Hit Length (bp) | E Value t             | Number of Reads |
|------------------|-------------------------------------|--------------|----------------|-----------------|-----------------------|-----------------|
| NC_013859.1      | <i>Azospirillum</i> sp.             | pAB510e      | 95             | 90              | $8.0 \times 10^{-35}$ | 59              |
| NC_009753.1      | <i>Paracoccus methylutens</i>       | pMTH1        | 97             | 93              | $2.0 \times 10^{-41}$ | 57              |
| NC_008790.1      | <i>Campylobacter jejuni</i>         | pTet         | 96             | 97              | $7.0 \times 10^{-40}$ | 50              |
| NC_008697.1      | <i>Nocardioides</i> sp.             | pNOCA01      | 95             | 91              | $2.0 \times 10^{-35}$ | 44              |
| NC_009429.1      | <i>Rhodobacter sphaeroides</i>      | pRSPA01      | 95.65          | 91              | $2.0 \times 10^{-35}$ | 40              |
| NC_014107.1      | <i>Enterobacter cloacae</i> subsp.  | pECL_A       | 95             | 91              | $3.0 \times 10^{-38}$ | 37              |
| NC_004059.1      | <i>Bacillus thuringiensis</i>       | pUIBI-1      | 95             | 90              | $1.0 \times 10^{-33}$ | 37              |
| NC_014811.1      | <i>Mycobacterium</i> sp.            | pMSPYR101    | 96.94          | 92              | $2.0 \times 10^{-40}$ | 36              |
| NC_008826.1      | <i>Methylibium petroleiphilum</i>   | RPME01       | 95             | 92              | $6.0 \times 10^{-36}$ | 34              |
| NC_014633.1      | <i>Ilyobacter polytropus</i>        | pILYOP01     | 95             | 90              | $8.0 \times 10^{-35}$ | 33              |
| NC_012527.1      | <i>Deinococcus deserti</i>          | plasmid 1    | 95             | 92              | $2.0 \times 10^{-35}$ | 32              |
| NC_008703.1      | <i>Mycobacterium</i> sp.            | pMKMS01      | 95             | 90              | $2.0 \times 10^{-36}$ | 30              |
| NC_012780.1      | <i>Eubacterium eligens</i>          | unnamed      | 95             | 92              | $1.0 \times 10^{-37}$ | 27              |
| NC_006821.1      | <i>Bacillus thuringiensis</i>       | pBMBt1       | 95.96          | 98              | $1.0 \times 10^{-38}$ | 27              |
| NC_014309.1      | <i>Ralstonia solanacearum</i>       | RCFBPv3_mp   | 95             | 90              | $3.0 \times 10^{-34}$ | 26              |
| NC_008308.1      | <i>Sphingomonas</i> sp.             | pCAR3        | 95             | 91              | $2.0 \times 10^{-36}$ | 25              |
| NC_009507.1      | <i>Sphingomonas wittichii</i>       | pSWIT01      | 95             | 93              | $3.0 \times 10^{-38}$ | 24              |
| NC_008704.1      | <i>Mycobacterium</i> sp.            | pMKMS02      | 95             | 98              | $3.0 \times 10^{-38}$ | 22              |
| NC_002637.1      | <i>Mannheimia haemolytica</i>       | pMHSCS1      | 96             | 93              | $7.0 \times 10^{-40}$ | 22              |
| NC_007800.1      | <i>Bibersteinia trehalosi</i>       | pCCK13698    | 97             | 99              | $2.0 \times 10^{-41}$ | 22              |
| NC_007486.1      | <i>Rhodococcus erythropolis</i>     | pREC1        | 96.67          | 90              | $2.0 \times 10^{-35}$ | 22              |
| NC_009339.1      | <i>Mycobacterium gilvum</i>         | pMFLV01      | 95             | 93              | $5.0 \times 10^{-37}$ | 21              |
| NC_007959.1      | <i>Nitrobacter hamburgensis</i>     | plasmid 1    | 95             | 95              | $5.0 \times 10^{-37}$ | 20              |
| NC_011961.1      | <i>Thermomicrobium roseum</i>       | unnamed      | 95             | 90              | $8.0 \times 10^{-35}$ | 20              |
| NC_014908.1      | <i>Alicycliphilus denitrificans</i> | pALIDE01     | 95             | 90              | $5.0 \times 10^{-37}$ | 20              |
| NC_006824.1      | <i>Azoarcus</i> sp.                 | plasmid 2    | 95             | 92              | $1.0 \times 10^{-37}$ | 17              |
| NC_008242.1      | <i>Mesorhizobium</i> sp.            | plasmid 1    | 95             | 91              | $6.0 \times 10^{-36}$ | 17              |

Table S3. Cont.

| Accession Number | Bacterial Host                         | Plasmid Name | Identity (%) t | Hit Length (bp) | E Value t              | Number of Reads |
|------------------|--|--------------|----------------|-----------------|------------------------|-----------------|
| NC_014144.1      | <i>Thiomonas</i> sp.                   | pTHI         | 96             | 94              | $7.0 \times 10^{-40}$  | 17              |
| NC_009717.1      | <i>Xanthobacter autotrophicus</i>      | pXAUT01      | 95.65          | 92              | $2.0 \times 10^{-35}$  | 17              |
| NC_014801.1      | <i>Campylobacter jejuni</i>            | pTet         | 97             | 91              | $2.0 \times 10^{-41}$  | 17              |
| NC_009426.1      | <i>Novosphingobium aromaticivorans</i> | pNL1         | 95             | 90              |                        | 16              |
| NC_012586.1      | <i>Sinorhizobium fredii</i>            | pNGR234b     | 95             | 91              | $4.0 \times 10^{-33}$  | 16              |
| NC_013963.1      | <i>Bacillus</i> sp.                    | pBS-01       | 98             | 100             | $6.0 \times 10^{-36}$  | 15              |
| NC_013280.1      | <i>Uncultured bacterium</i>            | pTRACA22     | 96.94          | 97              | $3.0 \times 10^{-43}$  | 15              |
| NC_003430.1      | <i>Uncultured bacterium</i>            | pB4          | 95             | 99              | $2.0 \times 10^{-40}$  | 15              |
| NC_008272.1      |  | pKJK5        | 95             | 100             | $3.0 \times 10^{-38}$  | 14              |
| NC_012811.1      | <i>Methylobacterium extorquens</i>     | megaplasmid  | 95             | 93              | $3.0 \times 10^{-38}$  | 14              |
| NC_014155.1      | <i>Thiomonas intermedia</i>            | pTINT02      | 95             | 99              | $6.0 \times 10^{-36}$  | 13              |
| NC_002698.1      | <i>Shigella flexneri</i>               | pWR501       | 95             | 96              | $3.0 \times 10^{-38}$  | 13              |
| NC_010076.1      | <i>Bacillus thuringiensis</i>          | pBtoxis      | 96             | 100             | $3.0 \times 10^{-38}$  | 12              |
| NC_008766.1      | <i>Acidovorax</i> sp.                  | pAOVO02      | 95             | 95              | $7.0 \times 10^{-40}$  | 12              |
| NC_008043.1      | <i>Ruegeria</i> sp.                    | mega plasmid | 95             | 90              | $3.0 \times 10^{-38}$  | 12              |
| NC_008712.1      | <i>Arthrobacter aurescens</i>          | TC1          | 95             | 91              | $1.0 \times 10^{-33}$  | 12              |
| NC_011419.1      | <i>Escherichia coli</i>                | pSE11-1      | 99             | 100             | $8.0 \times 10^{-35}$  | 11              |
| NC_010983.1      | <i>Alpha proteobacterium</i>           | pAP3.9       | 95             | 99              | $7.0 \times 10^{-45}$  | 11              |
| NC_010848.1      | <i>Flavobacterium</i> sp.              | pOAD2        | 96             | 100             | $3.0 \times 10^{-38}$  | 11              |
| NC_006143.1      | <i>Aeromonas punctata</i>              | pFBAOT6      | 98.02          | 99              | $7.0 \times 10^{-40}$  | 11              |
| NC_008608.1      | <i>Pelobacter propionicus</i>          | pPRO2        | 98             | 100             | $3.0 \times 10^{-43}$  | 11              |
| NC_010488.1      | <i>Escherichia coli</i>                | pSMS35_130   | 95             | 92              | $3.0 \times 10^{-43}$  | 11              |
| NC_013855.1      | <i>Azospirillum</i> sp.                | pAB510a      | 95             | 90              | $3.00 \times 10^{-38}$ | 11              |
| NC_009739.1      | <i>Pseudomonas aeruginosa</i>          | pMATVIM-7    | 95             | 93              | $3.0 \times 10^{-34}$  | 10              |
| NC_007100.1      | <i>Pseudomonas aeruginosa</i>          | Rms149       | 96             | 92              | $3.0 \times 10^{-38}$  | 10              |
| NC_013857.1      | <i>Azospirillum</i> sp.                | pAB510c      | 95             | 92              | $5.0 \times 10^{-37}$  | 10              |

Table S3. Cont.

| Accession Number | Bacterial Host                               | Plasmid Name | Identity (%) t | Hit Length (bp) | E Value t              | Number of Reads |
|------------------|--|--------------|----------------|-----------------|------------------------|-----------------|
| NC_005873.1      | <i>Oligotropha carboxidovorans</i>           | pHCG3        | 95             | 94              | $1.0 \times 10^{-37}$  | 10              |
| NC_002091.1      | <i>Bacillus thuringiensis</i>                | pTX14-1      | 95             | 92              | $5.0 \times 10^{-37}$  | 9               |
| NC_014231.1      | <i>Escherichia coli</i>                      | pKC394       | 98             | 95              | $3.0 \times 10^{-43}$  | 9               |
| NC_013191.1      | <i>Candidatus Accumulibacter phosphatis</i>  | pAph03       | 95             | 99              | $3.0 \times 10^{-38}$  | 8               |
| NC_005793.1      | <i>Achromobacter denitrificans</i>           | pEST4011     | 95             | 95              | $3.0 \times 10^{-38}$  | 8               |
| NC_009508.1      | <i>Sphingomonas wittichii</i>                | pSWIT02      | 95.92          | 98              | $3.0 \times 10^{-38}$  | 8               |
| NC_010558.1      | <i>Escherichia coli</i>                      | pIP1206      | 100            | 100             | $2.00 \times 10^{-46}$ | 8               |
| NC_006365.1      | <i>Legionella pneumophila</i>                | pLPP         | 95.74          | 92              | $2.0 \times 10^{-36}$  | 8               |
| NC_009669.1      | <i>Ochrobactrum anthropi</i>                 | pOANT01      | 95             | 97              | $1.0 \times 10^{-37}$  | 7               |
| NC_010625.1      | <i>Burkholderia phymatum</i>                 | pBPHY01      | 95             | 93              | $6.0 \times 10^{-36}$  | 7               |
| NC_007141.1      | <i>Campylobacter jejuni</i>                  | pTet         | 95             | 97              | $1.0 \times 10^{-37}$  | 7               |
| NC_010189.1      | <i>Naegleria gruberi</i>                     | unnamed      | 95             | 100             | $3.0 \times 10^{-38}$  | 7               |
| NC_008688.1      | <i>Paracoccus denitrificans</i>              | plasmid 1    | 95             | 92              | $2.0 \times 10^{-35}$  | 7               |
| NC_009475.1      | <i>Bradyrhizobium</i> sp.                    | pBBta01      | 95             | 98              | $3.0 \times 10^{-38}$  | 7               |
| NC_014007.1      | <i>Sphingobium japonicum</i>                 | pCHQ1        | 95             | 92              | $2.0 \times 10^{-35}$  | 7               |
| NC_002679.1      | <i>Mesorhizobium loti</i>                    | pMLa         | 95             | 95              | $3.0 \times 10^{-38}$  | 6               |
| NC_005241.1      | <i>Cupriavidus necator</i>                   | pHG1         | 95             | 94              | $2.0 \times 10^{-36}$  | 6               |
| NC_003123.1      | <i>Aeromonas salmonicida salmonicida</i>     | pRAS3.1      | 100            | 100             | $2.0 \times 10^{-46}$  | 6               |
| NC_008055.1      | <i>Uncultured bacterium in freshwater</i>    | QKH54        | 98             | 100             | $3.0 \times 10^{-43}$  | 6               |
| NC_004957.1      |  | p121BS       | 99             | 100             | $7.0 \times 10^{-45}$  | 6               |
| NC_002089.1      |  | pIE1107      | 99             | 100             | $7.00 \times 10^{-45}$ | 6               |
| NC_003042.1      | <i>Clostridium perfringens</i>               | pCP13        | 97             | 99              | $2.0 \times 10^{-41}$  | 6               |
| NC_012855.1      | <i>Ralstonia pickettii</i>                   | pRp12D01     | 96             | 100             | $7.00 \times 10^{-40}$ | 6               |
| NC_007972.2      | <i>Cupriavidus metallidurans</i>             | PMOL28       | 95.88          | 91              | $3 \times 10^{-38}$    | 6               |
| NC_007960.1      | <i>Nitrobacter hamburgensis</i>              | plasmid 2    | 95.05          | 98              | $3.00 \times 10^{-38}$ | 6               |
| NC_006385.1      | <i>Uncultured bacterium activated sludge</i> | pRSB101      | 97             | 98              | $2.0 \times 10^{-41}$  | 6               |
| NC_004956.1      | <i>Pseudomonas</i> sp.                       | pADP-1       | 99             | 97              | $7.0 \times 10^{-45}$  | 6               |

Table S3. Cont.

| Accession Number | Bacterial Host                       | Plasmid Name | Identity (%) t | Hit Length (bp) | E Value t              | Number of Reads |
|------------------|--------------------------------------|--------------|----------------|-----------------|------------------------|-----------------|
| NC_008010.2      | <i>Deinococcus geothermalis</i>      | pDGEO01      | 95.56          | 90              | $1.0 \times 10^{-33}$  | 5               |
| NC_010937.1      | <i>Clostridium perfringens</i>       | pCW3         | 96             | 100             | $7.0 \times 10^{-40}$  | 5               |
| NC_010643.1      | <i>Providencia rettgeri</i>          | R7K          | 99             | 100             | $7.0 \times 10^{-45}$  | 5               |
| NC_013513.1      | <i>Paracoccus aminophilus</i>        | pAMI3        | 95             | 100             | $3.0 \times 10^{-38}$  | 5               |
| NC_014557.1      | <i>Bacillus</i> sp.                  | pBS-02       | 100            | 100             | $2.0 \times 10^{-46}$  | 5               |
| NC_003122.1      | <i>Uncultured bacterium in soil</i>  | pSB102       | 95.65          | 92              | $2.00 \times 10^{-35}$ | 5               |
| NC_006363.1      | <i>Nocardia farcinica</i>            | pNF2         | 97             | 92              | $2.0 \times 10^{-41}$  | 5               |
| NC_009957.1      | <i>Dinoroseobacter shibae</i>        | pDSHI03      | 97.03          | 100             | $2 \times 10^{-41}$    | 5               |
| NC_005307.1      | <i>Gordonia westfalica</i>           | pKB1         | 95.96          | 99              | $3.0 \times 10^{-39}$  | 5               |
| NC_012556.1      | <i>Enterobacter cloacae</i>          | pEC-IMPQ     | 100            | 92              | $4.00 \times 10^{-42}$ | 5               |
| NC_007337.1      | <i>Ralstonia eutropha</i>            | plasmid 1    | 97.96          | 98              | $2.00 \times 10^{-41}$ | 5               |
| NC_013278.1      | <i>Uncultured bacterium</i>          | pTRACA18     | 96.94          | 98              | $2.0 \times 10^{-40}$  | 4               |
| NC_010891.1      | <i>Pseudomonas</i> sp.               | pCT14        | 99             | 92              | $4.0 \times 10^{-42}$  | 4               |
| NC_010864.1      | <i>Pediococcus acidilactici</i>      | pEOC01       | 95             | 100             | $3.00 \times 10^{-38}$ | 4               |
| NC_013190.1      | <i>Candidatus Accumulibacter</i>     | pAph02       | 96             | 98              | $7.0 \times 10^{-40}$  | 4               |
| NC_013279.1      | <i>Uncultured bacterium</i>          | pTRACA20     | 97             | 99              | $2.0 \times 10^{-41}$  | 4               |
| NC_010466.1      | <i>Leuconostoc citreum</i>           | pLCK2        | 95             | 100             | $3.00 \times 10^{-38}$ | 4               |
| NC_005707.1      | <i>Bacillus cereus</i>               | pBc10987     | 95             | 94              | $3.0 \times 10^{-38}$  | 4               |
| NC_013783.1      | <i>Lactococcus lactis</i>            | pAR141       | 97.06          | 100             | $4.00 \times 10^{-42}$ | 4               |
| NC_008758.1      | <i>Polaromonas naphthalenivorans</i> | pPNAP02      | 95.65          | 92              | $2.00 \times 10^{-35}$ | 4               |
| NC_013858.1      | <i>Azospirillum</i> sp.              | pAB510d      | 95.05          | 92              | $3.00 \times 10^{-38}$ | 4               |
| NC_011339.1      | <i>Bacillus cereus</i>               | pH308197_258 | 100            | 100             | $2.0 \times 10^{-46}$  | 3               |
| NC_007352.1      | <i>Staphylococcus saprophyticus</i>  | pSSP2        | 98.99          | 99              | $3.0 \times 10^{-44}$  | 3               |
| NC_003037.1      | <i>Sinorhizobium meliloti</i>        | pSymA        | 96             | 97              | $7.0 \times 10^{-40}$  | 3               |
| NC_011982.1      | <i>Agrobacterium vitis</i>           | pTiS4        | 96             | 100             | $7.0 \times 10^{-40}$  | 3               |
| NC_004945.1      | <i>Corynebacterium glutamicum</i>    | pCG4         | 99             | 100             | $7.0 \times 10^{-45}$  | 3               |
| NC_014154.1      | <i>Thiomonas intermedia</i>          | pTINT01      | 95.88          | 97              | $3.0 \times 10^{-38}$  | 3               |

Table S3. Cont.

| Accession Number | Bacterial Host                           | Plasmid Name | Identity (%) t | Hit Length (bp) | E Value t              | Number of Reads |
|------------------|--|--------------|----------------|-----------------|------------------------|-----------------|
| NC_007773.1      | <i>Clostridium perfringens</i>           | pCPF5603     | 100            | 100             | $2.0 \times 10^{-46}$  | 3               |
| NC_010330.1      | <i>Enterococcus faecium</i>              | pRI1         | 98             | 100             | $3.0 \times 10^{-43}$  | 3               |
| NC_010996.1      | <i>Rhizobium etli</i>                    | pB           | 95.65          | 92              | $2.00 \times 10^{-35}$ | 3               |
| NC_010529.1      | <i>Cupriavidus taiwanensis</i>           | pRALTA       | 95.74          | 91              | $2.0 \times 10^{-36}$  | 3               |
| NC_012848.1      | <i>Rhizobium leguminosarum</i>           | pR132501     | 95             | 92              | $2.0 \times 10^{-35}$  | 3               |
| NC_008269.1      | <i>Rhodococcus jostii</i>                | pRHL1        | 95             | 97              | $3.0 \times 10^{-38}$  | 3               |
| NC_008765.1      | <i>Acidovorax</i> sp.                    | pAOVO01      | 100            | 100             | $2.00 \times 10^{-46}$ | 3               |
| NC_011604.1      | <i>Salmonella enterica</i>               | pWES-1       | 95.05          | 90              | $3.0 \times 10^{-34}$  | 3               |
| NC_006625.1      | <i>Klebsiella pneumoniae</i>             | pK2044       | 98             | 100             | $3.00 \times 10^{-43}$ | 3               |
| NC_009779.1      | <i>Cronobacter sakazakii</i>             | pESA2        | 98             | 100             | $3.00 \times 10^{-43}$ | 3               |
| NC_009129.1      | <i>Corynebacterium</i> sp.               | pLEW279b     | 98             | 100             | $3.00 \times 10^{-43}$ | 3               |
| NC_014726.1      | <i>Enterococcus faecalis</i>             | pTW9         | 97             | 100             | $2.00 \times 10^{-41}$ | 3               |
| NC_014005.1      | <i>Sphingobium japonicum</i>             | pUT1         | 95.05          | 99              | $3.00 \times 10^{-38}$ | 3               |
| NC_006872.1      | <i>Clostridium perfringens</i>           | pBCNF5603    | 98             | 100             | $3.00 \times 10^{-43}$ | 3               |
| NC_007502.1      | Uncultured bacterium in river sediments  | pB8          | 97             | 96              | $6.0 \times 10^{-41}$  | 3               |
| NC_013452.1      | <i>Staphylococcus aureus</i>             | pT181        | 100            | 100             | $2.00 \times 10^{-46}$ | 3               |
| NC_011371.1      | <i>Rhizobium leguminosarum</i>           | pRLG204      | 95             | 97              | $3.0 \times 10^{-38}$  | 3               |
| NC_012849.1      | <i>Ralstonia pickettii</i>               | pRp12D02     | 95             | 95              | $3 \times 10^{-38}$    | 3               |
| NC_003078.1      | <i>Sinorhizobium meliloti</i>            | pSymB        | 95             | 96              | $1.0 \times 10^{-37}$  | 3               |
| NC_006352.1      | Uncultured bacterium activated sludge    | pTB11        | 95.92          | 98              | $1.00 \times 10^{-38}$ | 3               |
| NC_014035.1      | <i>Rhodobacter capsulatus</i>            | pRCB133      | 95.7           | 93              | $6.0 \times 10^{-36}$  | 2               |
| NC_009620.1      | <i>Sinorhizobium medicae</i>             | pSMED01      | 95             | 100             | $3.0 \times 10^{-38}$  | 2               |
| NC_002143.1      | <i>Comamonas testosteroni</i>            | pPT1         | 98.97          | 97              | $3.0 \times 10^{-43}$  | 2               |
| NC_006388.1      | Uncultured bacterium in activated sludge | pB3          | 96             | 100             | $7.0 \times 10^{-40}$  | 2               |
| NC_009340.1      | <i>Mycobacterium gilvum</i>              | pMFLV02      | 96             | 98              | $7.0 \times 10^{-40}$  | 2               |
| NC_004341.2      | <i>Weissella cibaria</i>                 | pKLCB        | 95.05          | 101             | $3.0 \times 10^{-38}$  | 2               |
| NC_009651.1      | <i>Klebsiella pneumoniae</i>             | pKPN5        | 100            | 100             | $2.0 \times 10^{-46}$  | 2               |

Table S3. Cont.

| Accession Number | Bacterial Host                     | Plasmid Name | Identity (%) t | Hit Length (bp) | E Value t              | Number of Reads |
|------------------|------------------------------------|--------------|----------------|-----------------|------------------------|-----------------|
| NC_010938.1      | <i>Tetragenococcus halophilus</i>  | pSKPB18      | 100            | 100             | $2.0 \times 10^{-46}$  | 2               |
| NC_012473.1      | <i>Bacillus cereus</i>             | p03BB102_179 | 96             | 100             | $7.0 \times 10^{-40}$  | 2               |
| NC_008382.1      | <i>Rhizobium leguminosarum</i> bv. | pRL7         | 95             | 98              | $3.0 \times 10^{-38}$  | 2               |
| NC_011073.1      | <i>Bacteroides fragilis</i>        | pBFP35       | 100            | 100             | $2.0 \times 10^{-46}$  | 2               |
| NC_005566.1      | <i>Staphylococcus epidermidis</i>  | pSK639       | 98             | 100             | $3.0 \times 10^{-43}$  | 2               |
| NC_013176.1      | <i>Pseudomonas putida</i>          | pW2          | 99             | 100             | $2.0 \times 10^{-45}$  | 2               |
| NC_012661.1      | <i>Haemophilus parasuis</i>        | pHN61        | 100            | 100             | $2.0 \times 10^{-46}$  | 2               |
| NC_002114.1      | <i>Nitrosomonas</i> sp.            | pAYL         | 96             | 100             | $7.0 \times 10^{-40}$  | 2               |
| NC_001446.1      | <i>Bacillus thuringiensis</i>      | pTX14-3      | 95             | 100             | $1.00 \times 10^{-37}$ | 2               |
| NC_007771.1      | <i>Staphylococcus chromogenes</i>  | pLNU4        | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_015053.1      | <i>Bifidobacterium longum</i>      | p157F-NC1    | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_007956.1      | <i>Bacillus subtilis</i>           | pLS30        | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_001766.1      | <i>Bacillus subtilis</i>           | pTA1060      | 98             | 100             | $3.00 \times 10^{-43}$ | 2               |
| NC_015187.1      | <i>Acidiphilum multivorum</i>      | pACMV2       | 97             | 100             | $2.00 \times 10^{-41}$ | 2               |
| NC_011887.1      | <i>Methylobacterium nodulans</i>   | pMNOD02      | 95.56          | 90              | $3.00 \times 10^{-34}$ | 2               |
| NC_014558.1      | <i>Lactobacillus plantarum</i>     | pST-III      | 95             | 100             | $3.00 \times 10^{-38}$ | 2               |
| NC_010283.1      | <i>Bacillus thuringiensis</i>      | pFR55        | 97             | 100             | $2.00 \times 10^{-41}$ | 2               |
| NC_010997.1      | <i>Rhizobium etli</i>              | pC           | 95             | 100             | $3.00 \times 10^{-38}$ | 2               |
| NC_008352.1      | <i>Staphylococcus chromogenes</i>  | pLNU8        | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_010180.1      | <i>Bacillus weihenstephanensis</i> | pBWB401      | 96             | 98              | $7.00 \times 10^{-40}$ | 2               |
| NC_006827.2      | <i>Enterococcus faecalis</i>       | pCF10        | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_013034.2      | <i>Staphylococcus aureus</i>       | pKKS825      | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_008505.1      | <i>Lactococcus lactis</i>          | plasmid 3    | 96             | 100             | $7.00 \times 10^{-40}$ | 2               |
| NC_012521.1      | <i>Rhodococcus opacus</i>          | pROB02       | 95             | 100             | $3.00 \times 10^{-38}$ | 2               |
| NC_010290.1      | <i>Enterococcus faecium</i>        | pJS33        | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_005073.1      | <i>Rhodococcus erythropolis</i>    | pBD2         | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_011652.1      | <i>Lactobacillus sakei</i>         | pYC2         | 99             | 100             | $7.00 \times 10^{-45}$ | 2               |

Table S3. Cont.

| Accession Number | Bacterial Host                     | Plasmid Name | Identity (%) t | Hit Length (bp) | E Value t              | Number of Reads |
|------------------|------------------------------------|--------------|----------------|-----------------|------------------------|-----------------|
| NC_005570.1      | <i>Yersinia enterocolitica</i>     | p29807       | 97.03          | 100             | $2 \times 10^{-41}$    | 2               |
| NC_011892.1      | <i>Methylobacterium nodulans</i>   | pMNOD01      | 95             | 100             | $3.00 \times 10^{-38}$ | 2               |
| NC_009128.1      | <i>Corynebacterium</i> sp.         | pLEW279a     | 100            | 95              | $1.00 \times 10^{-43}$ | 2               |
| NC_013856.1      | <i>Azospirillum</i> sp.            | pAB510b      | 95.65          | 92              | $2.00 \times 10^{-35}$ | 2               |
| NC_008384.1      | <i>Rhizobium leguminosarum</i>     | pRL11        | 95.79          | 95              | $5.00 \times 10^{-37}$ | 2               |
| NC_005244.2      | <i>Pseudomonas</i> sp.             | pND6-1       | 96             | 96              | $3.00 \times 10^{-39}$ | 2               |
| NC_007770.1      | <i>Staphylococcus haemolyticus</i> | pLNU3        | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_009841.1      | <i>Bacillus thuringiensis</i>      | pBMB67       | 98             | 100             | $3.00 \times 10^{-43}$ | 2               |
| NC_007950.1      | <i>Polaromonas</i> sp.             | plasmid 2    | 95             | 100             | $3.00 \times 10^{-38}$ | 2               |
| NC_010423.1      | <i>Streptococcus pyogenes</i>      | pRW35        | 99             | 100             | $7.00 \times 10^{-45}$ | 2               |
| NC_002575.1      | <i>Agrobacterium rhizogenes</i>    | pRi1724      | 95             | 93              | $2.0 \times 10^{-35}$  | 2               |
| NC_014172.1      | <i>Bacillus thuringiensis</i>      | pBMB171      | 95.05          | 100             | $3 \times 10^{-38}$    | 2               |
| NC_007792.1      | <i>Staphylococcus aureus</i>       | pUSA03       | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_008506.1      | <i>Lactococcus lactis</i>          | plasmid 4    | 98             | 100             | $3.00 \times 10^{-43}$ | 2               |
| NC_009794.1      | <i>Citrobacter koseri</i>          | pCKO2        | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_014959.1      | <i>Enterococcus faecium</i>        | pS177        | 95             | 100             | $3.00 \times 10^{-38}$ | 2               |
| NC_014475.1      | <i>Enterococcus faecalis</i>       | pWZ1668      | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_010802.1      | <i>Burkholderia multivorans</i>    | pTGL1        | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_011996.1      | <i>Macrococcus caseolyticus</i>    | pMCCL2       | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_009430.1      | <i>Rhodobacter sphaeroides</i>     | pRSPA02      | 95             | 100             | $3.00 \times 10^{-38}$ | 2               |
| NC_005249.1      | <i>Klebsiella pneumoniae</i>       | pLVPK        | 100            | 100             | $2.00 \times 10^{-46}$ | 2               |
| NC_010309.1      | <i>Acinetobacter venetianus</i>    | pAV1         | 100            | 98              | $2.0 \times 10^{-45}$  | 1               |
| NC_007971.2      | <i>Cupriavidus metallidurans</i>   | pMOL30       | 98.99          | 99              | $3.0 \times 10^{-44}$  | 1               |
| NC_012520.1      | <i>Rhodococcus opacus</i>          | pROB01       | 101            | 2               | $7.0 \times 10^{-40}$  | 1               |
| NC_003124.1      | <i>Aeromonas salmonicida</i>       | pRAS3.2      | 100            | 100             | $2.0 \times 10^{-46}$  | 1               |
| NC_008490.1      | <i>Escherichia coli</i>            | pMG828-5     | 100            | 100             | $2.0 \times 10^{-46}$  | 1               |
| NC_008354.1      | <i>Staphylococcus chromogenes</i>  | pLNU9        | 100            | 100             | $2.0 \times 10^{-46}$  | 1               |

Table S3. Cont.

| Accession Number | Bacterial Host                     | Plasmid Name | Identity (%) t | Hit Length (bp) | E Value t              | Number of Reads |
|------------------|------------------------------------|--------------|----------------|-----------------|------------------------|-----------------|
| NC_009132.1      | <i>Escherichia coli</i>            | pLEW517      | 100            | 91              | $2.0 \times 10^{-41}$  | 1               |
| NC_014385.1      | <i>Escherichia coli</i>            | pEC_L46      | 97.94          | 97              | $6.00 \times 10^{-41}$ | 1               |
| NC_009671.1      | <i>Ochrobactrum anthropi</i>       | pOANT03      | 98.99          | 99              | $3.00 \times 10^{-44}$ | 1               |
| NC_004768.1      | <i>Bifidobacterium longum</i>      | pNAC3        | 96             | 100             | $7.00 \times 10^{-40}$ | 1               |
| NC_007791.1      | <i>Staphylococcus aureus</i>       | pUSA02       | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_013442.1      | <i>Gordonia bronchialis</i>        | pGBRO01      | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_005076.1      | <i>Staphylococcus sciuri</i>       | pSCFS1       | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_006979.1      | <i>Streptococcus pyogenes</i>      | pSM19035     | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_014164.1      | <i>Lactobacillus acidipiscis</i>   | pLAC1        | 98             | 100             | $3.00 \times 10^{-43}$ | 1               |
| NC_002137.1      | <i>Lactococcus lactis cremoris</i> | pNZ4000      | 98             | 100             | $3.00 \times 10^{-43}$ | 1               |
| NC_004954.1      | <i>Micrococcus</i> sp.             | pSD10        | 97             | 100             | $2.00 \times 10^{-41}$ | 1               |
| NC_008246.1      | <i>Sphingobium yanoikuyae</i>      | pYAN-1       | 98             | 100             | $3.00 \times 10^{-43}$ | 1               |
| NC_007336.1      | <i>Ralstonia eutropha</i>          | megaplasmid  | 96             | 100             | $7.00 \times 10^{-40}$ | 1               |
| NC_013860.1      | <i>Azospirillum</i> sp.            | pAB510f      | 95.92          | 98              | $1.00 \times 10^{-38}$ | 1               |
| NC_008501.1      | <i>Streptococcus thermophilus</i>  | plasmid 2    | 95.92          | 98              | $3.00 \times 10^{-38}$ | 1               |
| NC_008538.1      | <i>Arthrobacter</i> sp.            | plasmid 2    | 95.92          | 98              | $1.00 \times 10^{-38}$ | 1               |
| NC_011960.1      | <i>Rhodobacter sphaeroides</i>     | pRSKD131B    | 96.91          | 97              | $7.0 \times 10^{-40}$  | 1               |
| NC_008507.1      | <i>Lactococcus lactis</i>          | plasmid 5    | 99             | 100             | $7.00 \times 10^{-45}$ | 1               |
| NC_007763.1      | <i>Rhizobium etli</i>              | p42b         | 96.04          | 101             | $7.00 \times 10^{-40}$ | 1               |
| NC_006427.1      | <i>Enterococcus faecium</i>        | pJB01        | 99             | 100             | $7.00 \times 10^{-45}$ | 1               |
| NC_005088.1      | <i>Delftia acidovorans</i>         | pUO1         | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_007103.1      | <i>Bacillus cereus</i>             | pE33L466     | 98             | 100             | $3.00 \times 10^{-43}$ | 1               |
| NC_000914.2      | <i>Sinorhizobium fredii</i>        | pNGR234a     | 96             | 100             | $7.00 \times 10^{-40}$ | 1               |
| NC_004992.1      |                                    | pCI411       | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_005703.1      | <i>Bacillus mycoides</i>           | pBMY1        | 96.04          | 101             | $7.00 \times 10^{-40}$ | 1               |
| NC_015184.1      | <i>Agrobacterium</i> sp.           | pAspH13-3a   | 96.74          | 92              | $5.00 \times 10^{-37}$ | 1               |
| NC_006822.1      | <i>Leuconostoc citreum</i>         | pIH01        | 100            | 100             | $2.0 \times 10^{-46}$  | 1               |

Table S3. Cont.

| Accession Number | Bacterial Host                        | Plasmid Name | Identity (%) t | Hit Length (bp) | E Value t              | Number of Reads |
|------------------|---------------------------------------|--------------|----------------|-----------------|------------------------|-----------------|
| NC_013657.1      | <i>Lactococcus lactis</i>             | pKF147A      | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_014312.1      | <i>Klebsiella pneumoniae</i>          | pKP048       | 99             | 100             | $7.0 \times 10^{-45}$  | 1               |
| NC_008760.1      | <i>Polaromonas naphthalenivorans</i>  | pPNAP04      | 96             | 100             | $7.00 \times 10^{-40}$ | 1               |
| NC_007768.1      | <i>Staphylococcus chromogenes</i>     | pLNU1        | 97             | 100             | $2.0 \times 10^{-41}$  | 1               |
| NC_012858.1      | <i>Rhizobium leguminosarum</i>        | pR132502     | 96.84          | 95              | $1.00 \times 10^{-38}$ | 1               |
| NC_003922.1      | <i>Xanthomonas axonopodis</i> pv.     | pXAC64       | 97             | 100             | $2.00 \times 10^{-41}$ | 1               |
| NC_007772.1      | <i>Clostridium perfringens</i>        | pCPF4969     | 100            | 99              | $6.0 \times 10^{-46}$  | 1               |
| NC_004566.1      | <i>Lactobacillus fermentum</i>        | pLME300      | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_002075.1      | <i>Bacillus subtilis</i>              | p1414        | 99             | 100             | $7.0 \times 10^{-45}$  | 1               |
| NC_010398.1      | <i>Acinetobacter baumannii</i>        | p3ABSDF      | 99             | 100             | $7.00 \times 10^{-45}$ | 1               |
| NC_010401.1      | <i>Acinetobacter baumannii</i>        | p1ABAYE      | 97             | 100             | $2.00 \times 10^{-41}$ | 1               |
| NC_011355.1      | <i>Mycobacterium liflandii</i>        | pMUM002      | 97             | 100             | $2.00 \times 10^{-41}$ | 1               |
| NC_014843.1      | <i>Escherichia coli</i>               | p3521        | 98             | 100             | $3.00 \times 10^{-43}$ | 1               |
| NC_004320.1      | <i>Corynebacterium efficiens</i>      | pCE3         | 98.97          | 97              | $3.00 \times 10^{-43}$ | 1               |
| NC_005023.1      | <i>Acidithiobacillus ferrooxidans</i> | pTF5         | 99             | 100             | $7.00 \times 10^{-45}$ | 1               |
| NC_007621.1      | <i>Staphylococcus saprophyticus</i>   | pSES22       | 99             | 100             | $7.00 \times 10^{-45}$ | 1               |
| NC_011743.1      | <i>Escherichia fergusonii</i>         | pEFER        | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_010540.1      | <i>Lactococcus garvieae</i>           | pKL0018      | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_009227.1      | <i>Burkholderia vietnamiensis</i>     | pBVIE02      | 95.83          | 96              | $5.00 \times 10^{-37}$ | 1               |
| NC_004981.2      | <i>Lactococcus lactis</i>             | pCL2.1       | 95.92          | 98              | $1.00 \times 10^{-38}$ | 1               |
| NC_009139.1      | <i>Yersinia ruckeri</i>               | pYR1         | 100            | 100             | $2.0 \times 10^{-46}$  | 1               |
| NC_004532.1      | <i>Lactobacillus reuteri</i>          | pAE78        | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_014133.1      | <i>Leuconostoc kimchii</i>            | LkipL4719    | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_006826.1      | <i>Sphingomonas xenophaga</i>         | pSx-Qyy      | 96             | 100             | $7.00 \times 10^{-40}$ | 1               |
| NC_000938.1      | <i>Streptococcus thermophilus</i>     | pER36        | 97             | 100             | $2.00 \times 10^{-41}$ | 1               |
| NC_009666.2      | <i>Lactobacillus plantarum</i>        | pM4          | 99             | 100             | $7.00 \times 10^{-45}$ | 1               |
| NC_005703.1      | <i>Bacillus mycoides</i>              | pBMY1        | 95.05          | 101             | $3.00 \times 10^{-38}$ | 1               |

Table S3. Cont.

| Accession Number | Bacterial Host                     | Plasmid Name | Identity (%) t | Hit Length (bp) | E Value t              | Number of Reads |
|------------------|------------------------------------|--------------|----------------|-----------------|------------------------|-----------------|
| NC_010998.1      | <i>Rhizobium etli</i>              | pA           | 95             | 100             | $3.00 \times 10^{-38}$ | 1               |
| NC_010933.1      | <i>Bacillus cereus</i>             | pBC210       | 99             | 100             | $7.00 \times 10^{-45}$ | 1               |
| NC_009621.1      | <i>Sinorhizobium medicae</i>       | pSMED02      | 95             | 100             | $3.00 \times 10^{-38}$ | 1               |
| NC_004847.1      | <i>Lactococcus lactis cremoris</i> | pHP003       | 97             | 100             | $2.00 \times 10^{-41}$ | 1               |
| NC_002682.1      | <i>Mesorhizobium loti</i>          | pMLb         | 95.79          | 95              | $5.00 \times 10^{-37}$ | 1               |
| NC_011136.1      | <i>Lactobacillus plantarum</i>     | pLR1         | 99             | 100             | $7.00 \times 10^{-45}$ | 1               |
| NC_009435.1      | <i>Lactococcus lactis</i>          | pGdh442      | 98.99          | 99              | $3.00 \times 10^{-44}$ | 1               |
| NC_008385.1      | <i>Burkholderia cepacia</i>        | plasmid 1    | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |
| NC_009083.1      | <i>Acinetobacter baumannii</i>     | pAB1         | 95.96          | 99              | $3.00 \times 10^{-39}$ | 1               |
| NC_010603.1      | <i>Lactobacillus reuteri</i>       | pLR581       | 95             | 100             | $3.00 \times 10^{-38}$ | 1               |
| NC_009349.1      | <i>Aeromonas salmonicida</i>       | plasmid 4    | 99             | 100             | $7.00 \times 10^{-45}$ | 1               |
| NC_011617.1      | <i>Klebsiella pneumoniae</i>       | pKP96        | 100            | 100             | $2.00 \times 10^{-46}$ | 1               |

**Table S4.** Matched high-throughput sequencing reads of plasmids in sludge cultured with 20mg/L tetracycline of Day 6 against NCBI Plasmid Genome Database.

| Accession Number | Bacterial Host                              | Plasmid Name    | Identity (%) t | Hit lenGTh (bp) n | E Value t             | Number of Reads |
|------------------|---|-----------------|----------------|-------------------|-----------------------|-----------------|
| NC_013193.1      | <i>Candidatus Accumulibacter phosphatis</i> | pAph01          | 95             | 90                | $1.0 \times 10^{-33}$ | 764             |
| NC_014641.1      | <i>Achromobacter xylosoxidans</i>           | pA81            | 95.05          | 91                | $1.0 \times 10^{-37}$ | 733             |
| NC_003296.1      | <i>Ralstonia solanacearum</i>               | pGMI1000MP      | 95             | 90                | $3.0 \times 10^{-34}$ | 684             |
| NC_014911.1      | <i>Alicycliphilus denitrificans</i>         | pALIDE02        | 95             | 93                | $1.0 \times 10^{-37}$ | 280             |
| NC_007974.2      | <i>Cupriavidus metallidurans</i>            | megaplasmid     | 95             | 90                | $1.0 \times 10^{-33}$ | 229             |
| NC_011092.1      | <i>Salmonella enterica</i> subsp.           | pCVM19633_110   | 96             | 90                | $1.0 \times 10^{-37}$ | 219             |
| NC_006823.1      | <i>Azoarcus</i> sp.                         | plasmid 1       | 95             | 90                | $8.0 \times 10^{-35}$ | 219             |
| NC_010510.1      | <i>Methylobacterium radiotolerans</i>       | pMRAD01         | 95             | 90                | $1.0 \times 10^{-33}$ | 215             |
| NC_011667.1      | <i>Thauera</i> sp.                          | pTha01          | 95             | 90                | $6.0 \times 10^{-36}$ | 207             |
| NC_013365.1      | <i>Escherichia coli</i>                     | pO111_1         | 95             | 94                | $3.0 \times 10^{-38}$ | 203             |
| NC_003350.1      | <i>Pseudomonas putida</i>                   | pWW0            | 95             | 91                | $8.0 \times 10^{-35}$ | 203             |
| NC_010394.1      | <i>Mycobacterium abscessus</i>              | unnamed plasmid | 95             | 97                | $1.0 \times 10^{-37}$ | 179             |
| NC_014167.1      | <i>Corynebacterium resistens</i>            | pJA144188       | 97             | 97                | $2.0 \times 10^{-41}$ | 162             |
| NC_014211.1      | <i>Nocardiopsis dassonvillei</i> subsp.     | pNDAS01         | 95             | 90                | $3.0 \times 10^{-34}$ | 161             |
| NC_014208.1      | <i>Klebsiella oxytoca</i>                   | pKOX105         | 95.1           | 94                | $1.0 \times 10^{-38}$ | 148             |
| NC_009140.1      | <i>Salmonella enterica</i> subsp.           | pSN254          | 95             | 90                | $6.0 \times 10^{-36}$ | 133             |
| NC_006994.1      | <i>Pasteurella multocida</i>                | pCCK381         | 95             | 92                | $3.0 \times 10^{-38}$ | 131             |
| NC_014107.1      | <i>Enterobacter cloacae</i> subsp.          | pECL_A          | 95             | 90                | $6.0 \times 10^{-36}$ | 101             |
| NC_013859.1      | <i>Azospirillum</i> sp.                     | pAB510e         | 95             | 90                | $3.0 \times 10^{-34}$ | 98              |
| NC_014621.1      | <i>Ketogulonicigenium vulgare</i>           | pYP1            | 95             | 90                | $3.0 \times 10^{-34}$ | 89              |
| NC_009426.1      | <i>Novosphingobium aromaticivorans</i>      | pNL1            | 95             | 90                | $6.0 \times 10^{-36}$ | 76              |
| NC_010935.1      | <i>Comamonas testosteroni</i>               | pCNB            | 95             | 95                | $3.0 \times 10^{-38}$ | 73              |
| NC_008308.1      | <i>Sphingomonas</i> sp.                     | pCAR3           | 95             | 90                | $3.0 \times 10^{-34}$ | 72              |
| NC_014908.1      | <i>Alicycliphilus denitrificans</i>         | pALIDE01        | 95             | 94                | $3.0 \times 10^{-38}$ | 64              |
| NC_004604.2      | <i>Bacillus megaterium</i>                  | pBM400          | 95             | 90                | $1.0 \times 10^{-33}$ | 62              |
| NC_009429.1      | <i>Rhodobacter sphaeroides</i>              | pRSPA01         | 95             | 91                | $8.0 \times 10^{-35}$ | 62              |
| NC_012527.1      | <i>Deinococcus deserti</i>                  | plasmid 1       | 95             | 90                | $3.0 \times 10^{-34}$ | 61              |

Table S4. Cont.

| Accession Number | Bacterial Host                              | Plasmid Name | Identity (%) t | Hit Length (bp) n | E Value t             | Number of Reads |
|------------------|---|--------------|----------------|-------------------|-----------------------|-----------------|
| NC_006143.1      | <i>Aeromonas punctata</i>                   | pFBAOT6      | 96.04          | 98                | $7.0 \times 10^{-40}$ | 51              |
| NC_006824.1      | <i>Azoarcus</i> sp.                         | plasmid 2    | 95             | 97                | $3.0 \times 10^{-38}$ | 51              |
| NC_002698.1      | <i>Shigella flexneri</i>                    | pWR501       | 95             | 100               | $3.0 \times 10^{-38}$ | 45              |
| NC_013190.1      | <i>Candidatus Accumulibacter phosphatis</i> | pAph02       | 95             | 90                | $6.0 \times 10^{-36}$ | 42              |
| NC_010488.1      | <i>Escherichia coli</i>                     | pSMS35_130   | 96             | 97                | $7.0 \times 10^{-40}$ | 42              |
| NC_008826.1      | <i>Methylibium petroleiphilum</i>           | RPME01       | 95             | 94                | $3.0 \times 10^{-38}$ | 41              |
| NC_011602.1      | <i>Escherichia coli</i>                     | pE2348-2     | 99             | 90                | $6.0 \times 10^{-41}$ | 40              |
| NC_009508.1      | <i>Sphingomonas wittichii</i>               | pSWIT02      | 95             | 97                | $3.0 \times 10^{-38}$ | 39              |
| NC_008697.1      | <i>Nocardiooides</i> sp.                    | pNOCA01      | 95             | 95                | $5.0 \times 10^{-37}$ | 39              |
| NC_014811.1      | <i>Mycobacterium</i> sp.                    | pMSPYR101    | 97             | 90                | $6.0 \times 10^{-41}$ | 38              |
| NC_014633.1      | <i>Ilyobacter polytropus</i>                | pILYOP01     | 95             | 90                | $3.0 \times 10^{-34}$ | 38              |
| NC_008272.1      | <i>IncP-1</i>                               | pKJK5        | 95             | 92                | $1.0 \times 10^{-37}$ | 37              |
| NC_009753.1      | <i>Paracoccus methylutens</i>               | pMTH1        | 99             | 100               | $7.0 \times 10^{-45}$ | 32              |
| NC_008043.1      | <i>Ruegeria</i> sp.                         | mega plasmid | 95             | 95                | $5.0 \times 10^{-37}$ | 32              |
| NC_003430.1      | <i>Uncultured bacterium</i>                 | pB4          | 95             | 100               | $3.0 \times 10^{-38}$ | 31              |
| NC_009507.1      | <i>Sphingomonas wittichii</i>               | pSWIT01      | 98             | 99                | $3.0 \times 10^{-43}$ | 31              |
| NC_013857.1      | <i>Azospirillum</i> sp.                     | pAB510c      | 95             | 92                | $2.0 \times 10^{-35}$ | 30              |
| NC_008703.1      | <i>Mycobacterium</i> sp.                    | pMKMS01      | 95             | 91                | $2.0 \times 10^{-36}$ | 29              |
| NC_003123.1      | <i>Aeromonas salmonicida</i>                | pRAS3.1      | 96             | 96                | $7.0 \times 10^{-40}$ | 26              |
| NC_014144.1      | <i>Thiomonas</i> sp.                        | pTHI         | 95             | 97                | $3.0 \times 10^{-38}$ | 26              |
| NC_007100.1      | <i>Pseudomonas aeruginosa</i>               | Rms149       | 98             | 97                | $3.0 \times 10^{-43}$ | 25              |
| NC_011961.1      | <i>Thermomicrobium roseum</i>               | unnamed      | 95             | 91                | $8.0 \times 10^{-35}$ | 25              |
| NC_013509.1      | <i>Edwardsiella tarda</i>                   | pEIB202      | 99             | 100               | $7.0 \times 10^{-45}$ | 24              |
| NC_014155.1      | <i>Thiomonas intermedia</i>                 | pTINT02      | 96             | 90                | $3.0 \times 10^{-39}$ | 24              |
| NC_012586.1      | <i>Sinorhizobium fredii</i>                 | pNGR234b     | 95             | 92                | $5.0 \times 10^{-37}$ | 23              |
| NC_008704.1      | <i>Mycobacterium</i> sp.                    | pMKMS02      | 95             | 99                | $3.0 \times 10^{-38}$ | 22              |
| NC_008608.1      | <i>Pelobacter propionicus</i>               | pPRO2        | 95             | 97                | $3.0 \times 10^{-38}$ | 22              |

Table S4. Cont.

| Accession Number | Bacterial Host                                  | Plasmid Name | Identity (%) t | Hit Length (bp) n | E Value t             | Number of Reads |
|------------------|---|--------------|----------------|-------------------|-----------------------|-----------------|
| NC_006352.1      | <i>Uncultured bacterium activated sludge</i>    | pTB11        | 95             | 99                | $3.0 \times 10^{-38}$ | 22              |
| NC_007486.1      | <i>Rhodococcus erythropolis</i>                 | pREC1        | 99             | 99                | $7.0 \times 10^{-45}$ | 22              |
| NC_007972.2      | <i>Cupriavidus metallidurans</i>                | pMOL28       | 96.81          | 94                | $3.0 \times 10^{-38}$ | 22              |
| NC_010558.1      | <i>Escherichia coli</i>                         | pIP1206      | 96             | 93                | $7.0 \times 10^{-40}$ | 21              |
| NC_009669.1      | <i>Ochrobactrum anthropi</i>                    | pOANT01      | 95             | 91                | $6.0 \times 10^{-36}$ | 20              |
| NC_010891.1      | <i>Pseudomonas</i> sp.                          | pCT14        | 96             | 100               | $7.0 \times 10^{-40}$ | 19              |
| NC_014154.1      | <i>Thiomonas intermedia</i>                     | pTINT01      | 95             | 95                | $5.0 \times 10^{-37}$ | 17              |
| NC_011419.1      | <i>Escherichia coli</i>                         | pSE11-1      | 97.98          | 99                | $1.0 \times 10^{-42}$ | 15              |
| NC_009339.1      | <i>Mycobacterium gilvum</i>                     | pMFLV01      | 95             | 92                | $5.0 \times 10^{-37}$ | 14              |
| NC_014007.1      | <i>Sphingobium japonicum</i>                    | pCHQ1        | 95             | 90                | $3.0 \times 10^{-34}$ | 14              |
| NC_009717.1      | <i>Xanthobacter autotrophicus</i>               | pXAUT01      | 95             | 97                | $3.0 \times 10^{-38}$ | 14              |
| NC_009779.1      | <i>Cronobacter sakazakii</i>                    | pESA2        | 95             | 99                | $3.0 \times 10^{-38}$ | 13              |
| NC_014309.1      | <i>Ralstonia solanacearum</i>                   | RCFBPv3_mp   | 95             | 90                | $3.0 \times 10^{-34}$ | 12              |
| NC_014231.1      | <i>Escherichia coli</i>                         | pKC394       | 96             | 99                | $7.0 \times 10^{-40}$ | 12              |
| NC_003122.1      | <i>Uncultured bacterium in soil</i>             | pSB102       | 98.97          | 97                | $3.0 \times 10^{-43}$ | 12              |
| NC_008712.1      | <i>Arthrobacter aurescens</i>                   | TC1          | 95.65          | 92                | $2.0 \times 10^{-35}$ | 11              |
| NC_013855.1      | <i>Azospirillum</i> sp.                         | pAB510a      | 95             | 90                | $1.0 \times 10^{-33}$ | 10              |
| NC_008010.2      | <i>Deinococcus geothermalis</i>                 | pDGE001      | 95.05          | 91                | $2.0 \times 10^{-36}$ | 10              |
| NC_007353.2      | <i>Sphingomonas</i> sp.                         | pA1          | 95             | 100               | $3.0 \times 10^{-38}$ | 10              |
| NC_005241.1      | <i>Cupriavidus necator</i>                      | pHG1         | 95             | 90                | $3.0 \times 10^{-34}$ | 10              |
| NC_013858.1      | <i>Azospirillum</i> sp.                         | pAB510d      | 95             | 90                | $3.0 \times 10^{-34}$ | 9               |
| NC_001740.1      |   | RSF1010      | 98             | 100               | $3.0 \times 10^{-43}$ | 9               |
| NC_012811.1      | <i>Methylobacterium extorquens</i>              | megaplasmid  | 95             | 92                | $2.0 \times 10^{-35}$ | 9               |
| NC_008242.1      | <i>Mesorhizobium</i> sp.                        | plasmid 1    | 95             | 93                | $1.0 \times 10^{-37}$ | 8               |
| NC_012556.1      | <i>Enterobacter cloacae</i>                     | pEC-IMPQ     | 95             | 100               | $3.0 \times 10^{-38}$ | 8               |
| NC_007502.1      | <i>Uncultured bacterium in river sediments</i>  | pB8          | 97.98          | 91                | $7.0 \times 10^{-40}$ | 7               |
| NC_009139.1      | <i>Yersinia ruckeri</i>                         | pYR1         | 96             | 100               | $7.0 \times 10^{-40}$ | 7               |
| NC_006388.1      | <i>Uncultured bacterium in activated sludge</i> | pB3          | 96.77          | 93                | $1.0 \times 10^{-37}$ | 7               |

Table S4. Cont.

| Accession Number | Bacterial Host                               | Plasmid Name | Identity (%) t | Hit Length (bp) n | E Value t             | Number of Reads |
|------------------|--|--------------|----------------|-------------------|-----------------------|-----------------|
| NC_009475.1      | <i>Bradyrhizobium</i> sp.                    | pBBta01      | 95             | 98                | $3.0 \times 10^{-38}$ | 6               |
| NC_008765.1      | <i>Acidovorax</i> sp.                        | pAOVO01      | 95.96          | 99                | $3.0 \times 10^{-39}$ | 6               |
| NC_012849.1      | <i>Ralstonia pickettii</i>                   | pRp12D02     | 95.56          | 90                | $1.0 \times 10^{-33}$ | 6               |
| NC_013191.1      | <i>Candidatus Accumulibacter phosphatis</i>  | pAph03       | 97             | 100               | $2.0 \times 10^{-41}$ | 6               |
| NC_009739.1      | <i>Pseudomonas aeruginosa</i>                | pMATVIM-7    | 97             | 95                | $2.0 \times 10^{-41}$ | 6               |
| NC_006625.1      | <i>Klebsiella pneumoniae</i>                 | pK2044       | 97             | 100               | $2.0 \times 10^{-41}$ | 6               |
| NC_008766.1      | <i>Acidovorax</i> sp.                        | pAOVO02      | 95.05          | 100               | $3.0 \times 10^{-38}$ | 6               |
| NC_005873.1      | <i>Oligotropha carboxidovorans</i>           | pHCG3        | 95             | 97                | $3.0 \times 10^{-38}$ | 6               |
| NC_009128.1      | <i>Corynebacterium</i> sp.                   | pLEW279a     | 97             | 97                | $2.0 \times 10^{-41}$ | 6               |
| NC_006385.1      | <i>Uncultured bacterium activated sludge</i> | pRSB101      | 97             | 100               | $2.0 \times 10^{-41}$ | 6               |
| NC_002679.1      | <i>Mesorhizobium loti</i>                    | pMLa         | 95             | 90                | $6.0 \times 10^{-36}$ | 6               |
| NC_009621.1      | <i>Sinorhizobium medicae</i>                 | pSMED02      | 95             | 90                | $3.0 \times 10^{-34}$ | 6               |
| NC_002524.1      | <i>Uncultured eubacterium</i>                | pIE1115      | 100            | 100               | $2.0 \times 10^{-46}$ | 6               |
| NC_007960.1      | <i>Nitrobacter hamburgensis</i>              | plasmid 2    | 95             | 91                | $8.0 \times 10^{-35}$ | 5               |
| NC_009671.1      | <i>Ochrobactrum anthropi</i>                 | pOANT03      | 95             | 100               | $3.0 \times 10^{-38}$ | 5               |
| NC_013176.1      | <i>Pseudomonas putida</i>                    | pW2          | 99             | 100               | $7.0 \times 10^{-45}$ | 5               |
| NC_015187.1      | <i>Acidiphilium multivorum</i>               | pACMV2       | 95             | 92                | $2.0 \times 10^{-35}$ | 5               |
| NC_007680.1      | <i>Uncultured bacterium</i>                  | pTP6         | 95             | 100               | $3.0 \times 10^{-38}$ | 4               |
| NC_009838.1      | <i>Escherichia coli</i>                      | pAPEC-O1-R   | 100            | 100               | $2.0 \times 10^{-46}$ | 4               |
| NC_010912.1      | <i>Avibacterium paragallinarum</i>           | pYMH5        | 99             | 100               | $7.0 \times 10^{-45}$ | 4               |
| NC_009349.1      | <i>Aeromonas salmonicida</i> subsp.          | plasmid 4    | 100            | 100               | $2.0 \times 10^{-46}$ | 4               |
| NC_008688.1      | <i>Paracoccus denitrificans</i>              | plasmid 1    | 95             | 100               | $3.0 \times 10^{-38}$ | 4               |
| NC_011617.1      | <i>Klebsiella pneumoniae</i>                 | pKP96        | 100            | 100               | $2.0 \times 10^{-46}$ | 4               |
| NC_004956.1      | <i>Pseudomonas</i> sp.                       | pADP-1       | 99             | 96                | $3.0 \times 10^{-44}$ | 4               |
| NC_008055.1      | <i>Uncultured bacterium in freshwater</i>    | QKH54        | 95.96          | 94                | $5.0 \times 10^{-37}$ | 4               |
| NC_012723.1      | <i>Burkholderia glumae</i>                   | bglu_1p      | 95             | 93                | $1.0 \times 10^{-37}$ | 4               |
| NC_011892.1      | <i>Methylobacterium nodulans</i>             | pMNOD01      | 95.74          | 94                | $2.0 \times 10^{-36}$ | 4               |
| NC_014035.1      | <i>Rhodobacter capsulatus</i>                | pRCB133      | 96             | 97                | $7.0 \times 10^{-40}$ | 4               |

Table S4. Cont.

| Accession Number | Bacterial Host                       | Plasmid Name | Identity (%) t | Hit lengTh (bp) n | E Value t             | Number of Reads |
|------------------|--------------------------------------|--------------|----------------|-------------------|-----------------------|-----------------|
| NC_007336.1      | <i>Ralstonia eutropha</i>            | megaplasmid  | 96             | 90                | $6.0 \times 10^{-36}$ | 4               |
| NC_010189.1      | <i>Naegleria gruberi</i>             | unnamed      | 95             | 96                | $1.0 \times 10^{-37}$ | 4               |
| NC_008573.1      | <i>Shewanella</i> sp.                | plasmid 1    | 99             | 100               | $7.0 \times 10^{-45}$ | 3               |
| NC_008758.1      | <i>Polaromonas naphthalenivorans</i> | pPNAP02      | 95.88          | 97                | $3.0 \times 10^{-38}$ | 3               |
| NC_007800.1      | <i>Bibersteinia trehalosi</i>        | pCCK13698    | 100            | 100               | $2.0 \times 10^{-46}$ | 3               |
| NC_008269.1      | <i>Rhodococcus jostii</i>            | pRHL1        | 95.65          | 92                | $2.0 \times 10^{-35}$ | 3               |
| NC_007763.1      | <i>Rhizobium etli</i>                | p42b         | 95.79          | 95                | $5.0 \times 10^{-37}$ | 3               |
| NC_004771.1      | <i>Pasteurella multocida</i>         | pJR1         | 97             | 100               | $2.0 \times 10^{-41}$ | 3               |
| NC_003078.1      | <i>Sinorhizobium meliloti</i>        | pSymB        | 95.88          | 97                | $3.0 \times 10^{-38}$ | 3               |
| NC_004973.1      | <i>Uncultured eubacterium</i>        | pIE1130      | 100            | 100               | $2.0 \times 10^{-46}$ | 3               |
| NC_007950.1      | <i>Polaromonas</i> sp.               | plasmid 2    | 95             | 90                | $6.0 \times 10^{-36}$ | 3               |
| NC_008357.1      | <i>Pseudomonas aeruginosa</i>        | pBS228       | 96             | 100               | $7.0 \times 10^{-40}$ | 3               |
| NC_009227.1      | <i>Burkholderia vietnamensis</i>     | pBVIE02      | 96.91          | 97                | $7.0 \times 10^{-40}$ | 3               |
| NC_008244.1      | <i>Mesorhizobium</i> sp.             | plasmid 3    | 95             | 100               | $3.0 \times 10^{-38}$ | 3               |
| NC_005793.1      | <i>Achromobacter denitrificans</i>   | pEST4011     | 98             | 100               | $3.0 \times 10^{-43}$ | 3               |
| NC_005909.1      | <i>Pseudomonas alcaligenes</i>       | pRA2         | 100            | 100               | $2.0 \times 10^{-46}$ | 3               |
| NC_007337.1      | <i>Ralstonia eutropha</i>            | plasmid 1    | 100            | 100               | $2.0 \times 10^{-46}$ | 3               |
| NC_008757.1      | <i>Polaromonas naphthalenivorans</i> | pPNAP01      | 95             | 90                | $1.0 \times 10^{-37}$ | 3               |
| NC_008385.1      | <i>Burkholderia cepacia</i>          | plasmid 1    | 95.88          | 97                | $3.0 \times 10^{-38}$ | 3               |
| NC_009651.1      | <i>Klebsiella pneumoniae</i> subsp.  | pKPN5        | 100            | 100               | $2.0 \times 10^{-46}$ | 3               |
| NC_003922.1      | <i>Xanthomonas axonopodis</i> pv.    | pXAC64       | 97.98          | 99                | $1.0 \times 10^{-42}$ | 3               |
| NC_009341.1      | <i>Mycobacterium gilvum</i>          | pMFLV03      | 96             | 100               | $7.0 \times 10^{-40}$ | 3               |
| NC_008384.1      | <i>Rhizobium leguminosarum</i> bv.   | pRL11        | 95.05          | 98                | $3.0 \times 10^{-38}$ | 2               |
| NC_011143.1      | <i>Phenylobacterium zucineum</i>     | unnamed      | 95.92          | 98                | $1.0 \times 10^{-38}$ | 2               |
| NC_013856.1      | <i>Azospirillum</i> sp.              | pAB510b      | 95.05          | 90                | $3.0 \times 10^{-34}$ | 2               |
| NC_000914.2      | <i>Sinorhizobium fredii</i>          | pNGR234a     | 96             | 100               | $3.0 \times 10^{-39}$ | 2               |
| NC_010643.1      | <i>Providencia rettgeri</i>          | R7K          | 99             | 100               | $7.0 \times 10^{-45}$ | 2               |
| NC_010529.1      | <i>Cupriavidus taiwanensis</i>       | pRALTA       | 95             | 100               | $3.0 \times 10^{-38}$ | 2               |

Table S4. Cont.

| Accession Number | Bacterial Host                     | Plasmid Name | Identity (%) t | Hit Length (bp) n | E Value t             | Number of Reads |
|------------------|------------------------------------|--------------|----------------|-------------------|-----------------------|-----------------|
| NC_010606.1      | <i>Acinetobacter baumannii</i>     | pACICU2      | 99             | 100               | $7.0 \times 10^{-45}$ | 2               |
| NC_003227.1      | <i>Corynebacterium glutamicum</i>  | pTET3        | 99             | 100               | $7.0 \times 10^{-45}$ | 2               |
| NC_008379.1      | <i>Rhizobium leguminosarum</i> bv. | pRL9         | 96.84          | 95                | $1.0 \times 10^{-38}$ | 2               |
| NC_009957.1      | <i>Dinoroseobacter shibae</i>      | pDSHI03      | 96             | 100               | $7.0 \times 10^{-40}$ | 2               |
| NC_011982.1      | <i>Agrobacterium vitis</i>         | pTiS4        | 95             | 100               | $3.0 \times 10^{-38}$ | 2               |
| NC_010998.1      | <i>Rhizobium etli</i>              | pA           | 97             | 100               | $2.0 \times 10^{-41}$ | 2               |
| NC_014356.1      | <i>Escherichia coli</i>            | pQ7          | 100            | 100               | $2.0 \times 10^{-46}$ | 2               |
| NC_006363.1      | <i>Nocardia farcinica</i>          | pNF2         | 97             | 100               | $2.0 \times 10^{-41}$ | 2               |
| NC_011371.1      | <i>Rhizobium leguminosarum</i> bv. | pRLG204      | 96.94          | 98                | $2.0 \times 10^{-40}$ | 2               |
| NC_010625.1      | <i>Burkholderia phymatum</i>       | pBPHY01      | 95             | 100               | $1.0 \times 10^{-37}$ | 2               |
| NC_004808.2      | <i>Streptomyces rochei</i>         | pSLA2-L      | 95.79          | 95                | $2.0 \times 10^{-36}$ | 2               |
| NC_013718.1      | <i>Citrobacter rodentium</i>       | pCROD2       | 100            | 100               | $2.0 \times 10^{-46}$ | 2               |
| NC_010727.1      | <i>Methylobacterium populi</i>     | pMPOP01      | 100            | 100               | $2.0 \times 10^{-46}$ | 2               |
| NC_013281.1      | <i>Uncultured bacterium</i>        | pTRACA30     | 100            | 100               | $2.0 \times 10^{-46}$ | 2               |
| NC_003037.1      | <i>Sinorhizobium meliloti</i>      | pSymA        | 95.65          | 92                | $2.0 \times 10^{-35}$ | 2               |
| NC_009620.1      | <i>Sinorhizobium medicae</i>       | pSMED01      | 95.96          | 99                | $3.0 \times 10^{-39}$ | 2               |
| NC_014801.1      | <i>Campylobacter jejuni</i> subsp. | pTet         | 100            | 100               | $2.0 \times 10^{-46}$ | 2               |
| NC_010865.1      | <i>Sinorhizobium meliloti</i>      | pSmeSM11b    | 95.05          | 92                | $2.0 \times 10^{-36}$ | 2               |
| NC_007491.1      | <i>Rhodococcus erythropolis</i>    | pREL1        | 97             | 100               | $2.0 \times 10^{-41}$ | 2               |
| NC_007959.1      | <i>Nitrobacter hamburgensis</i>    | plasmid 1    | 96.97          | 97                | $7.0 \times 10^{-40}$ | 2               |
| NC_014005.1      | <i>Sphingobium japonicum</i>       | pUT1         | 100            | 100               | $2.0 \times 10^{-46}$ | 2               |
| NC_010996.1      | <i>Rhizobium etli</i>              | pB           | 96             | 100               | $7.0 \times 10^{-40}$ | 2               |
| NC_014022.1      | <i>Yersinia pestis</i>             | pMT1         | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_007961.1      | <i>Nitrobacter hamburgensis</i>    | plasmid 3    | 96.77          | 93                | $1.0 \times 10^{-37}$ | 1               |
| NC_012690.1      | <i>Escherichia coli</i>            | peH4H        | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_006830.1      | <i>Achromobacter xylosoxidans</i>  | pA81         | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_001370.1      |                                    | pC30il       | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_009340.1      | <i>Mycobacterium gilvum</i>        | pMFLV02      | 95             | 100               | $3.0 \times 10^{-38}$ | 1               |

Table S4. Cont.

| Accession Number | Bacterial Host                                  | Plasmid Name | Identity (%) t | Hit Length (bp) n | E Value t             | Number of Reads |
|------------------|---|--------------|----------------|-------------------|-----------------------|-----------------|
| NC_004840.1      | <i>Uncultured bacterium</i>                     | pB10         | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_003292.1      | <i>Escherichia coli</i>                         | R46          | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_006821.1      | <i>Bacillus thuringiensis sv darmstadiensis</i> | pBMBt1       | 97             | 100               | $2.0 \times 10^{-41}$ | 1               |
| NC_010600.1      | <i>Acidithiobacillus caldus</i>                 | pTcM1        | 97             | 100               | $2.0 \times 10^{-41}$ | 1               |
| NC_011368.1      | <i>Rhizobium leguminosarum</i> bv.              | pRLG201      | 96.91          | 97                | $7.0 \times 10^{-40}$ | 1               |
| NC_009132.1      | <i>Escherichia coli</i>                         | pLEW517      | 100            | 95                | $1.0 \times 10^{-43}$ | 1               |
| NC_005244.2      | <i>Pseudomonas</i> sp.                          | pND6-1       | 95.96          | 99                | $3.0 \times 10^{-39}$ | 1               |
| NC_007971.2      | <i>Cupriavidus metallidurans</i>                | pMOL30       | 95.79          | 95                | $2.0 \times 10^{-36}$ | 1               |
| NC_014478.1      | <i>Klebsiella pneumoniae</i>                    | unnamed      | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_010847.2      | <i>Paracoccus aminophilus</i>                   | pAMI2        | 97.89          | 95                | $2.0 \times 10^{-40}$ | 1               |
| NC_008538.1      | <i>Arthrobacter</i> sp.                         | plasmid 2    | 96             | 100               | $7.0 \times 10^{-40}$ | 1               |
| NC_010370.1      | <i>Laribacter hongkongensis</i>                 | pHLHK22      | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_009469.1      | <i>Acidiphilium cryptum</i>                     | pACRY03      | 96.91          | 97                | $7.0 \times 10^{-40}$ | 1               |
| NC_007764.1      | <i>Rhizobium etli</i>                           | p42c         | 95             | 100               | $3.0 \times 10^{-38}$ | 1               |
| NC_003124.1      | <i>Aeromonas salmonicida</i>                    | pRAS3.2      | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_012848.1      | <i>Rhizobium leguminosarum</i> bv.              | pR132501     | 95.6           | 91                | $3.0 \times 10^{-34}$ | 1               |
| NC_005088.1      | <i>Delftia acidovorans</i>                      | pUO1         | 100            | 98                | $2.0 \times 10^{-45}$ | 1               |
| NC_007765.1      | <i>Rhizobium etli</i>                           | p42e         | 95             | 100               | $1.0 \times 10^{-37}$ | 1               |
| NC_006671.1      | <i>Escherichia coli</i>                         | pAPEC-O2-R   | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_012220.1      | <i>Lactobacillus plantarum</i>                  | pLD1         | 99             | 100               | $7.0 \times 10^{-45}$ | 1               |
| NC_014918.1      | <i>Mesorhizobium ciceri</i>                     | pMESCI01     | 96             | 100               | $7.0 \times 10^{-40}$ | 1               |
| NC_008459.1      | <i>Bordetella pertussis</i>                     | pBP136       | 97             | 100               | $2.0 \times 10^{-41}$ | 1               |
| NC_009427.1      | <i>Novosphingobium aromaticivorans</i>          | pNL2         | 95             | 100               | $1.0 \times 10^{-37}$ | 1               |
| NC_007507.1      | <i>Xanthomonas campestris</i> pv.               | pXCV183      | 98             | 100               | $3.0 \times 10^{-43}$ | 1               |
| NC_013860.1      | <i>Azospirillum</i> sp.                         | pAB510f      | 95             | 100               | $3.0 \times 10^{-38}$ | 1               |
| NC_010997.1      | <i>Rhizobium etli</i>                           | pC           | 95.7           | 93                | $2.0 \times 10^{-35}$ | 1               |
| NC_004041.2      | <i>Rhizobium etli</i>                           | p42d         | 98.95          | 95                | $4.0 \times 10^{-42}$ | 1               |
| NC_008790.1      | <i>Campylobacter jejuni</i> subsp.              | pTet         | 98             | 100               | $3.0 \times 10^{-43}$ | 1               |

Table S4. Cont.

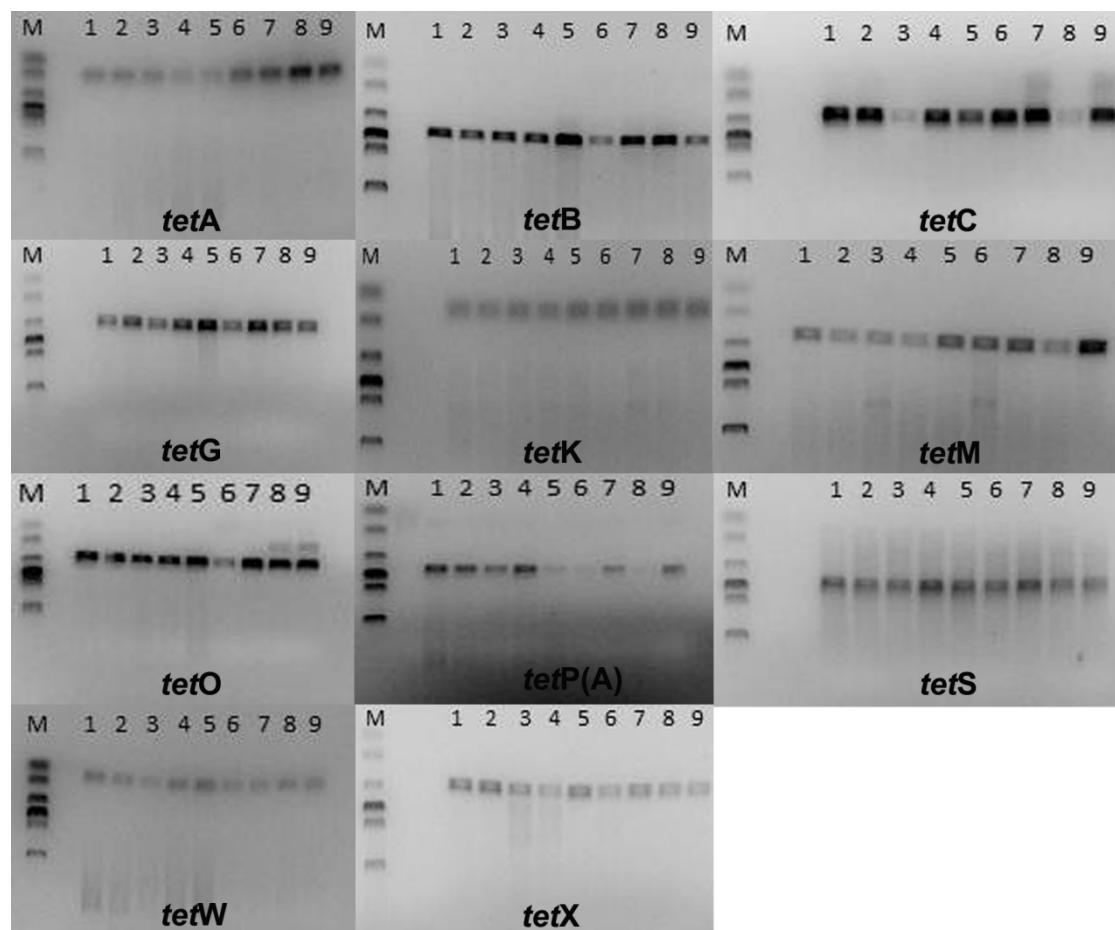
| Accession Number | Bacterial Host                        | Plasmid Name | Identity (%) t | Hit Length (bp) n | E Value t             | Number of Reads |
|------------------|---------------------------------------|--------------|----------------|-------------------|-----------------------|-----------------|
| NC_012987.1      | <i>Methylobacterium extorquens</i>    | p1METDI      | 96.04          | 101               | $7.0 \times 10^{-40}$ | 1               |
| NC_004059.1      | <i>Bacillus thuringiensis</i>         | pUIBI-1      | 98             | 100               | $3.0 \times 10^{-43}$ | 1               |
| NC_011984.1      | <i>Agrobacterium vitis</i>            | pAtS4c       | 97.96          | 98                | $4.0 \times 10^{-42}$ | 1               |
| NC_010518.1      | <i>Methylobacterium radiotolerans</i> | pMRAD05      | 95.79          | 95                | $5.0 \times 10^{-37}$ | 1               |
| NC_011960.1      | <i>Rhodobacter sphaeroides</i>        | pRSKD131B    | 98             | 100               | $3.0 \times 10^{-43}$ | 1               |
| NC_005307.1      | <i>Gordonia westfalica</i>            | pKB1         | 95             | 100               | $1.0 \times 10^{-37}$ | 1               |
| NC_007949.1      | <i>Polaromonas</i> sp.                | plasmid 1    | 95.96          | 99                | $3.0 \times 10^{-39}$ | 1               |
| NC_011355.1      | <i>Mycobacterium liflandii</i>        | pMUM002      | 97             | 100               | $2.0 \times 10^{-41}$ | 1               |
| NC_013201.1      | <i>Halomicrombium mukohataei</i>      | pHmuk01      | 95             | 100               | $3.0 \times 10^{-38}$ | 1               |
| NC_006816.1      | <i>Salmonella enterica</i> subsp.     | pU302L       | 96             | 100               | $3.0 \times 10^{-39}$ | 1               |
| NC_009650.1      | <i>Klebsiella pneumoniae</i> subsp.   | pKPN4        | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_006628.1      | <i>Laribacter hongkongensis</i>       | pHLHK8       | 96.88          | 96                | $3.0 \times 10^{-39}$ | 1               |
| NC_014615.1      | <i>Escherichia coli</i>               | pETN48       | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_011981.1      | <i>Agrobacterium vitis</i>            | pAtS4e       | 96             | 100               | $7.0 \times 10^{-40}$ | 1               |
| NC_004939.1      | <i>Corynebacterium striatum</i>       | pTP10        | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_014312.1      | <i>Klebsiella pneumoniae</i>          | pKP048       | 98.94          | 94                | $2.0 \times 10^{-41}$ | 1               |
| NC_011887.1      | <i>Methylobacterium nodulans</i>      | pMNOD02      | 95.74          | 94                | $2.0 \times 10^{-36}$ | 1               |
| NC_009230.1      | <i>Burkholderia vietnamiensis</i>     | pBVIE01      | 97             | 100               | $2.0 \times 10^{-41}$ | 1               |
| NC_005249.1      | <i>Klebsiella pneumoniae</i>          | pLVPK        | 100            | 100               | $2.0 \times 10^{-46}$ | 1               |
| NC_007926.1      | <i>Pseudomonas putida</i>             | NAH7         | 95.05          | 101               | $1.0 \times 10^{-37}$ | 1               |

**Table S5.** PCR primers of the 15 tetracycline resistance genes detected in this study.

| Gene           | Primer(5'-3')  | Fragment Size | Annealing Temperature |
|----------------|--|---------------|-----------------------|
| <i>tetA</i>    | Forward GCTACATCCTGCTTGCCTTC<br>Reverse CATAGATGCCGTGAAGAGG    | 210           | 55.5                  |
| <i>tetB</i>    | Forward TTGGTTAGGGCAAGTTTG<br>Reverse GTAATGGCCAATAACACCG      | 659           | 56                    |
| <i>tetC</i>    | Forward CTTGAGAGCCTCAACCCAG<br>Reverse ATGGTCGTCATCTACCTGCC    | 418           | 55                    |
| <i>tetD</i>    | Forward AAACCATTACGGCATTCTGC<br>Reverse GACCGGATACACCATCCATC   | 787           | 56                    |
| <i>tetE</i>    | Forward AAACCACATCCTCCATACGC<br>Reverse AAATAGGCCACAACCGTCAG   | 278           | 55                    |
| <i>tetG</i>    | Forward GCTCGGTGGTATCTCTGCTC<br>Reverse AGCAACAGAACATGGGAACAC  | 468           | 55                    |
| <i>tetK</i>    | Forward TCGATAGGAACAGCAGTA<br>Reverse CAGCAGATCCTACTCCTT       | 169           | 55                    |
| <i>tetL</i>    | Forward TCGTTAGCGTGCTGTCATT<br>Reverse GTATCCCACCAATGTAGCCG    | 267           | 55                    |
| <i>tetM</i>    | Forward GTGGACAAAGGTACAACGAG<br>Reverse CGGTAAAGTCGTACACAC     | 406           | 55                    |
| <i>tetO</i>    | Forward AACTAGGCATTCTGGCTCAC<br>Reverse TCCCACTGTTCCATATCGTCA  | 515           | 50.3                  |
| <i>tetS</i>    | Forward CATAGACAAGCCGTGACC<br>Reverse ATGTTTTGGAACGCCAGAG      | 667           | 56                    |
| <i>tetP(A)</i> | Forward CTTGGATTGCGGAAGAAGAG<br>Reverse ATATGCCATTAAACCACGC    | 676           | 55                    |
| <i>tetQ</i>    | Forward TTATACTCCTCCGGCATCG<br>Reverse ATCGGTTCGAGAACATGTCCAC  | 904           | 55                    |
| <i>tetW</i> *  | Forward GAGAGCCTGCTATATGCCAGC<br>Reverse GGGCGTATCCACAATGTTAAC | 168           | 64                    |
| <i>tetX</i>    | Forward CAATAATTGGTGGTGGACCC<br>Reverse TTCTTACCTTGGACATCCCG   | 468           | 55                    |

The primers used in this study were designed according to Ng *et al.* (2001) and Lee *et al.* (1993). Ng, L.-K.; Martin, I.; Alfa, M.; Mulvey, M. Multiplex PCR for the detection of tetracycline resistant genes. *Mol. Cell. Probe.* **2001**, *15*, 209–215. \* Lee, C.; Langlois, B.E.; Dawson, K.A. Detection of tetracycline resistance determinants in pig isolates from three herds with different histories of antimicrobial exposure. *Appl. Environ. Microbiol.* **1993**, *59*, 1467–1472.

**Figure S1.** Occurrence patterns of 11 *tet* genes in activated sludge of Day 0 and Day 6 analyzed by electrophoresis of PCR products. M: DNA Marker (DL2000, TaKaRa, Shiga, Japan).



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