Draft Genome Sequences of Isolates from Sediments of the River Elbe That Are Highly Tolerant to Diclofenac

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ABSTRACT Here, we report the genome sequences of one Achromobacter and four Pseudomonas strains isolated from sediments of the River Elbe which are highly tolerant toward the xenobiotic target compound diclofenac, a nonsteroidal anti-inflammatory drug (NSAID) and emerging contaminant.

Diclofenac, an over-the-counter medication used in many countries, has a yearly production volume of about 90 tons in Germany and 800 tons in India. Insufficient removal by wastewater treatment plants has led to an increasing occurrence of diclofenac, as well as that of other pharmaceuticals and personal care products (PPCPs), in surface waters such as the River Elbe in Germany (1). Moreover, its toxic effects toward microbes as well as higher organisms (2, 3) have converted diclofenac into an emerging contaminant.

Isolates were obtained from enrichment cultures of sediment samples from the River Elbe downstream of the Hamburg harbor (4) with 0.5 mM diclofenac as the sole carbon and energy source. Bacteria growing in the presence of diclofenac were isolated and identified by sequencing the corresponding 16S rRNA genes. A MIC(s) study with various concentrations of diclofenac in lysogeny broth (LB) medium showed that the isolates RW405 and RW409 grew at concentrations greater than 1,500 mg/liter, but RW407, RW408, and RW410 grew only in a range between 750 and 1,200 mg/liter.

To characterize each isolate, pure cultures of each strain were grown overnight at 30°C in LB medium under agitation. Total genomic DNA was extracted using the Wizard genomic DNA purification kit (Promega). The quality and quantity of the DNA were assessed with the Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific). To obtain the draft genome of each isolate, 300-bp paired-end sequencing libraries were prepared using the Illumina Nextera XT DNA library version 3 sample preparation kit, and sequencing was performed with the Illumina MiSeq platform at the Center for Scientific Instrumentation of the University of Granada (Spain). Sequence reads were assessed for quality using FastQC (Babraham Bioinformatics, Q/H11022) and Trimomatic (version 1.2.10) and VelvetOptimizer (version 2.2.5) (6) within a customized workflow on Galaxy (http://galaxy-mel.genome.edu.au/galaxy/). The contigs obtained were further annotated with the Rapid Annotations using Subsystems Technology (RAST) server version 2.0 (7), and for submission to GenBank, gene annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (8).

Genome sizes vary between 5,862 and 7,346 Mbp and GC contents from 61.8 to 67.6% (Table 1). Taxonomy was determined by comparing 16S rRNA gene sequences in EzBioCloud (9) and the rpoD, recA, and gyrB genes (10) with a BLAST search (11). As possible mechanisms for diclofenac tolerance, the isolates harbored between 35 and 46 genes related to efflux mechanisms for multidrug resistance and between 143 and 240 genes related to the metabolism of aromatic compounds, which constitute between 0.6 and 0.7% and 2.4 and 3.8% of the total number of genes, respectively. Pairwise
similarity between the genomes as determined by average nucleotide identity (ANI) with the OrthoANIu tool (12) ranged between the pseudomonad genomes from 76.6 to 82% and between the pseudomonads and the Achromobacter strain from 69.2 to 70.7%, which are all well below the 95 to 96% species threshold (13).

**Data availability.** The whole-genome sequences have been deposited in DDBJ/ENA/GenBank under the accession numbers listed in Table 1. The versions described in this paper are the first versions.

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**TABLE 1** Characteristics and accession numbers of genomes of the diclofenac-tolerant bacterial isolates

| Isolate | Bacterial species* | Genome size (bp) | No. of contigs | N50 (bp) | Total no. of genes | G+C content (%) | No. of genes related to efflux systems* | No. of genes related to metabolism of aromatic compounds* | GenBank accession no. |
|---------|-------------------|------------------|---------------|----------|-------------------|----------------|----------------------------------------|----------------------------------------------------------|---------------------|
| RW405   | *Pseudomonas putida* | 5,862,946        | 33            | 127,964  | 5,612             | 61.8           | 35                                     | 152                                                           | QHJ0000000000      |
| RW407   | *Pseudomonas citronellolis* | 7,346,097        | 108           | 38,667   | 6,724             | 67.4           | 37                                     | 256                                                           | QGL0000000000      |
| RW408   | *Achromobacter xylosoxidans* | 6,532,250        | 49            | 57,962   | 6,060             | 67.6           | 44                                     | 143                                                           | QHH0000000000      |
| RW409   | *Pseudomonas chlororaphis*  | 7,154,805        | 42            | 130,324  | 6,555             | 62.5           | 46                                     | 171                                                           | QHP0000000000      |
| RW410   | *Pseudomonas aeruginosa*  | 6,529,102        | 74            | 120,648  | 6,289             | 66.2           | 42                                     | 166                                                           | QGS0000000000      |

*Strain identification by ≥99% similarity with 16S rRNA and ≥97% with the *rpoD*, *recA*, and *gyrB* genes.

*According to annotation with the RAST server.