Complete Genome Sequences of Four Atrazine-Degrading Bacterial Strains, *Pseudomonas* sp. Strain ADPe, *Arthrobacter* sp. Strain TES, *Variovorax* sp. Strain 38R, and *Chelatobacter* sp. Strain SR38

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**ABSTRACT** We report here the complete genome sequences of four atrazine-degrading bacteria. Their genomes will serve as references for determining the genetic changes that have occurred during an evolution experiment.

We recently set up an evolution experiment on a four-species bacterial consortium to determine whether liquid medium supplemented with the herbicide atrazine and/or different N and C sources could support the coexistence of multiple species (1). Here, we provide the full genome sequences of the four ancestral atrazine-degrading strains, namely, *Pseudomonas* sp. strain ADPe (2, 3), *Variovorax* sp. strain 38R (4), *Arthrobacter* sp. strain TES (5), and *Chelatobacter* sp. strain SR38 (6).

The strains were all derived from ancestors isolated from atrazine-contaminated soils and were stored at −80°C in glycerol (30%). Multiple colonies were picked and grown to late exponential phase on mineral salt medium containing atrazine as the sole nitrogen source. Genomic DNA was extracted using the Qiagen genomic DNA extraction kit. Oxford Nanopore Technologies (ONT) libraries were generated from 2 μg fragmented DNA with a ligation sequencing kit (SQK-LSK109) and sequenced on the GridION platform using an R9.5 flow cell. Illumina paired-end (PE) reads were prepared using the TruSeq Nano DNA LT library preparation kit (Illumina). Briefly, DNA was fragmented by sonication and adaptors were ligated. Eight cycles of PCR were applied to amplify the libraries. Library quality was assessed using Fragment Analyzer (Advanced Analytical Technologies, Inc.), and libraries were quantified by quantitative PCR using the Kapa library quantification kit (Roche). Sequencing was performed on a HiSeq instrument (Illumina) using a PE read length of 2 × 150 bp with the Illumina HiSeq 3000 reagent kits.

Fast5 files from ONT sequencing were obtained with ONT MinKNOW software (v1.10.24-1) and were base called with ONT Albacore sequencing pipeline software (v2.1.10). Adaptors were trimmed using Porechop v0.2.1 (7), and reads with a quality score of <7 and size of <3,000 bp were discarded using NanoFilt v2.2.0 (8). Nanopore reads were then assembled using Canu v1.7 (9) with the “minReadLength=3000” and “genomeSize” options. A first polishing step and circularization were performed on the assembly using Pilon v1.22 (10) and Circulator v1.5.1 (11), respectively. Illumina PE reads were processed with Trim Galore v0.4.0 (https://github.com/FelixKrueger/TrimGalore) to trim adaptor sequences and were mapped on the assembly using BWA-MEM v0.7.12 (12) and SAMtools v1.3.1 (13). The mapping was finally used to improve the polishing with two rounds with Pilon v1.22 with the following option:
mindepth, 25. For each strain, taxonomic classification was inferred using MiGA (14) and the maximum average amino acid identity (AAI) found between its chromosome and all of the reference genomes in the NCBI RefSeq database. Default parameters were used for all software unless otherwise noted. Detailed statistics regarding the assembly of the four genomes are given in Table 1.

Taxonomic classification of Pseudomonas sp. ADPe confirmed that it may belong to the species Pseudomonas citronellolis (P = 0.03) (15). The chromosome contains five 16S rRNAs. The sequence of its circular plasmid is similar to that of pADPe (99% overall similarity) except that a 22.3-kb sequence containing atzA and atzB and surrounded by insertion sequences (ISs) is deleted.

Variovorax sp. 38R most likely belongs to the genus Variovorax (P = 0.0049) and probably belongs to the species Variovorax paradoxus (P = 0.083). It contains atzA and atzB in two distinct regions, both delimited by ISs and exhibiting 99% similarity to Pseudomonas sp. strain ADP1.

Arthrobacter sp. TES most likely belongs to the same species as Arthrobacter sp. strain ZXY-2 (P = 0.0054). Atrazine-degrading genes trzN, atzB, and atzC are all located on the 205.8-kbp contig; trzN is found within a 9,081-bp region and atzB and atzC within a 36,366-bp region, both of which present 99% similarity to the atrazine-degrading plasmid pTC1 of Paenarthrobacter aurescens strain TC1 (GenBank accession number CP000475.1).

For Chelatobacter sp. SR38, AAI analysis performed on its chromosome reveals that it belongs to the genus Aminobacter and probably to the species Aminobacter aminoivorans (P = 0.035). A BLASTN analysis indicated that atzA is located on the 74-kb uncircularized contig while atzB, atzC, and trzD are on the 197-kb one. Interestingly, atzB is present in two copies on this contig.

These genomes will be used as references to analyze full genome resequencing of these strains to search for genetic changes that might have occurred in the time course of their evolution in a four-species atrazine-degrading bacterial consortium facing different environmental challenges.

Data availability. Raw data and assembled genomes have been deposited in the NCBI GenBank under the BioProject accession number PRJNA664737. GenBank accession numbers for the assembled genomes are given in Table 1.

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**TABLE 1** Assembly statistics and accession numbers for the strains in this study

| Strain                     | No. of reads | Nanopore read coverage (x) | N_{50} (nucleotides) | No. of contigs | Contig size (bp) | GenBank accession no. | GC content (%) |
|----------------------------|--------------|-----------------------------|----------------------|----------------|-------------------|-----------------------|----------------|
| Pseudomonas sp. ADPe       | 165,586      | 366                         | 21,830               | 2              | Chromosome        | 7,177,635             | CP062122       | 66.9          |
|                            |              |                             |                      |                | pADPe             | 62,583                | CP062123       | 64.7          |
| Variovorax sp. 38R         | 308,810      | 571                         | 15,149               | 1              | Chromosome        | 6,870,625             | CP062121       | 67.5          |
| Arthrobacter sp. TES       | 153,561      | 633                         | 25,143               | 4              | Chromosome        | 4,181,416             | CP062235       | 63.5          |
|                            |              |                             |                      |                | pTES1             | 351,150               | CP062236       | 62            |
|                            |              |                             |                      |                | pTES2             | 205,802               | CP062237       | 62.6          |
|                            |              |                             |                      |                | pTES3             | 48,156                | CP062238       | 60.9          |
| Chelatobacter sp. SR38     | 116,302      | 290                         | 23,740               | 9              | Chromosome        | 5,667,809             | CP062112       | 63.4          |
|                            |              |                             |                      |                | pSR1              | 560,213               | CP062113       | 63.2          |
|                            |              |                             |                      |                | pSR2              | 120,900               | CP062114       | 61.2          |
|                            |              |                             |                      |                | pSR3              | 170,880               | CP062115       | 58.8          |
|                            |              |                             |                      |                | pSR4              | 74,540                | CP062116       | 58.0          |
|                            |              |                             |                      |                | pSR5              | 163,970               | CP062117       | 60.4          |
|                            |              |                             |                      |                | pSR6              | 52,741                | CP062118       | 58.3          |
|                            |              |                             |                      |                | pSR7              | 359,029               | CP062119       | 60.2          |
|                            |              |                             |                      |                | pSR8              | 197,271               | CP062120       | 62.8          |
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