Research article

Acidovorax bellezanensis sp. nov., a novel bacterium from uranium mill tailings repository sites with selenium bioremediation capabilities

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ABSTRACT

A Gram-stain-negative bacterial strain designated Be4^T, belonging to the genus *Acidovorax*, was isolated from mining porewaters sampled in uranium mill tailings repository sites, located in Bellezane, near Bessines-sur-Gartempe (Limousin, France). Cells were facultative anaerobic, rod-shaped, non-endospore-forming and motile with flagella. The mean cell size was 1.25–1.31 μm long and 0.70–0.73 μm wide. Colonies were light yellow, opaque, circular, convex with smooth margins, and 1–2 mm in diameter. Growth occurs at 4–37 °C and between pH 5.5–9.0. It differed from its phylogenetically related strains by phenotypic and physiological characteristics such as growth at 4 °C, presence of acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucosidase enzymatic activities, and fermentation of L-xylose and esculin. The major fatty acids were $C_{16:0}$, $C_{16:1}$ ω7c/$C_{16:1}$ ω6c, $C_{17:0}$ cyclo and $C_{18:1}$ ω7c. Phylogenetic analysis based on 16S rRNA and 938 core genes, confirmed its placement within the genus *Acidovorax* as a novel species. Strain Be4^T showed highest 16S rRNA sequence similarity to *Acidovorax antarcticus* (98.2 %), *Acidovorax radicis* (97.9 %), *Acidovorax temperans* (97.8 %) and *Acidovorax facilis* (97.7 %). The genome of strain Be4^T is 5,041,667 bp size with a DNA G + C content of 65.15 %. By automatic annotation numerous sequences involved in the interaction with metals/metalloids including some genes related to Se uptake and selenite resistance were detected in its genome. The average nucleotide identity (ANI) values calculated from whole genome sequences between strain Be4^T and the most closely related strains *A. radicis* and *A. facilis* were below the threshold value of 95 %. Thus, the data from the phylogenetic, physiological, biochemical, and genomic analyses clearly indicates that strain Be4^T represents a novel species with the suggested name *Acidovorax bellezanensis* sp. nov. The type strain is *Acidovorax bellezanensis* Be4^T (=DSM116209^T = CECT30865^T). This novel

species, due to its unique isolation source, genomic analysis, and preliminary laboratory tests where it was able to reduce toxic Se(IV) to less harmful Se(0) in the form of nanoparticles, holds great potential for further investigation in bioremediation, particularly concerning Se.

1. Introduction

The genus *Acidovorax*, belonging to *Comamonadaceae* family (*Betaproteobacteria* class in the phylum *Pseudomonadota*), was first proposed when *Pseudomonas facilis* and *P. delafeldii* were re-classified as *Acidovorax facilis* and *A. delafeldii*, respectively. They were reported to belong to the acidovorans rRNA complex in rRNA superfamily III [1]. At the time of writing this work, the genus comprises 20 species with validly published names (https://lpsn.dsmz.de/genus/acidovorax) being *A. facilis* the type species [1]. *Acidovorax* species have been isolated from diverse habitats, such as plants: *A. lacteus* [2], *A. kalamii* [3], *A. anthurii* [4], *A. avenae*, *A. cattleyae*, *A. citrulli*, *A. konjaci* [5], *A. valerianellae* [6], *A. radicis* [7], *A. oryzae* [8]; sludges: *A. caeni* [9], *A. defluvii* [10]; soil: *A. soli* [11], *A. monticola* [12], *A. carolinensis* [13], *A. antarcticus* [14]; and clinical samples: *A. delafieldii*, *A. facilis*, *A. temperans* [1] and *A. wautersii* [15]. While many species of this genus are classified as phytopathogenic bacteria [1,4–6,9], others are known to participate in denitrification and contaminants biodegradation processes [16,17] as well as to sequester U(VI) [18]. Isolation, characterization and selection of microbial isolates offering high performance on contaminants removal/degradation emerges as a prerequisite for optimal design of bioremediation strategies. This screening process must be based on certain criteria like the ability to cope with a wide variety of constraining conditions (e.g. high concentrations of contaminants), or the presence of particular biochemical features (e.g. specific enzymatic activities), which allow an effective remediation process [19].

There are several features common to all members of this genus which can be summarized as follows: smooth, convex, and white to yellow colonies, aerobic, catalase and oxidase positive, motile with polar flagella, rod-shaped and Gram-stain-negative. At the chemotaxonomic level, species of the genus *Acidovorax* present summed feature 3 (C16: 1ω7c and/or C16: 1ω6c), C16:0, and summed feature 8 (C18: 1ω7c and/or C18: 1ω6c) as dominant fatty acids. DNA G-C content varies from 62.0 to 70.0 mol%.

This study describes the major characteristics of the *Acidovorax* isolate named Be4^T, isolated from mining porewaters sampled in France, and compare its properties with those of *A. antarcticus* 16-35-5^T, *A. facilis* DSM 649^T, *A. radicis* DSM 23535^T, *A. temperans* DSM 7270^T and *A. delafieldii* DSM 64^T. Based on the results obtained from phenotypic, genotypic, chemotaxonomic, and phylogenomic analyses, strain Be4^T is proposed as a novel species of the genus *Acidovorax*. We present strain Be4^T as the type strain of a new species with promising biotechnological capacities for which the name *Acidovorax bellezanensis* sp. nov. is proposed.

2. Material and methods

2.1. Morphological characterization and cell motility evaluation

Colony-level phenotypic characterization including pigmentation and morphology was conducted through direct observation using an optical microscope and cells grown for 3 days at 28 °C on R2A agar plates [20]. Cell-level morphological determinations such as shape, size, and the presence of cellular structures like flagella were performed using environmental scanning electron microscopy (ESEM, model FEI Quanta 650 FEG, Thermofisher-FEI) with cells from the early exponential phase grown on R2A broth.

Gram-staining, endospore detection and cell motility were investigated based on methods published by Smibert and Kreig [21].

2.2. Physiological characterization

The physiological growth parameters of this strain were investigated by monitoring growth for 5 days at different temperatures (4, 15, 28, 37, 40 and 42 °C) on TSA (tryptic soy agar medium) plates, NaCl concentrations (0, 0.5, 1.0, 1.5 % w/v, using a basal medium with the composition of 15 g/1 casein, 5 g/1 soybean meal, supplemented with the required amount of NaCl) and pH levels (5.0, 5.5, 6.5, 7.0, 8.0, 9.0, 10.0 and 11.0) set using different buffer systems: phosphate–citrate buffer (5.0–6.5), Tris–HCl buffer (7.0–9.0), NaHCO$_3$–NaOH buffer (9.5–11.0), and Na$_2$HPO$_4$–NaOH buffer (11.5–12.0). Anaerobic growth was assessed by inoculating TSA plates which were incubated in an anaerobic jar flushed with a gas mixture consisting of nitrogen (85 %), carbon dioxide (10 %) and hydrogen (5 %) using an Anoxomat system (MART). Other biochemical properties such as carbon source utilization, enzyme activities and additional physiological tests were investigated with API 50CH, API ZYM and API 20NE test strips according to the manufacturer's instructions (BioMerieux, France). Other determinations (oxidase and catalase activities) were carried out by classic methods such as described in Sánchez-Castro et al. [22].

2.3. Cellular fatty acid composition and identification

Cellular fatty acids were analyzed after conversion into fatty acid methyl esters by saponification, methylation and extraction using the method of Miller [23] and Kuykendall et al. [24]. The fatty acid methyl esters mixtures were separated by gas chromatography and detected by a flame ionisation detector using Sherlock Microbial Identification System (MIS) (MIDI, Microbial ID, Newark, DE 19711 U S A.). Peaks were automatically integrated and fatty acid names and percentages calculated by the MIS Standard Software (Microbial ID). Next, summed features were resolved and identities of fatty acids were confirmed by a GC-MS-based analysis using retention time

locking and mass spectral data. These analyses were carried out by the Identification Service, Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

2.4. Molecular analyses

Genomic DNA from strain Be4[T] was extracted for further analyses according to Martín-Platero et al. [25]. Be4[T] 16S rRNA gene was amplified using the universal bacterial primer set 27F (5′-GAGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACG GYTACCTTGTTACGACTT-3′). The PCR product purification and sequencing were carried out as outlined by Sánchez-Castro et al. [19]. For the phylogenetic reconstruction of the 16S rRNA gene, the sequence of the type strain Be4[T] was compared to the LTP_01_2022 database, which includes sequences of the type strains classified up to January 2022 [26] and is accessible at https://imedea.uib-csic. es/mmg/ltp/. The sequence alignment was performed using the SINA v1.3.1 aligner [27] within the ARB program package [28], and manually checked in order to improve the alignment. Phylogenetic analyses were then conducted using the ARB software package v6.0.6 [28] based on almost complete 16S sequences, utilizing the neighbor-joining [29] and RAxML [30] algorithms with different corrections (Jukes-Cantor and GTRGAMMA, respectively) as implemented in the ARB software package [28].

The draft genome sequence of strain Be4[T] was produced at the STAB VIDA Next Generation Sequencing Laboratory (Caparica, Portugal) using the lllumina Novaseq platform, generating 150bp paired-end sequencing reads. Initially, 1 μL of gDNA was assessed for integrity and purity through 1.5 % agarose gel electrophoresis followed by quantification using the Qubit ® 2.0 fluorometer. The sample integrity (>30 ng of DNA) was confirmed before library construction and sequencing. The raw sequence data underwent automatic initial treatment, namely adapters and low-quality base trimming. The quality of the data was evaluated using the Phred quality score at each cycle. The resulting genome was assembled *de novo* by STAB VIDA Next Generation Sequencing Laboratory (Caparica, Portugal) using an algorithm based on de Bruijn graphs [31]. The G + C content of genomic DNA was determined from the whole genome. Prodigal v2.6.3 [32] was used for gene prediction. The predicted protein sequences of the genomes included in the study were used for phylogenetic reconstruction based on single-copy core genes. Predicted proteins were compared using an all-versus-all BLAST (v2.2.31) and shared reciprocal best matches in all pairwise genome comparisons were identified using *rbm.rb* script [33] using a 40 % sequence similarity cut-off and over 50 % or more of the query sequence length. All single-copy core-genes were aligned individually using MUSCLE v3.8.31 [34]. Aligned proteins were concatenated using the script *Aln.cat.rb* [33] and the phylogenetic analyses conducted using the RAxML and neighbor-joining algorithms within the ARB v6.0.6 software [28]. Both algorithms were employed with the corrections PROTGAMMA [35] and Kimura [36], respectively. The average nucleotide identity (ANI) and average amino acid identity (AAI) between all genomes were calculated using the BLAST tool with *ani.rb* and *aai.rb* scripts [33], respectively.

The final genome assembly was functionally annotated through Rapid Annotation System Technology (RAST) server using the default RASTtk parameter [37].

2.5. Selenium tolerance test and microscopic observation

Selenium tolerance was tested by aerobically growing cells in R2A liquid and solid media supplemented with concentrations ranging from 1 to 16 mM, at 28 °C with shaking at 180 rpm. Sodium selenite (Na_2SeO_3) (Merck) was used as the selenite (Se(IV)) source. 1 M stock solution was prepared by dissolving it in distilled water which was then sterilized by filtration using 0.22-μm syringe filters (SartoriusTM).

The cellular location, size and structure of Se(IV) reduction products were studied using a high-angle annular dark field scanning transmission electron microscope (HAADF-STEM) FEI TITAN G2 80–300 (Centro de Instrumentación Científica, University of Granada, Spain). The samples were prepared for microscopy observation following the procedures described in Ruiz-Fresneda et al. [38].

3. Results and discussion

3.1. Isolation and cultivation conditions

A bacterial strain, designated Be4[T], was isolated from porewaters sampled at French uranium mill tailings repository sites (GPS coordinates 46°5′49.09″N - 1°23′28.51″E; [19,39]). Further details on sampling procedures are provided in Sánchez-Castro et al. [19]. The water samples were serially diluted with sterile deionized water and these dilutions were plated on several oligotrophic media such as R2A agar and incubated at 28 °C for 5–7 days to stimulate the growth of slow-growing oligotrophic species. Single colonies on these R2A agar plates were purified by transferring them onto new R2A plates and incubated again at 28 °C for 3–5 days. Subsequently, the isolate was routinely cultivated on R2A agar at 28–30 °C and preserved in glycerol (50 % v/v) at −80 °C.

Other bacterial species isolated in the same area demonstrated already a promising U(VI) phosphate biomineralization potential as basis of U bioremediation strategy, especially in oxidizing conditions which normally are favorable to the high mobility of this element [40,41].

3.2. Multidisciplinary Be4[T] strain characterization and Se bioremediation potential

3.2.1. Phenotypic characterization

At the morphological level, colonies grown on R2A plates at 28 °C for 3 days were observed to be light yellow colored and

approximately 1–2 mm in diameter. They were circular, smooth and convex. Cells were motile due to the presence of polar flagella, Gram-negative and non-endospore-forming. Using SEM microscopy, rod-shaped Be4[T] cells were found to be 1.25–1.31 μm long and 0.70–0.73 μm wide (Fig. 1), with numerous granules localized at the surface level (Fig. 1). The formation of external granules increases the surface/volume ratio of the cells, potentially enhancing their interaction with contaminants, and improving bioremediation efficiency.

The main physiological and biochemical features of strain Be4[T] along with closely related strains (*A. antarcticus* 16-35-5[T], *A. facilis* DSM 649, *A. radicis* DSM 23535, *A. temperans* DSM 7270 and *A. delafieldii* DSM 64) are listed in Table 1. Strain Be4[T] was positive for hydrolysis of gelatin and urea and fermentation of glycerol, D-ribose, L-xylose, D-mannitol, D-sorbitol and esculin, while tested negative for the rest of carbohydrates substrates included in this commercial test. Assimilation tests for capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid resulted negative in all cases. Enzymatic activities, such as catalase, oxidase, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucosidase were positive for strain Be4[T], while other were negative as lipase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl- β-glucosaminidase, α-mannosidase, arginine dihydrolase, urease, gelatinase and α-fucosidase. Be4[T] showed nitrate reduction activity but did not produce indole. Phosphatase activity, positive in Be4[T], is crucial in designing bioremediation strategies based on phosphate biomineralization processes [42,43]. These mechanisms are known to have numerous advantages for bioremediation purposes since they occur under oxidizing conditions and the resulting precipitates present low solubility and high chemical stability ([40,44,45]).

Strain Be4[T] did not require NaCl for its growth and could not tolerate additional NaCl than that included in the original formulation of the growing media employed. Growth of Be4[T] was observed at a pH range of 5.5–9.0 (optimum at 7.0) and temperatures of 4–37 °C (optimum at 15 °C). Growth in the absence of oxygen was also demonstrated. These growing conditions, especially anoxia and low temperature, were expected based on the sampling site where this isolate was obtained: 25-m depth porewaters [19]. In previous studies, Be4[T] was also reported as highly tolerant to certain heavy metals such as uranium (minimum inhibitory concentration higher than 4 mM) and nickel (minimum inhibitory concentration higher than 8 mM) [19], an important aspect for microbes used for bioremediation applications.

The fatty acid composition of strain Be4[T] was similar to those of the other related strains (Table 2), with some differences in some components like the presence of $C_{17:0\ cyclo}$ and a lower proportion of $C_{16:1}\ \omega7c/C_{16:1}\ \omega6c$ and $C_{18:1}\ \omega7c$. Strain Be4[T] had $C_{16:0}$, $C_{16:1}\ \omega7c/C_{16:1}\ \omega6c$, $C_{17:0}$ cyclo and $C_{18:1}\ \omega7c$ as major fatty acids (Table 2).
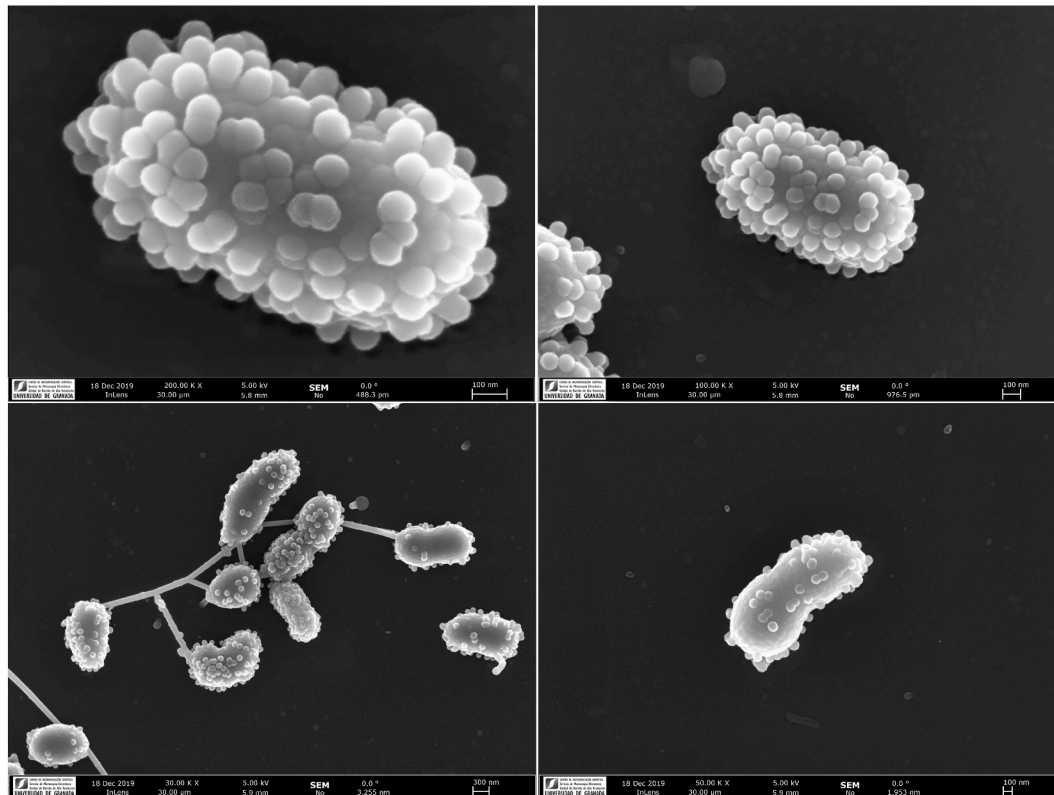

Fig. 1. Scanning electron microscopy image of Be4 cells cultured in R2A medium for 48 h at a temperature of 28 °C.

Table 1

Differential features of strain Be4 and closely related species of the genus *Acidovorax*

Taxa: 1, Be4[T]; 2, *A. radicis* (DSM 23535[T]); 3, *A. delafieldii* (DSM 64[T]); 4, *A. facilis* (DSM 649[T]); 5, *A. temperans* (DSM 7270[T]); and 6, *A. antarcticus* (16-35-5[T]). References: [1,7,9,14]. Data from present study. +, Positive reaction; -, negative reaction; w, weak positive.

Characteristic	1	2	3	4	5	6
Growth at						
4 °C	+	–	–	–	–	+
40 °C	–	+	+	+	+	–
0.5 % NaCl	–	+	+	+	+	+
1.0 % NaCl	–	+	+	+	+	+
Enzyme activity (API ZYM/20NE)						
Alkaline phosphatase	+		+	+	+	+
Acid phosphatase	+		–	–	–	+
Naphthol-AS-BI-phosphohydrolase	+		–	–	–	+
β-glucosidase	+		–	–	–	–
Carbohydrate Metabolism (API 50CH)						
L-Xylose	+		–	–	–	–
D-Fructose	–	+	+	w	+	–
Esculin	+		–	–	–	–

Table 2

Cellular fatty acid composition (%) of strain Be4 and closely related *Acidovorax* type strains. Taxa: 1, Be4[T]; 2, *A. radicis* (DSM 23535[T]); 3, *A. delafieldii* (DSM 64[T]); 4, *A. facilis* (DSM 649[T]); 5, *A. temperans* (DSM 7270[T]); and 6, *A. antarcticus* (16-35-5[T]).

Fatty acid	1	2	3[a]	4	5	6
$C_{10:0}$ 3OH	2.3	2.9	3.0	3.3	3.2	1.9
$C_{12:0}$	2.7	2.6	3.7	3.1	4.9	2.5
$C_{14:0}$	3.2	3.0	4.1	3.4	TR	1.2
$C_{16:0}$	30.5	29.6	26.2	29.9	25.7	35.2
$C_{16:1}$ ω7c/$C_{16:1}$ ω6c	29.4	41.7	38.0	40.9	44.4	25.2
$C_{17:0}$	TR	TR	TR	1.4	TR	TR
$C_{17:0\ cyclo}$	16.2	1.2	TR	TR	TR	19.0
$C_{18:1}$ ω7c	13.8	18.0	22.9	16.8	20.0	12.9

TR – Trace concentration.

[a] Data for 3, *A. delafieldii* (DSM 64[T]) were obtained from Ref. [3].
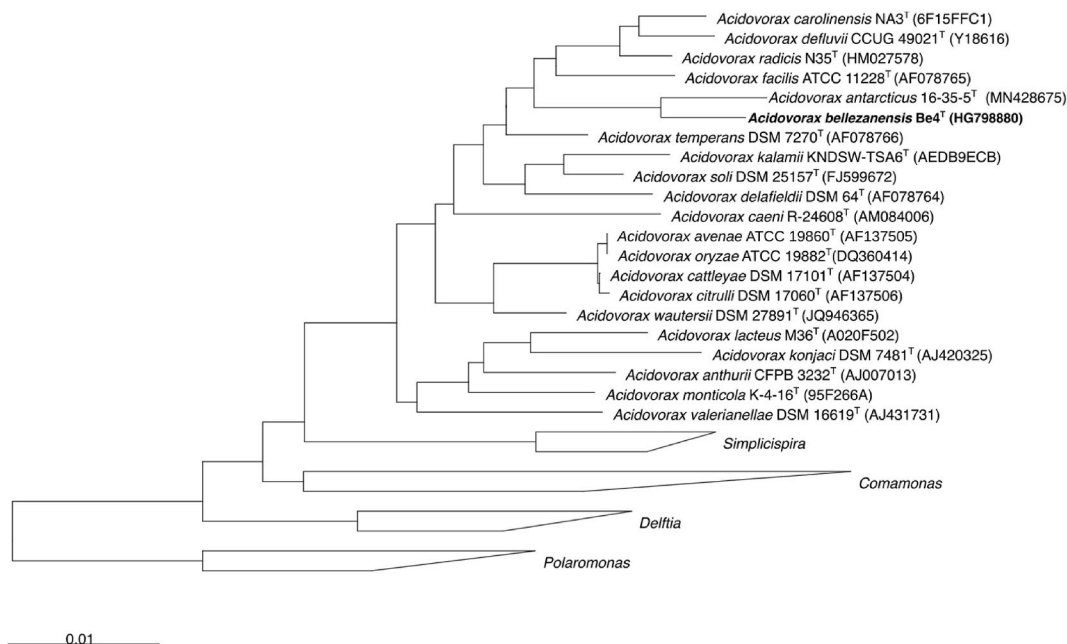
Fig. 2. Phylogenetic reconstruction based on 16S rRNA genes of strains of members of the genus *Acidovorax*, *Simplicispira*, *Comamonas* and *Delftia* available in the LTP_01_2022. This is a consensus tree between the neighbor-joining and RaxML reconstructed trees using the termini filter and taking into account all variable positions. Bar, 0.01 substitutions per nucleotide position.

3.2.2. Molecular characterization

An almost complete 16S rRNA gene sequence (1342 bp) of Be4^T was determined and deposited in the GenBank database (accession number HG798880). The phylogenetic tree based on 16S rRNA gene sequence indicated that the new strain represents a novel species within the genus *Acidovorax* (Fig. 2), sharing the highest sequence similarity with the closest relative *A. antarcticus* 16-35-5^T (98.2 %), *A. radicis* N35^T (97.9 %), *A. temperans* CCUG11779^T (97.8 %) and *A. facilis* CCUG2113^T (97.7 %) species. The similarity with the other *Acidovorax* species was lower than 97.3 % (Sup. Table S1). The novelty of the new isolate was further supported by the phylogenies based on the core single copy genes (i.e., all shared genes of the sequenced type strains of the genus *Acidovorax*, *Simplicispira*, *Comamonas*, *Delftia* and *Polaromonas*; Fig. 3). We observed different topologies comparing both 16S rRNA genes and core genes phylogenetic reconstruction. Based on core-genes phylogenetic genes, Be4^T formed an independent branch closely related to the type strain of *A. antarcticus* 16-35-5^T within the phylogenetic clade jointly with the members of the genus *Comamonas* and *Delftia*, including the type strain of the species *A. caeni* R-24608^T.

The genome of the strain Be4^T had a total genome length of 5,041,667 bps and G + C mol% value of 65.15 %, which were in accordance with those expected for the *Acidovorax* genus (Table 3). The whole genome project has been deposited at GenBank under the accession number JAODYH000000000. The ANI [46] and AAI [47] values comparing the new strain and their closest relative type strains confirmed that Be4^T represents a novel species (Sup. Table S2 and Sup. Table S3), which are well below the cut off values (95–96 % for ANI) recommended for species delineation. The ANI and AAI value with the close relative strain *A. antarcticus* 16-35-5^T was 84.45 % and 81.91 %, respectively. The percentage of genome shared between both strains was around 70 %. The ANI and AAI value of Be4^T and all the other type strains were always lower than 81 %.

Automatic annotation generated 4690 features potentially assigned to protein-encoding genes (open reading frames [ORFs]). A chromosome 5,039,301 bases long was identified and among the annotated genes, 52 % corresponded to RAST subsystems responsible for membrane transport (381), stress response (112) and resistance to antibiotics and toxic compounds (93). In *Acidovorax* sp. Be4^T genome annotation several glutathione S-transferases (GSTs) sequences were detected. The presence of GSTs has been previously reported in *Acidovorax* sp. strain KKS102 as novel zeta-like GST, an enzyme with potential in bioremediation of diverse classes of organochlorine pollutants [48]. Furthermore, other genes related to Se uptake and selenite resistance (*cysA*, *dedA*; [49]) were detected, suggesting its function in the biological detoxification of Se oxyanions. Additionally, the Be4^T genome annotation showed numerous sequences involved in the interaction with metals/metalloids such us chromium (*chrB* and *chrA*) copper (*cutA*, *cutE* and *corC*), cobalt-zinc-cadmium (*czcD*, *czcA*, *czcC*, *cusB*, *cusA*, *czrR* and *zraP*) and arsenic (*arsB*, *arsC* and *arsH*).

3.3. Novel species description of Be4^T

Based on phenotypic and genotypic results (morphology, growth characteristics, biochemical and chemotaxonomic characteristics, molecular systematic studies including 16S rRNA and core single copy genes sequence analyses, and genome-level ANI and AAI studies), strain Be4^T is considered to represent a novel species of the genus *Acidovorax*, for which the name *Acidovorax bellezanensis* (bel.le.za.nen'sis. N.L. masc. adj. *bellezanensis*, pertaining to Bellezane, a French mining site in the region of Limousin, from which this bacterium was isolated) sp. nov. is proposed.

Cells are Gram-stain-negative, aerobic, non-endospore-forming, motile rods with polar flagella. The mean size (n = 3) of the rod-
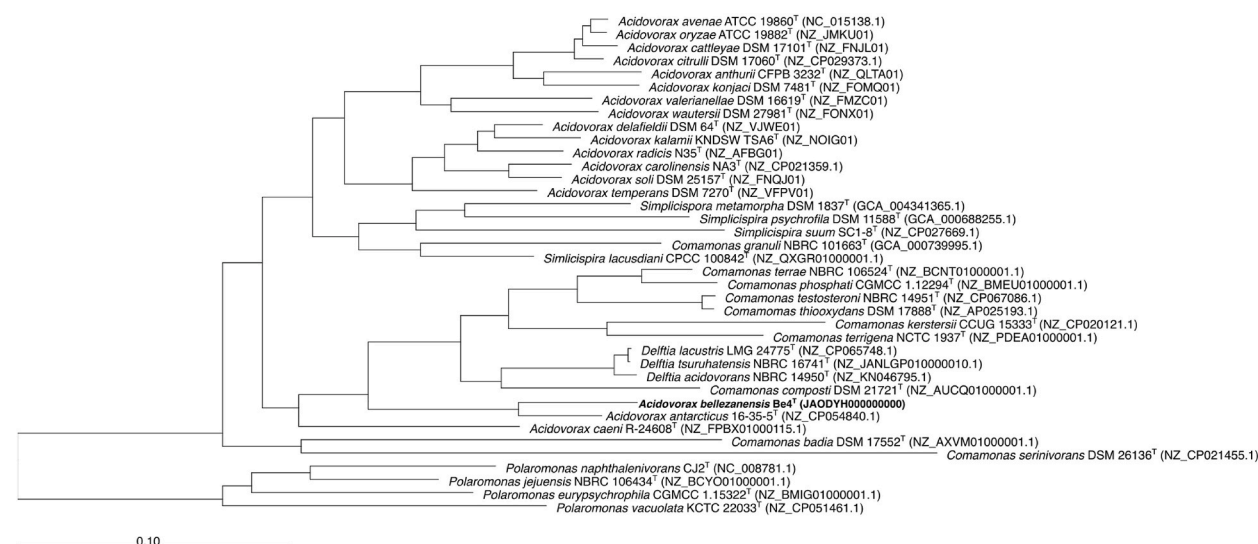

Fig. 3. Phylogenetic tree reconstruction based on neighbor-joining calculation for the 938 single copy core genes shared between the genomes affiliated to *Acidovorax*, *Simplicispira*, *Comamonas*, *Delftia* and *Polaromonas*. Genome sequence accession numbers are given in parentheses. Bar, 0.1 substitutions per amino-acid position.

Table 3
Comparative genomic analysis of *Acidovorax bellezanensis* Be4^T and some closely related species.

Species	Genome Acc.	Contigs	Length (pb)	G + C mol%	N50	Num. CDs
A. bellezanensis Be4^T	JAODYH000000000	43	5,041,667	65.1	599,371	4554
A. avenae	NC_015138	1	5,482,170	68.8		4788
A. caeni	CYIG01	152	4,114,188	63.2	125,181	3872
A. carolinensis	CP0213611	1	4,122,625	64.2		3911
A. delafieldii	LSHP01	150	4,007,395	64.4	47,754	4552
A. defluvii	QLTU0	83	4,114,188	63.2	125,181	3807
A. facilis	DCAIGKB01	66	4,982,623	64.6	190,917	4564
A. radicis	AFBG01	30	5,530,232	64.8	689,800	5055
A. soli	FNQJ01	90	4,130,223	64.6	98,697	3849
A. temperans	JXYQ01	141	4,475,784	62.8	90,180	4309
A. anthurii	QLTA01	130	5,469,042	68.8	78,738	4816
A. cattleyae	CP028290	1	5,618,386	68.9		5117
A. citrulli	CP029373	2	4,899,546	68.8	4,846,466	4412
A. kalamii	NOIG0	31	5,137,998	65.6	489,359	4642
A. konjaci	FOMQ01	36	5,077,528	68.1	369,808	4454
A. oryzae	DJMKU0	69	5,526,777	68.7	212,027	4885
A. valerianellae	DFMZC01	54	5,257,076	65.5	235,924	4707
A. wautersii	DFONX01	55	5,335,586	68.4	282,850	4721
C. badia	DAXVM01	24	3,681,020	65.9	474,156	3490
C. composti	DAUCQ01	55	4,634,066	63.2	251,975	4085
C. granuli	DBBJX01	34	3,505,986	68.5	249,089	3199
C. kerstersii	DCP020121	1	3,547,915	59.6		3250
C. phosphati	DBMEU01	70	4,095,457	65.1	157,100	3779
C. serinivorans	DCP021455	1	4,522,913	67.9		3842
C. terrae	DBCNT0	40	4,716,713	65.7	282,127	4254
C. terrigena	DPDEA01	1	4,710,225	65.0		4143
C. testosteroni	DCP067086	1	5,497,097	61.4		4900
C. thiooxydans	DAP025193	3	5,620,102	61.2	5,588,008	4757
D. acidovorans	DKN046795	1	6,777,958	66.6		5941
D. lacustris	DCP065748	2	7,274,508	66.1	6,904,082	6463
D. tsuruhatensis	DLCZH01	62	5,737,182	66.8	254,996	5944

shaped cells is 1.25–1.31 μm long and 0.70–0.73 μm wide. Colonies on R2A agar are light yellow colored, opaque, circular, convex with smooth margins and 1–2 mm in diameter after incubation on R2A agar plates at 28 °C for 3 days. Growth occurs at 4–37 °C (optimum 15 °C) and between pH 5.5–9.0 (optimum 7.0), and cells cannot tolerate additional NaCl in the culture media. Growth in the absence of oxygen was possible for this strain. Positive for hydrolysis of gelatin and urea, reduction of nitrate but negative for indole production. Also positive for enzymatic activities catalase, oxidase, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucosidase, but negative for lipase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl- β-glucosaminidase, α-mannosidase, arginine dihydrolase, urease, gelatinase and α-fucosidase. Be4^T was positive for fermentation of glycerol, D-ribose, L-xylose, D-mannitol, D-sorbitol and esculin, but negative for the rest of carbohydrate substrates. Assimilation tests for capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid were all negative. The major fatty acids present are $C_{16:0}$, $C_{16:1}$ ω7c/$C_{16:1}$ ω6c, $C_{17:0}$ cyclo and $C_{18:1}$ ω7c. The DNA G + C content of the type strain is 65.15 %.

The type strain Be4^T (=DSM116209^T = CECT30865^T) was isolated from mining porewaters in uranium mill tailings repository sites, located in Bellezane, near Bessines-sur-Gartempe (Limousin, France). The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene and whole genome sequence of the Be4T are HG798880 and JAODYH000000000, respectively.

3.4. Selenium interaction assays

In order to experimentally investigate the bioremediation potential of the studied strain, a series of preliminary interaction studies with Se(IV) were conducted.

Be4^T cells cultivated on solid R2A medium supplemented with Se(IV) exhibited the formation of reddish precipitates characteristic of Se(0) (Fig. 4A–D). This result suggested a bioremediation potential for the strain Be4^T, as it was able to reduce toxic Se from a tetravalent oxidation state (+IV) to a less mobile and less toxic elemental Se (zero-valent oxidation state). The studied strain demonstrated not only its capability to interact with this hazardous compound but also its ability to tolerate high concentrations of it, as indicated by the high minimum inhibitory concentration (MIC) value obtained (8 mM).

The application of high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) enabled the examination of the cellular localization and morphology of the Se reduction products synthesized by Be4 cells. A significant number of electron-dense accumulations, approximately 100 nm in size, were observed (Fig. 5A–C). These accumulations were primarily composed of Se, as indicated by both the energy-dispersive X-ray spectroscopy (EDX) analyses and EDX elemental mapping (Fig. 5D and E). Most of these Se nanostructures exhibited a hexagonal morphology, while some displayed irregular shapes. Concerning their localization, they were observed both intracellularly and extracellularly. The presence of a substantial number of lysed cells suggests
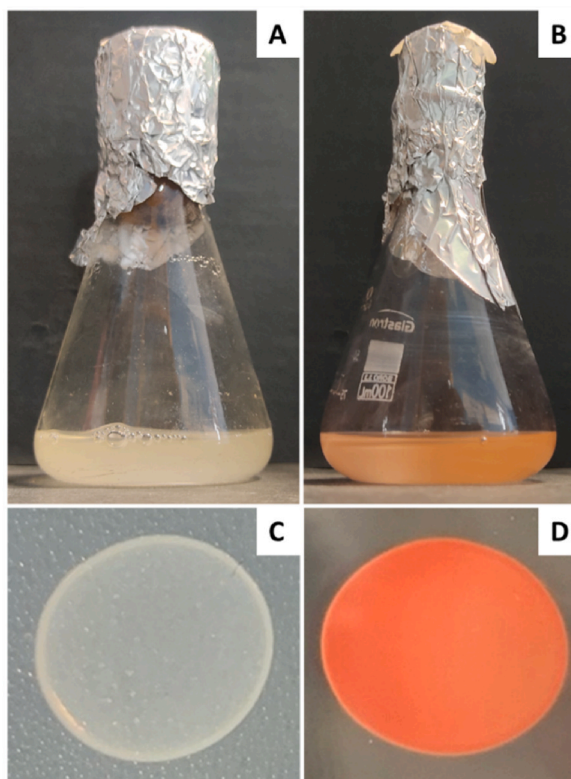
Fig. 4. Cell cultures on liquid and solid R2A medium of the strain *Acidovorax* sp. Be4[T] in the absence (A and C) and presence of Se(IV) (B and D) after incubation at 28 °C.

that the nanostructures are synthesized intracellularly by the bacteria for subsequent release into the extracellular space. However, further studies are needed to determine the exact formation process of these nanostructures.

4. Conclusions

The bacterial strain Be4[T] was demonstrated to be a novel species within the genus *Acidovorax* based on data obtained from phylogenetic, physiological, biochemical, and genomic analyses. The name *Acidovorax bellezanensis* nov. is proposed.

In addition, due to its unique isolation source (anthropized mill tailings), some bioremediation-focused genome-related analyses and laboratory-level tests were performed suggesting that this microbial strain possesses great potential for bioremediation of metals/ metalloids, and particularly of selenium. Moreover, the presence of phosphatase activity in this strain indicates a potential capacity for metal immobilization through biomineralization mechanisms which would result in low solubility minerals formation under oxidizing conditions.

Hence, this strain presents itself as a promising candidate for employment in both *in-situ* and *ex-situ* bioremediation strategies. Moreover, it exhibits the potential for recovering nanoparticles with significant prospects in various industrial and biomedical sectors, aligning with the principles of a sustainable circular economy concept.

Data availability statement

GenBank accession number for the 16S rRNA gene sequence of strain Be4T is HG798880. The Whole Genome of strain Be4T has been deposited at GenBank under the accession number JAODYH000000000.
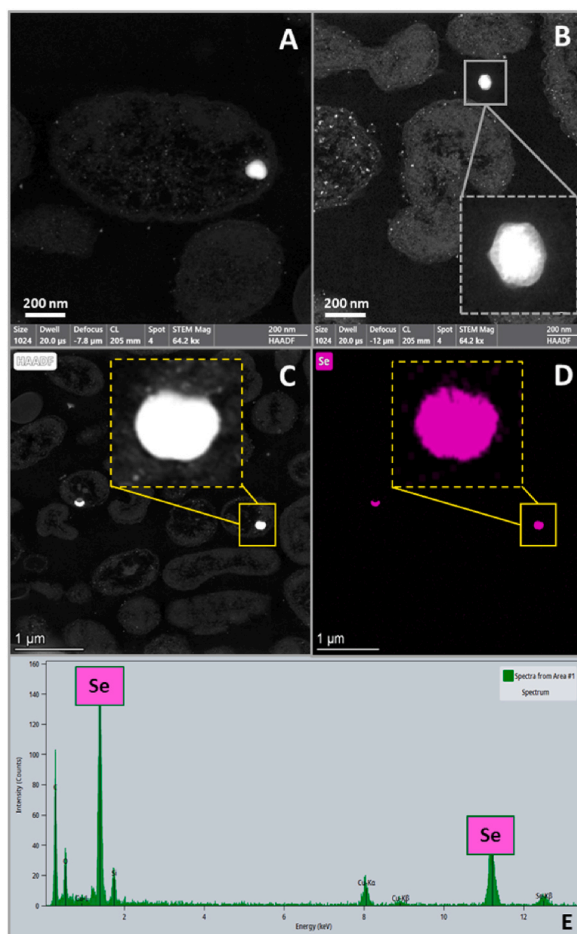
Fig. 5. HAADF-STEM micrographs of Se nanoparticles produced by *Acidovorax* sp. Be4[T] intra- and extracellularly (A–D). EDX-element mapping and spectra derived from a single nanoparticle are displayed in D and E, respectively.

CRediT authorship contribution statement

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33171.

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