

Genomic insights into arsenic and antibiotic resistance in *Comamonas thiooxydans* strains F1-6 and A7-5

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ABSTRACT: Two arsenic-resistant, Gram-negative, aerobic rod-shaped bacterial strains, F1-6 and A7-5, which were isolated from agricultural soil in Thailand, were systematically studied. They grew at 30 °C, pH 5–11, and in 3% (w/v) NaCl. Ubiquinone with eight isoprene units (Q-8) and the cellular fatty acids C_{16:0}, C_{18:1} ω7c, and C_{17:0} CYCLO were the major components. Phylogenetic analysis via 16S rRNA gene sequences revealed that strains F1-6 and A7-5 were affiliated with the genus *Comamonas* and closely related to *C. thiooxydans* DF2^T and *C. testosteroni* KCTC 2990^T, with 99.79% and 99.86% similarity, respectively. The average nucleotide identity and digital DNA-DNA hybridization values between F1-6 and *C. thiooxydans* DF2^T were 97.89% and 85.7%, respectively, whereas those between A7-5 and *C. thiooxydans* DF2^T were 97.23% and 81.3%, respectively. Thus, both the F1-6 and A7-5 were identified as *C. thiooxydans*. The draft genome sizes of F1-6 and A7-5 were 5.2 and 5.3 Mb, comprising 87 and 84 contigs, with DNA G+C contents of 61.5% and 61.4%, respectively. The genomes of both strains contained *ars* cluster genes and many genes for growth and resistance to heavy metals and antibiotics, similar to those of *C. testosteroni* ATCC 11996^T, *C. thiooxydans* CNB-1 substr. CNB-2 Chr, and *C. terrae* NBRC 106524^T. Nevertheless, the *acr3*, *qacG*, and *vanH* genes in the *vanO* cluster were found only in *C. terrae* NBRC 106524^T. This study provides more comprehensive insight into As-resistant bacteria and could be applied to the bioremediation of As and other heavy metals in the future.

KEYWORDS: *Comamonas*, genomic analysis, arsenic resistant, arsenic genes, antibiotic resistance

INTRODUCTION

The genus *Comamonas*, belonging to the family *Comamonadaceae* of the class Betaproteobacteria, was proposed by De Vos et al [1] after a polyphasic study. At the time of writing, the genus *Comamonas* encompasses 28 species with validly published names (<https://lpsn.dsmz.de/genus/comamonas>). *Comamonas* strains have been isolated from natural and polluted environments heavily contaminated by various complex organic compounds and heavy metals, as well as from hospital environments, including clinical samples. Arsenic (As) is a metalloid that occurs naturally in both aquatic and terrestrial environments, often because of the use of pesticides, metal smelters, and mining activities, which release toxins. The high toxicity of arsenic derivatives makes this element a serious public health problem for both humans and animals [2]. The trivalent species As(III), also known as the oxyanion arsenite (AsO₂³⁻), and the pentavalent species As(V), also known as arsenate (AsO₄³⁻), are

the most common chemical forms of arsenic. Arsenite is more toxic than arsenate because of its significant binding affinity for vicinal sulfhydryl groups in proteins. The toxicity of arsenate results from its capacity to compete with phosphate oxyanions for energy and transport activities, with the conversion of arsenate to arsenite being the main harmful consequence [3]. In many developing countries, including Bangladesh and India, arsenic-resistant bacteria such as *Bacillus* sp. and *Aneurinibacillus aneurinilyticus* have been isolated from groundwater contaminated with arsenic [4]. Similarly, *Agrobacterium/Rhizobium*, *Ochrobactrum*, and *Achromobacter* strains have been isolated from arsenic-rich groundwater in West Bengal, India [5]. Certain microorganisms can break down arsenic and participate in its metabolism via several pathways: 1) the arsenic resistance system, 2) arsenic methylation and related pathways, 3) the arsenite oxidation system, and 4) the arsenate reduction system [6]. The arsenic resistance system involves an *ars* operon, a group of genes widely distributed in both bacterial and

archaeal species, which may be regulated under different conditions. Examples include *E. coli*, the enteric pathogen *Yersinia* spp., *Acidiphilium multivorans* AIU 301, *Serratia marcescens*, *Halobacterium* sp. NRC-1, *Sinorhizobium* sp. M14, *Pseudomonas putida* KT2440, *Rhodospseudomonas palustris* CGA009, and *Comamonas testosteroni* CNB-1 [3, 7–10]. Understanding the genes associated with arsenic resistance in these strains is also achievable through genomic data. Currently, several researchers use various bioinformatics tools and technologies to examine genomes, functional genes, and molecular mechanisms. These efforts provide more information about genes associated with bacterial activity.

In Thailand, two identified arsenite-oxidizing *Comamonas* strains, F1-6 and A7-5, were isolated from soils with low arsenic contamination and showed low 16S rRNA gene sequence similarity (98.5% and 98.4%, respectively) [11]. They might be novel species; thus, genome analysis was performed for clear classification. Therefore, the goal of this study was to employ a polyphasic approach to analyze the genetic features of strains F1-6 and A7-5, including the detection of arsenic, heavy metal, and antibiotic resistance genes using bioinformatics technology.

MATERIALS AND METHODS

Phenotypic characterization

Strains F1-6 (KCTC 22605) and A7-5 (KCTC 22607) were phenotypically characterized by Gram staining, flagellar staining, colony appearance, cell morphology, spore formation, and pigmentation on TYEG (tryptone-yeast extract-glucose) agar medium at 30 °C for 24 h [11]. Catalase and oxidase activities, hydrolysis of casein, starch, and Tween 80, nitrate reduction, and deoxyribonuclease (DNase) activities were determined. The VP, indole test, citrate test, urease activity, amino acid decarboxylase (ADH, LDC, and ODC), tryptophan deaminase (TDA), ortho-nitrophenyl- β -D-galactopyranoside (ONPG), assimilation of carbohydrates, and hydrolysis of esculin were determined with commercial kit systems (API 20E, API 20NE, and API 50CH, bioMérieux, France). Growth in 3%, 4%, 4.5%, and 5% (w/v) NaCl at different pH values (3–11) and at different temperatures (4 °C, 30 °C, 37 °C, 40 °C, and 50 °C) was investigated in TYEG broth.

Antimicrobial susceptibility of strains

Antibiotic susceptibility was determined by a disc diffusion assay. The antibiotic discs contained ampicillin (10 μ g), bacitracin (10 U), carbenicillin (100 μ g), cephalothin (30 μ g), clindamycin (2 μ g), erythromycin (15 μ g), gentamicin (10 μ g), imipenem (10 μ g), kanamycin (30 μ g), novobiocin (5 μ g), penicillin (20 U), streptomycin (10 μ g), sulfonamide (300 μ g), tetracycline (30 μ g), tobramycin (10 μ g), and vancomycin (30 μ g). The plates were incubated at 30 °C

for 24 h, and the inhibition zones were measured.

Chemotaxonomy characterization

Ubiquinones (Q) of F1-6 and A7-5 were extracted from freeze-dried cells with chloroform:methanol (2:1) and purified using silica gel TLC (Merck No. 1.05744, Merck KGaA, Germany). The purified quinones were analyzed by HPLC [12]. Total cellular fatty acid analysis of F1-6 and A7-5 cells grown on tryptic soy agar (TSA, Difco™ & BBL™, USA) for 48 h at 30 °C was performed via the standard Microbial Identification System (MIDI Inc., Newark, USA) for automated GC analysis. Polar lipids of strain F1-6 were extracted and analyzed using two-dimensional TLC as described by Minnikin et al [13]. The detection of total lipids, aminolipids, phospholipids, glycolipids, and phosphatidylcholine were conducted using phosphomolybdic acid, ninhydrin, molybdenum blue, anisaldehyde, and Dragendorff's reagents, respectively.

Genotypic characterization

16S rRNA gene sequence

The 16S rRNA gene was amplified, purified, and analyzed as described previously [14]. The derived sequence was aligned with selected sequences from the GenBank/EMBL/DDBJ database using CLUSTAL_X version 1.83. The alignment was manually edited to remove gaps and ambiguous nucleotides prior to constructing the phylogenetic tree. The phylogenetic tree was constructed by the neighbor-joining method [15] in the MEGA program version 11 [16]. The confidence values of the branches of the phylogenetic tree were determined using bootstrap analyses [17] on the basis of 1,000 resamplings.

Genome and gene analyses

The genomic DNA of the arsenite-oxidizing *Comamonas* strains F1-6 and A7-5 was extracted using the GenepHlow™ Gel/PCR Kit (Geneaid Biotech Ltd., Taiwan). Whole-genome sequencing of these strains was performed by a MiSeq sequencer platform (Illumina) with 2 \times 250 bp paired-end reads at the Omics Sciences and Bioinformatics Center, Chulalongkorn University, Thailand. The quality of the raw reads was examined via FASTQC (Babraham Bioinformatics), and adapters and low-quality reads were removed using Trim Galore. The genome assembly was performed by Unicycler [18]. The F1-6 and A7-5 genomes were annotated and predicted via Prokka version 1.13 [19]. A genomic map was constructed by the CGView server [20]. The functional genes of F1-6 and A7-5 were categorized and analyzed by Rapid Annotation of Microbial Genomes using the Subsystem Technology (RAST) server (<http://rast.nmpdr.org/>) and SEED Viewer. Sequence information of coding sequence (CDS) regions of *Comamonas*

strains obtained from the GenBank database was annotated to generate an arsenic gene cluster linear map by DNAPlotter software [21]. The Comprehensive Antibiotic Resistance Database (CARD; <https://card.mcmaster.ca/analyze/rgi>), including BLAST and Resistance Gene Identifier (RGI) software, was used for the prediction of antimicrobial resistance genes. The overall genome-relatedness indices (OGRIs) of F1-6 and A7-5, including average nucleotide identity (ANI), were determined via BLASTn (ANiB) software using the JSpecies online service (<http://imedea.uib-csic.es/jspecies/>) and digital DNA-DNA hybridization (dddH) by the genome-to-genome distance calculator (GGDC 2.1) (<http://ggdc.dsmz.de/distcalc2.php>) with the recommended formula, which was compared with closely related type strains, including *C. thiooxydans* DF2^T, *C. testosteroni* ATCC 11996^T, *C. terrigena* NBRC 13299^T, *C. composti* DSM 21721^T, *C. aquatica* NBRC 14918^T, *C. kerstersii* 12322-1^T, *C. granuli* NBRC 101663^T, *C. jiangduensis* YW1^T, and *C. koreensis* KCTC 12005^T.

RESULTS AND DISCUSSION

Phenotypic characterization

Strains F1-6 and A7-5 were Gram-negative, short rods with polar flagella (Fig. S1). Colonies on TYEG agar were circular, 1–2.5 mm in diameter, with entire margins and no pigment. Colonies of F1-6 were white with a convex elevation, whereas those of A7-5 were colorless with an umbonate elevation. They grew at 30 °C in 3% (w/v) NaCl and at pH 5–11 but did not grow at pH 3 or at 4 °C, 40 °C, or 50 °C in 5% NaCl. These strains exhibited positive reactions for oxidase, catalase, urease, citrate, nitrate reduction, and assimilation of potassium gluconate, adipic acid, and malic acid, but negative reactions for indole, decarboxylation, and hydrolysis of lysine, ornithine, arginine, casein, starch, gelatin, Tween 80, DNase, H₂S production, oxidation of glycerol, potassium 2-ketogluconate, potassium 5-ketogluconate, capric acid, trisodium citrate, and phenylacetic acid. They could not produce acid or assimilate from different sugar/sugar alcohol such as glucose, mannitol, inositol, sorbitol, rhamnose, melibiose, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, dulcitol, inositol, D-mannitol, and D-sorbitol (Table S1). Their differential characteristics and DNA G+C contents of strains F1-6 and A7-5 and related *Comamonas* species are shown in Table S1.

Antimicrobial susceptibility of strains

Strains F1-6 and A7-5, *C. thiooxydans* JCM 1480^T, and *C. testosteroni* KCTC 2990^T were sensitive to carbenicillin, erythromycin, imipenem, kanamycin, sulfonamide, and tobramycin but resistant to ampicillin, cephalothin, clindamycin, penicillin, and streptomycin. Strain F1-6 was sensitive to novobiocin,

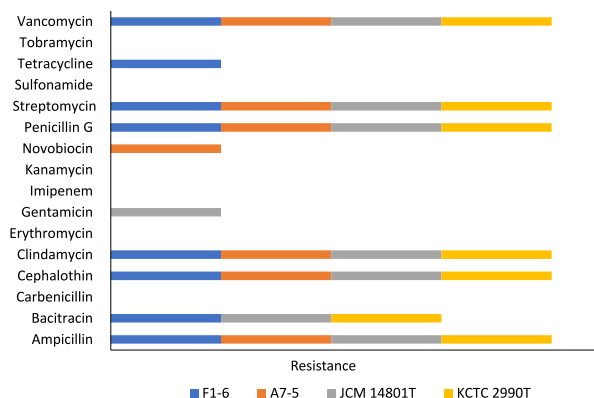


Fig. 1 Antibiotic resistance of *Comamonas* strains to 16 antibiotics.

whereas A7-5 was resistant; however, F1-6 was resistant to bacitracin and tetracycline, whereas A7-5 was inhibited by these antibiotics (Fig. 1). Among the sixteen antibiotics, which can be divided into six major classes, most strains were resistant to antibiotics in the β -lactam, polypeptide, and lincosamide groups but not resistant to antibiotics in the aminoglycoside, sulfonamide, aminocoumarin, macrolide, or tetracycline groups (Table S2). Strains resistant to three or more antibiotics are considered multiantibiotic-resistant strains [22]. The multiantibiotic resistance spectra of F1-6 and A7-5 were resistant to eight and six antibiotics, respectively.

Chemotaxonomy characterization

Strains F1-6 and A7-5 contained the ubiquinones Q-8 (88.1%, 94.7%), Q-9 (8.9%, 3.1%), and Q-7 (3.0%, 2.2%). Their major cellular fatty acids were C_{16:0}, C_{18:1} ω 7c, and C_{17:0} CYCLO, whereas those of *C. kerstersii* LMG 3475^T were C_{16:0}, C_{18:1} ω 9c, C_{10:0} 3-OH, C_{12:0}, and C_{14:0} [23,24]. The profiles of the cellular fatty acids of strains F1-6, A7-5, and related *Comamonas* species are shown in Table S3. According to the results of the chemotaxonomic analysis, the major cellular fatty acids were palmitic acid (C_{16:0}), cyclopropane-substituted methylene-hexadecanoic fatty acid (C_{17:0} CYCLO), and cis-vaccenic acid (C_{18:1} ω 7c), with ubiquinone Q-8 as the major quinone. The polar lipids of strain F1-6 were phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and three unidentified polar lipids (L1–L3) (Fig. S2), as reported in other *Comamonas* species: *C. jiangduensis* KACC 16697^T, *C. humi* JCM 19903^T, *C. fluminis* KACC 22237^T, and *C. endophytica* JCM 35331^T.

Genotypic characterization

A 16S rRNA gene sequence comparison between F1-6, A7-5, and the type strains of *Comamonas* species re-

vealed that these two strains presented the highest 16S rRNA gene sequence similarity with both *C. testosteroni* KCTC 2990^T (ATCC 1196^T) and *C. thiooxydans* DSM 17888^T at 99.79%, whereas A7-5 presented 99.86% similarity. These strains shared sequence similarity with other related strains ranging from 97.86% to 96.39%, including *C. guangdongensis* CY01^T, *C. fluminis* CJ34^T, *C. suwonensis* EJ-4^T, *C. odontotermitis* Dant 3-8^T, *C. terrae* NBRC 106524^T, *C. sediminis* S3^T, and *C. koreensis* KCTC 12005^T. In the 16S rRNA gene sequence-based phylogenetic tree reconstructed using the neighbor-joining method, strains F1-6 (GenBank accession no. GQ497243) and A7-5 (GenBank accession no. GQ497242) were placed in a monophyletic cluster of *Comamonas* species and were closely related to *C. testosteroni* KCTC 2990^T (ATCC 1196^T) and *C. thiooxydans* DSM 17888^T, with a bootstrap value of 100 (Fig. S3). Therefore, to explain their relationship with species of the genus *Comamonas*, a phylogenetic analysis based on the whole genomes of the two strains was carried out.

Genomic features and comparison of whole-genome sequences

To clarify the classification and understanding of the arsenic resistance of strains F1-6 and A7-5, this review begins with an analysis of their whole-genome sequences and the gene systems that respond to arsenic stress. The draft genome size of strain F1-6 was 5.2 Mb with 87 contigs and a G+C content of 61.5%, whereas the draft genome size of A7-5 was 5.3 Mb with 84 contigs and a G+C content of 61.4%. These sizes are similar to those of the genomes of *C. testosteroni* ATCC 11996^T (5.4 Mb) and *C. thiooxydans* DF2^T (5.6 Mb). According to Prokka's predictions and annotations, F1-6 contained 4,809 genes, and A7-5 contained 4,919 genes, with 69 and 67 RNA genes, respectively (Table 1). The phylogenomic tree, which is based on genome data, revealed that F1-6 and A7-5 were closely related to the *C. thiooxydans* DSM 17888^T and *C. testosteroni* strains (Fig. 2). Circular genome mapping for F1-6 and *C. testosteroni* ATCC 11996^T are displayed in Fig. 3, indicating that the genomes of F1-6 and ATCC 11996^T were highly similar in terms of structural features, core gene locations, and important metabolic, reproductive, and survival genes and included genes involved in arsenic resistance. A comparison of the genomes of F1-6 and A7-5 with those of *C. thiooxydans* DF2^T revealed ANI values of 97.89% and 97.23%, respectively, and dDDH values of 85.70% and 81.3%, respectively (Table 1). According to the ANI and dDDH criteria [25], F1-6 and A7-5 were members of the same species as *C. thiooxydans* DF2^T and were identified as *C. thiooxydans*. This finding suggests that the study and comparison of whole-genome sequences using bioinformatics tools have significantly enhanced the classification of these bacteria.

Arsenic resistance genes

According to Chitpirom et al [11], strains F1-6 and A7-5 are resistant to arsenic, which exists in various forms, including As(III), As(V), MMA, DMA, and trimethylarsine oxide, in the natural environment. Bacteria mitigate arsenic toxicity through biochemical degradation, detoxification mechanisms, and redox transformation [26]. The *ars* operon (*arsR*, *arsD*, *arsA*, *arsB*, *arsC*, and *arsH*) encodes an influx/efflux system involved in arsenic resistance mechanisms [27]. Before efflux, arsenate (AsV) is reduced to arsenite (AsIII) by the enzyme encoded by *arsC*, while the *arsB* gene encodes an AsIII efflux pump that exports AsIII from the cell [28]. The *arsH* gene, involved in arsenic methylation and detoxification of organoarsenicals, encodes an NADPH-dependent MAs(III) oxidase that oxidizes trivalent organoarsenical compounds to their pentavalent derivatives [29] and is generally found in Gamma proteobacteria but is absent in Gram-positive bacteria [30]. Several studies indicate that some *Comamonas* strains possess the *ars* operon and are resistant to arsenic. For example, the *ars*(*RPBC*) operon on the pCNB1 plasmid of *C. testosteroni* [10, 31], the arsenite transporter genes *arsB* and *acr3*(2) in *Comamonas* spp. TS32, TS35, TS37, and TS38 from arsenic-contaminated soil [32], and the *arsB* gene in *Comamonas* sp. CHW-7 and MHW-11 from polluted groundwater in Bangladesh [33] have been documented. The arsenic resistance gene clusters of F1-6, A7-5, and various *Comamonas* strains, including closely related strains such as *C. thiooxydans* DF2^T and *C. testosteroni* ATCC 11996^T, and arsenite-oxidizing bacteria such as *C. thiooxydans* CNB-1 substr. CNB-2 Chr and *C. terrae* NBRC 106524^T [31, 34] revealed that F1-6, A7-5, and other *Comamonas* strains contained the *arsB*, *arsC*, and *arsH* genes. These genes are highly similar but located in distinct positions in each genome sequence. Additionally, the *acr3* gene, an arsenite transporter gene for inorganic arsenic efflux [35], was detected in *C. terrae* NBRC 106524^T but not in other strains (Fig. 4). According to Cai et al [32], the *acr3* group is present in microorganisms from high (MIC > 20 mM) and intermediate arsenic-contaminated sites. Consequently, based on the gene analysis findings obtained, it could be ensured that both F1-6 and A7-5 strains are arsenite-oxidizing bacteria, and arsenic resistance genes are widely distributed among *Comamonas* strains, indicating their potential role in reducing arsenic in the environment. The functional genes identified by the RAST server revealed that both F1-6 and A7-5 had 64 RNA genes and 5,084 and 5,173 protein-coding sequences (CDSs), respectively. These include genes related to amino acids, carbohydrates, membrane transport, cofactors, vitamins, pigments, DNA metabolism, fatty acids, lipids, isoprenoids, motility, secondary metabolites, ammonia assimilation, and phosphorus metabolism, which are crucial for growth and reproduction.

Table 1 ANI values (percentages) and digital (*in silico*) DNA-DNA hybridization (dDDH) values between the draft genomes of strains F1-6, A7-5, and *Comamonas* species.

Species	Strain	Accession no.	ANI (%)		dDDH (%)		Genome size (Mb)	DNA G+C content (%)	No. of contigs	RNA genes
			F1-6	A7-5	F1-6	A7-5				
<i>Comamonas</i> sp.	F1-6	JBCPWN000000000	100	97.43	100	80.3	5.2	61.5	87	69
<i>Comamonas</i> sp.	A7-5	JBCPWO000000000	97.43	100	80.3	100	5.3	61.4	84	67
<i>C. thiooxydans</i>	DF2 ^T	AWTP000000000	97.89	97.23	85.7	81.3	5.6	61.0	186	83
<i>C. testosteroni</i>	ATCC 11996 ^T	AHIL01000000	93.75	93.87	55.7	56.7	5.4	61.5	63	73
<i>C. terrigena</i>	NBRC 13299 ^T	AP019749	76.94	76.80	24.0	24.0	4.7	65.0	1	107
<i>C. composti</i>	DSM 21721 ^T	AUCQ000000000	76.84	76.74	22.7	22.8	4.6	63.5	66	74
<i>C. aquatica</i>	NBRC 14918 ^T	BBJR000000000	76.26	76.20	23.4	23.5	3.7	65.0	103	71
<i>C. kerstersii</i>	12322-1	LPXH000000000	75.26	75.10	22.6	22.5	3.0	59.5	41	89
<i>C. granuli</i>	NBRC 101663 ^T	BBJX000000000	75.02	74.84	21.2	21.3	3.5	68.5	34	55
<i>C. jiangduensis</i>	YW1 ^T	JARXOV000000000	74.82	74.67	22.3	22.5	3.6	59.0	67	82
<i>C. koreensis</i>	KCTC 12005 ^T	JAJNCT000000000	74.82	74.69	22.1	22.1	5.1	63.0	53	78

* The number of RNA genes was determined by Prokka.

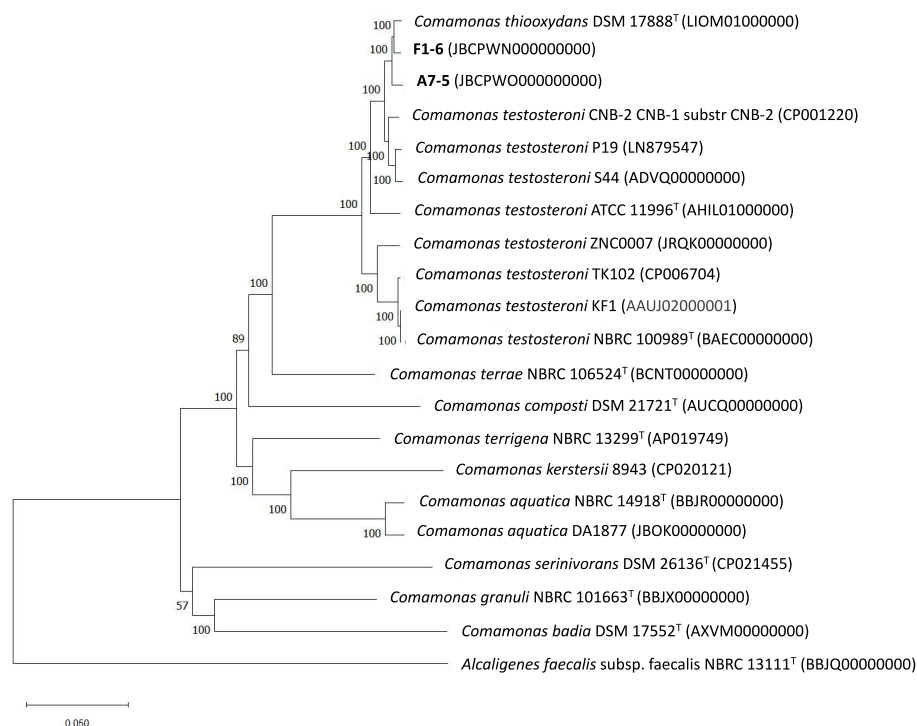


Fig. 2 Phylogenomic tree reconstructed by autoMLST on the basis of conserved core genes indicating the phylogenetic position of strains F1-6, A7-5, and *Comamonas* species. *Alcaligenes faecalis* subsp. *faecalis* NBRC 1311^T (BBJQ00000000) was used as an outgroup. Bootstrap values were expressed as a percentage of 1,000 replications. Bar, 0.050 substitutions per site.

Antibiotic resistance genes

Additionally, we identified antibiotic and toxic compound resistance genes. F1-6 and A7-5 contained 48 and 52 genes, respectively, associated with resistance to Cr compounds, Cu tolerance, Co-Zn-Cd resistance, and multidrug efflux systems (Fig. S4a,c and Fig. S5). These findings correlate with the presence of heavy metal resistance genes such as Co-Zn-Cd resistant protein (*czcA*), copper resistance protein (*copC*), and Co-

Zn-Cd efflux transporters (*czcB* and *czcC*). Antibiotic resistance gene profiles generated from Prokka annotations (Fig. S4b,d) revealed that both F1-6 and A7-5 include genes related to heavy metal resistance similar to those reported in *Comamonas* strains [10, 36, 37]. Additionally, the genomes of F1-6 and A7-5 include genes for bile hydrolysis, such as the *bai* operon, which encodes enzymes that convert primary bile acids into secondary bile acids (Fig. S4). These findings support

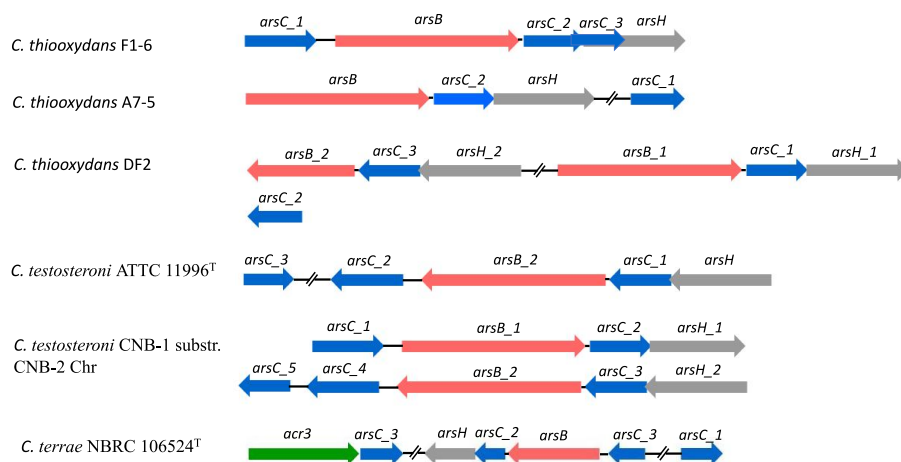


Fig. 4 Gene arrangements of arsenic gene clusters from different *Comamonas* strains. Arrows of different colors represent the following genes: *arsB*, As(III) efflux pump protein; *arsC*, As(V) reductase; *arsH*, putative flavoprotein; *acr3*, putative membrane protein.

tion in arsenic-contaminated soils or in the presence of metal pollutants, and could be beneficial for future bioremediation efforts.

CONCLUSION

In this study, a polyphasic approach was used for the characterization and identification of arsenite-oxidizing strains F1-6 and A7-5. The results revealed that strains F1-6 and A7-5 belong to the same species as *Comamonas thiooxydans*. The draft genome sizes were 5.2 Mb for F1-6 and 5.3 Mb for A7-5. Both genomes contained an *ars* gene cluster, which is functionally associated with arsenic resistance, similar to other *Comamonas* strains. Additionally, heavy metal and multiantibiotic resistance genes were identified in the genomes of F1-6 and A7-5, indicating their ability to survive in diverse environments. The genomic descriptions of *C. thiooxydans* F1-6 and *C. thiooxydans* A7-5 provided in this work will be crucial for guiding their potential applications in the future.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at <https://dx.doi.org/10.2306/scienceasia1513-1874.2026.028>.

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Appendix A. Supplementary data

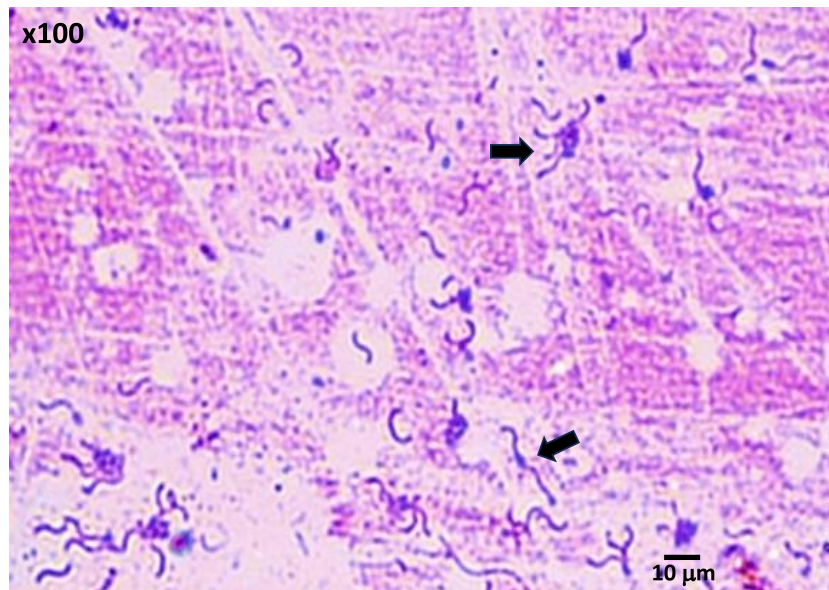


Fig. S1 Photomicrograph of flagella of strain F1-6 grown in NB, at 25 °C for 24 h. Image was taken under light microscopy at 100 × magnification.

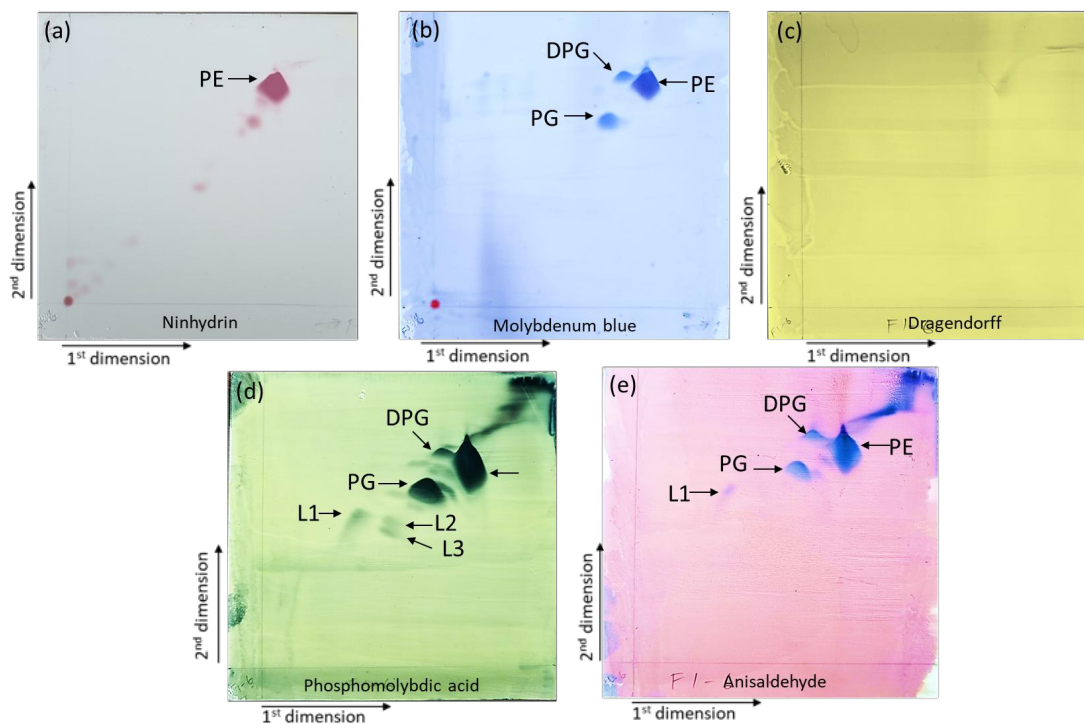


Fig. S2 Polar lipid profiles of strain F1-6 examined by two-dimensional TLC and stained with ninhydrin for amino lipids (a), molybdenum blue for phospholipids (b), dragendorff's reagent for choline-containing phospholipids (c), phosphomolybdic acid for total lipids (d), and anisaldehyde for glycolipids (e). DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; L1, L2, and L3, unidentified polar lipids.

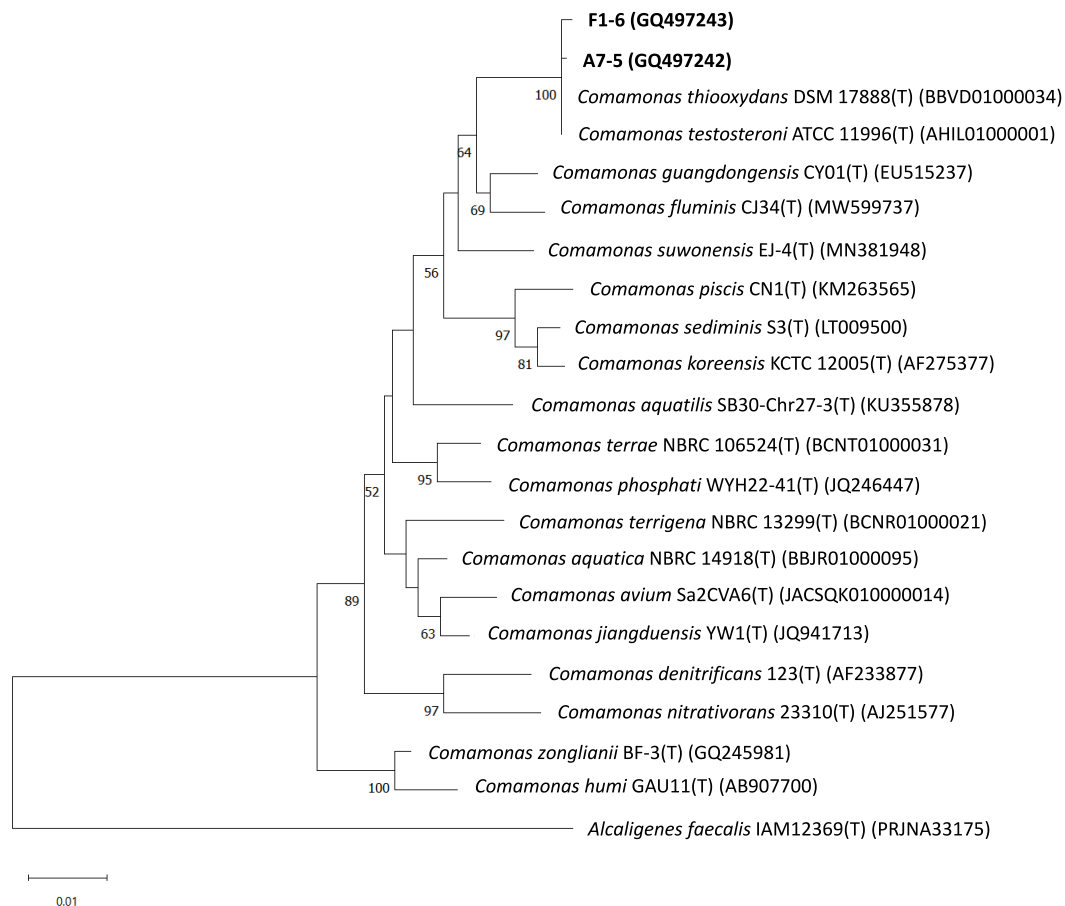


Fig. S3 Neighbor-joining tree of 16S rRNA gene sequences showing the phylogenetic relationships between strain F1-6, *Comamonas* species, and related taxa. Based on 1,000 resamplings, bootstrap percentages above 58% are shown. Bar, 0.01 substitutions per nucleotide position.

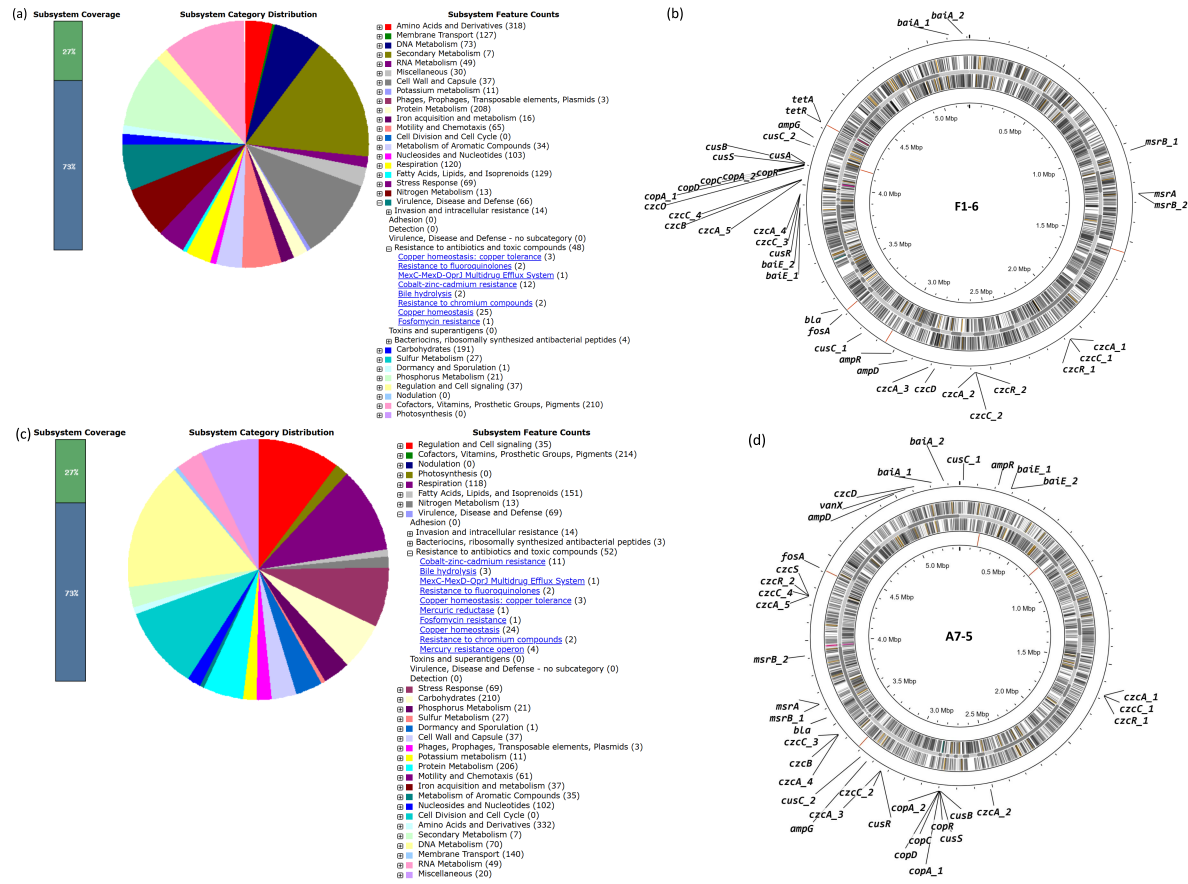


Fig. S4 Overview of the subsystem category distribution of major protein-coding genes predicted in the genomes of strains F1-6 and A7-5. RAST annotation of the whole-genome sequences of strains F1-6 (a) and A7-5 (c). The pie chart shows the counts of genes related to each subsystem. The bar graph (on the left) shows the subsystem coverage, the green bar represents the percentage of proteins that could be annotated in the SEED subsystem (25%), and the blue bar represents the proteins that were not annotated in the SEED subsystem (75%). Circular genomic map of strains F1-6 (b) and A7-5 (d) with antibiotic resistance-related genes: *amp*, *bla*, *msr*, *tet*, *vanX*, and *fosA*; heavy metal resistance-related genes: *czc*, *cus*, and *cop* genes; and bile hydrolysis genes: *baiA* and *baiE*.

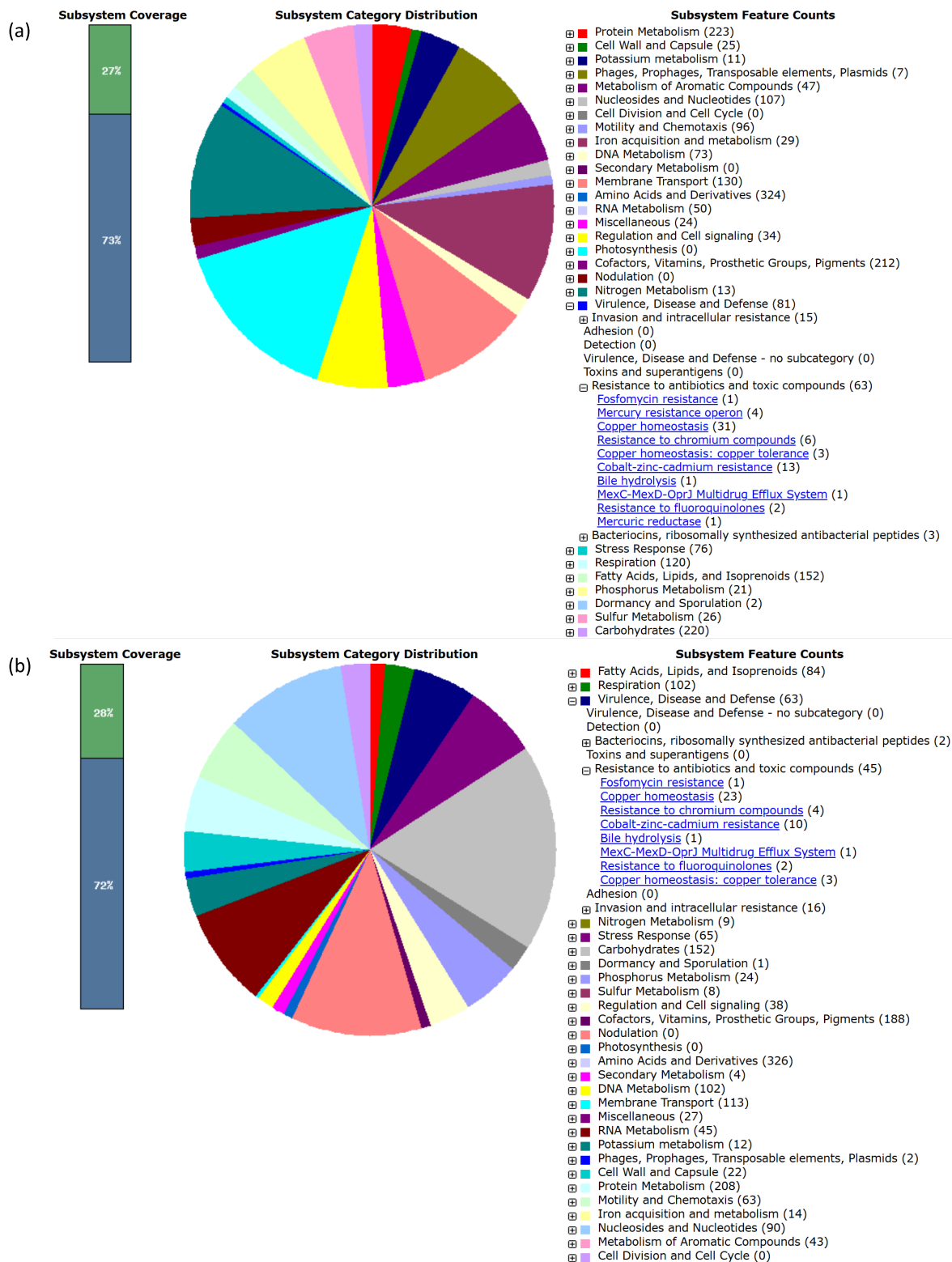


Fig. S5 Overview of subsystem category distribution of major protein-coding genes predicted in the *C. testosteroni* ATCC 11996^T and *C. terrae* NBRC 106524^T genomes. RAST annotation of the whole-genome sequences of strains ATCC 11996^T (a) and NBRC 106524^T (b). The pie chart shows the counts of genes related to each subsystem. The bar graph (on the left) determines the subsystem coverage, the green bar represents the percentage of proteins that could be annotated in the SEED subsystem (25%), and the blue bar represents the proteins that were not annotated in the SEED subsystem (75%).

Table S1 Phenotypic characteristics of strains F1-6, A7-5, and related *Comamonas* species.

Characteristic	F1-6	A7-5	DSM 17888 ^{Tb}	KCTC 2990 ^T
Cell shape	Rd	Rd	Rd	Rd
Flagella	P	P	P	P
Growth at 4 °C	-	-	-	-
Growth at 30 °C	+	+	+	+
Growth at 40 °C	-	-	+	-
Growth at 50 °C	-	-	-	-
Growth at pH 3	-	-	-	+
Growth at pH 5	+	+	+	+
Growth in 3% (w/v) NaCl	+	+	ND	+
Growth in 5% (w/v) NaCl	-	-	ND	-
Catalase	+	+	+	+
Oxidase	+	+	+	+
Urease	+	+	+	-
Nitrate reduction	+	+	+	+
Tween 80 hydrolysis	-	-	+	+
Citrate utilization	+	+	w	+
Hydrolysis of				
Tween 80	-	-	ND	+
Starch	-	-	+	-
Casein	-	-	ND	-
DNA	-	-	ND	-
Gelatin	-	+	-	+
Oxidation of				
Glycerol	-	-	ND	-
Erythritol	-	-	ND	-
D-Arabinose	-	-	ND	-
L-Arabinose	-	-	ND	-
D-Ribose	-	-	ND	-
D-Xylose	-	-	ND	-
L-Xylose	-	-	ND	-
D-Adonitol	-	-	ND	-
Methyl-βD-Xylopyranoside	-	-	ND	-
D-Galactose	-	-	ND	-
D-Glucose	-	-	ND	+w
D-Fructose	-	-	ND	-
D-Mannose	-	-	ND	-
L-Sorbose	-	-	ND	-
L-Rhamnose	-	-	ND	-
Dulcitol	-	-	ND	-
Inositol	-	-	ND	-
D-Mannitol	-	-	ND	-
D-Sorbitol	-	-	ND	-
Methyl-αD-Mannopyranoside	-	-	ND	-
Methyl-αD-Glucopyranoside	-	-	ND	-
N-Acetylglucosamine	-	-	ND	-
Amygdalin	-	+w	ND	-
Arbutin	-	+	ND	+
Esculin ferric citrate	+	+	ND	+
Salicin	-	-	ND	-
D-Cellobiose	-	-	ND	-
D-Maltose	-	-	ND	-
D-Lactose (bovine origin)	-	-	ND	-
D-Melibiose	-	-	ND	-
D-Saccharose (sucrose)	-	-	ND	-
D-Trehalose	-	-	ND	-
Inulin	-	-	ND	-
D-Melezitose	-	-	ND	-
D-Raffinose	-	-	ND	-
Amidon (starch)	-	-	ND	-
Glycogen	-	-	ND	-
Xylitol	-	-	ND	-
Gentiobiose	-	-	ND	-
D-Turanose	-	-	ND	-
D-Lyxose	-	-	ND	-
D-Tagatose	-	-	ND	-

+, positive; w, weakly positive; -, negative; Rd, rods; P, polar flagellum; R, resistant; S, sensitive; NA, no data. ^aaccording to Tamaoka et al [38]; ^bNarayan et al [39] and this study.

Table S1 Continue.

Characteristic	F1-6	A7-5	DSM 17888 ^{Tb}	KCTC 2990 ^T
Oxidation of				
D-Fucose	-	-	ND	-
L-Fucose	-	-	ND	-
D-Arabitol	-	-	ND	-
L-Arabitol	-	-	ND	-
Potassium gluconate	-	-	ND	-
Potassium 2-ketogluconate	-	-	ND	-
Potassium 5-ketogluconate	-	-	ND	-
ONPG	-	+	ND	+
Amino acid decarboxylation of				
ADH	-	-	ND	-
LDC	-	-	ND	-
ODC	-	-	ND	-
Citrate	+	+	+	+
H ₂ S production	-	-	+	-
Urease	+	+	+	+w
Tryptophan deaminase (TDA)	-	-	-	-
Indole	-	-	-	-
VP	+	-	-	-
Acid production from				
Sorbitol	-	-	ND	-
Rhamnose	-	-	ND	-
Sucrose	-	+w	ND	-
Melibiose	-	-	ND	-
Amygdalin	-	+w	ND	-
Arabinose	-	-	ND	-
Assimilation of				
Arbutin	-	+	-	+
Glucose	-	-	+	+
Inositol	-	-	-	-
Mannitol	-	-	-	-
Tagatose	-	-	-	-
Caprate	-	-	-	+
Malate	-	-	-	+
Citrate	+	+	+	w
Gluconate	-	-	-	+
Susceptibility to:				
Ampicillin	R	R	R	R
Bacitracin	R	S	R	R
Cephalothin	R	R	R	R
Novobiocin	S	R	S	S
Penicillin G	R	R	R	R
Streptomycin	R	R	R	R
G+C content (mol %)	62.7	64.5	61.4	62.5 ^a
% Major ubiquinone	Q-8 (88.1)	Q-8 (94.7)		Q-8 (>90) ^a

+, positive; w, weakly positive; -, negative; Rd, rods; P, polar flagellum; R, resistant; S, sensitive; NA, no data. ^a according to Tamaoka et al [38]; ^b Narayan et al [39] and this study.

Table S2 Zone diameter (mm)^a of antimicrobial disk susceptibility test for *Comamonas* species.

Antibiotic	Concentration	F1-6	A7-5	JCM 14801 ^T	KCTC 2990 ^T
β-Lactam					
Ampicillin	10 µg	0	0	0	0
Carbenicillin	100 µg	9.5 (±0.71)	15.5 (±0.71)	19.5 (±0.71)	29.5 (±0.71)
Cephalothin	30 µg	0	0	0	0
Imipenem	10 µg	34 (±1.41)	33 (±1.41)	30.5 (±0.71)	32.5 (±0.71)
Penicillin G	20 U	0	0	0	0
Aminoglycoside					
Gentamicin	10 µg	9.5 (±0.71)	9.5 (±0.71)	0	8.5 (±0.71)
Kanamycin	30 µg	19 (±1.41)	17 (±1.41)	14.5 (±0.71)	21 (±1.41)
Streptomycin	10 µg	0	0	0	0
Tobramycin	10 µg	13.5 (±0.71)	14 (±1.41)	7.5 (±0.71)	11.5 (±0.71)
Polypeptide					
Bacitracin	10 U	0	8.5 (±0.71)	0	0
Vancomycin	30 µg	0	0	0	0
Aminocoumarin					
Novobiocin	5 µg	10.5 (±0.71)	0	9.5 (±0.71)	11.5 (±0.71)
Lincosamide					
Clindamycin	2 µg	0	0	0	0
Macrolide					
Erythromycin	15 µg	16.5 (±0.71)	13 (±1.41)	10.5 (±0.71)	10.5 (±0.71)
Sulfonamide					
Sulfamonomethoxime	300 µg	31 (±1.41)	27 (±1.41)	27 (±1.41)	30.5 (±0.71)
Tetracycline					
Tetracycline	30 µg	0	28 (±1.41)	25.5 (±0.71)	26 (±1.41)

^a Values are expressed as the means of two determinations.

Table S3 Cellular fatty acid composition of strains F1-6, A7-5, and related *Comamonas* species. Strains: 1, F1-6; 2, A7-5; 3, *C. thiooxydans* S23^T [40]; 4, *C. testosteroni* KCTC 2990^T.

Fatty acid	1	2	3	4
C _{12:0}	3.3	3.2	4.5	2.4
C _{14:0}	0.5	tr	tr	tr
C _{16:0}	28.1	30.7	25.7	25.38
Saturated branched-chain				
C _{17:0} CYCLO	27.6	22.4	3.4	0.98
Unsaturated branched-chain				
C _{19:0} CYCLO ω8 ^c	2.7	0.7	ND	tr
Hydroxylated fatty acid				
C _{10:0} 3-OH	4.9	4.3	5.8	4.5
C _{14:0} 2-OH	0.7	0.8	tr	0.5
C _{16:0} 2-OH	3.4	3.2	4.6	3.22
C _{16:1} 2-OH	1.6	1.7	1.8	1.25
Summed feature 3 ^a	14.4	25.8	35.5	44.82
Summed feature 8 ^b	19.2	18.8	15.5	15.58

^aC_{16:1} ω7c/C_{16:1} ω6c; ^bC_{18:1} ω7c/C_{18:1} ω6c; tr, trace (< 0.5%); ND, not detected.

Table S4 Results of antimicrobial resistance gene detection in strains F1-6, A7-5, *C. thiooxydans* DF2^T, *C. testosteroni* ATCC 11996^T, *C. terrae* NBRC 106524^T, and *C. thiooxydans* CNB-1 substr. CNB-2 Chr using the CARD database.

Resistance gene *	Strain	Antimicrobial resistance (AMR) gene family	Drug class mechanism	Resistance
<i>FosA8</i>	F1-6, A7-5, ATCC 11996 ^T , NBRC 106524 ^T	Fosfomycin thiol transferase	Phosphonic acid inactivation	Antibiotic
<i>tet(A)</i>	F1-6	Major facilitator superfamily (MFS) antibiotic efflux pump	Tetracycline	Antibiotic efflux
<i>adeF</i>	F1-6, A7-5, DF2 ^T , ATCC 11996 ^T , NBRC 106524 ^T , CNB-1 substr. CNB-2 Chr	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Fluoroquinolone antibiotic, tetracycline	Antibiotic efflux
<i>qacJ</i>	F1-6, A7-5, DF2 ^T , ATCC 11996 ^T , CNB-1 substr. CNB-2 Chr	Small multidrug resistance (SMR) antibiotic efflux pump	Disinfecting agent and antiseptic	Antibiotic efflux
<i>qacG</i>	NBRC 106524 ^T	Small multidrug resistance (SMR) antibiotic efflux pump	Disinfecting agent and antiseptic	Antibiotic efflux
<i>vanH</i> gene in <i>vanO</i> cluster	NBRC 106524 ^T	<i>vanH</i> , Glycopeptide resistance gene cluster	Glycopeptide antibiotic target alteration	Antibiotic

* Strict, complete genes from only the CARD database.