Rugosibacter aromaticivorans gen. nov., sp. nov., a bacterium within the family *Rhodocyclaceae*, isolated from contaminated soil, capable of degrading aromatic compounds

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Abstract

A bacterial strain designated Ca6^T was isolated from polycyclic aromatic hydrocarbon (PAH)-contaminated soil from the site of a former manufactured gas plant in Charlotte, NC, USA, and linked phylogenetically to the family *Rhodocyclaceae* of the class *Betaproteobacteria*. Its 16S rRNA gene sequence was highly similar to globally distributed environmental sequences, including those previously designated 'Pyrene Group 1' demonstrated to grow on the PAHs phenanthrene and pyrene by stable-isotope probing. The most closely related described relative was *Sulfuritalea hydrogenivorans* strain sk43H^T (93.6 % 16S rRNA gene sequence identity). In addition to a limited number of organic acids, Ca6^T was capable of growth on the monoaromatic compounds benzene and toluene, and the azaarene carbazole, as sole sources of carbon and energy. Growth on the PAHs phenanthrene and pyrene was also confirmed. Optimal growth was observed aerobically under mesophilic temperature, neutral pH and low salinity conditions. Major fatty acids present included summed feature 3 (C$_{16:1}\omega 7c$ or C$_{16:1}\omega 6c$) and C$_{16:0}$. The DNA G+C content of the single chromosome was 55.14 mol% as determined by complete genome sequencing. Due to its distinct genetic and physiological properties, strain Ca6^T is proposed as a member of a novel genus and species within the family *Rhodocyclaceae*, for which the name *Rugosibacter aromaticivorans* gen. nov., sp. nov. is proposed. The type strain of the species is Ca6^T (=ATCC TSD-59^T=DSM 103039^T).

In a prior experiment that used stable-isotope probing (SIP) to reveal bacteria involved in the removal of the polycyclic aromatic hydrocarbon (PAH) pyrene in an aerobic, slurry-phase bioreactor treating contaminated soil from a former manufactured gas plant (MGP) in Charlotte, NC, USA, we identified three distinct groups of 16S rRNA genes derived from uncharacterized Proteobacteria. One of these groups was a monophyletic cluster associated with the family *Rhodocyclaceae* of the class *Betaproteobacteria* designated 'Pyrene Group 1' (PG1) [1]. Subsequent culture-independent experiments suggested that members of PG1 could also degrade the three-ring PAH phenanthrene [2]. A metagenome constructed from a mixed culture dominated by members of PG1 contained multiple genes for PAH degradation, some of which were heterologously expressed in *Escherichia coli* and their products shown to transform multiple PAHs [3].

16S rRNA gene sequences highly similar to those designated as PG1 have since been recovered from a variety of environments, suggesting widespread distribution of organisms associated with the clade in both water and soil. DNA sequences highly similar to both the 16S rRNA gene and functional genes associated with PG1 have also been detected in geographically distant soils contaminated by PAHs [4, 5]. Other PG1 16S rRNA gene sequences have appeared in samples contaminated with trichloroethylene [6], copper [7] and asphalts [8], and from such diverse locations as oilfields [9] to pristine water environments [10, 11]. In our own laboratory, PG1 sequences were among the most abundant in the bacterial community of a contaminated soil recovering from chemical oxidation as part of a remediation scheme [12]. In each of these instances, however, no isolates were obtained to represent the group.

The most closely related characterized isolate to organisms represented by SIP- and environmentally-derived PG1 sequences is *Sulfuritalea hydrogenivorans* strain sk43H^T, a facultatively autotrophic freshwater bacterium [13]. Cultivation efforts in our lab using a modified medium derived from the aerobic growth medium of strain sk43H^T resulted in the successful isolation of a strain from the bioreactor-

treated soil from the MGP site in Charlotte, NC, representing PG1 and designated Ca6^T. The modified sk43H^T isolation medium used to isolate strain Ca6^T originally contained 5 mM sodium-potassium phosphate buffer (pH 7.0) and 5 mM NH_4NO_3 (collectively referred to as 'reactor buffer'), 1 mM $MgSO_4 . 7H_2O$, 1 mM $CaCl_2 . 2 H_2O$, 1 ml trace element solution l^{-1} [containing per litre; 12.5 ml HCl (25 %), 2.1g $FeSO_4 . 7 H_2O$, 30 mg H_3BO_3, 100 mg $MnCl_2 . 4 H_2O$, 190 mg $CoCl_2 . 6 H_2O$, 24 mg $NaCl_2 . 6 H_2O$, 2 mg $CuCl_2 . 2H_2O$, 144 mg $ZnSO_4 . 7H_2O$, 36 mg $Na_2MoO_4 . 2 H_2O$] and 1 ml selenium-tungsten solution l^{-1} (containing per litre; 0.4 g NaOH, 6 mg $Na_2SeO_3 . 5H_2O$, 8 mg $Na_2WO_4 . 2H_2O$). After autoclaving for 15 min at 250 $^\circ$C, the following filter-sterilized solutions (0.2 µm pore size) were added aseptically to the cooled media (per l): 1 ml vitamin solution (containing 4 mg *p*-aminobenzoic acid, 1 mg biotin, 10 mg nicotinic acid, 5 mg calcium pantothenate, 15 mg pyridoxine hydrochloride, in 100 ml of a 10 mM phosphate buffer, pH 7.1), 1 ml thiamine solution (containing 10 mg thiamine in 100 ml of 25 mM sodium phosphate buffer, pH 3.4), 1 ml vitamin B12 solution (containing 5 mg cyanocobalamin in 100 ml distilled water) and 1.5 ml thiosulfate solution (24.8 g $Na_2S_2O_3 . 5H_2O$ in 100 ml distilled water, stored under N_2 gas). Carbon was added before autoclaving as either 0.02 % pyrene dissolved in acetone to the flask, allowing the solvent to evaporate prior to adding other components, or as 0.2 % sodium pyruvate. Subsequent tests of strain Ca6^T grown on the isolation medium excluding individual components indicated that only the reactor buffer, $MgSO_4$, trace element solution and a carbon source were required for growth at a rate equivalent to that of the complete medium. We observed that growth in the liquid medium was slower and to a lower cell density in the absence of amended $MgSO_4$, and that no growth at all occurred in the absence of the trace element solution. All subsequent tests used a medium containing only the required components: reactor buffer, $MgSO_4$ and trace element solution (referred to as sRB1 medium, 'supplemented reactor buffer') with sodium pyruvate as a carbon source unless otherwise noted. For solid media ('sRB1-agar'), 1.5 % agar (Acros Organics) was added to liquid medium prior to autoclaving.

The optimal growth conditions of strain Ca6^T in sRB1 medium with pyruvate as a carbon source were determined for a range of temperatures, pH and salinities. Optimal growth was defined as the maximum growth rate during the exponential growth phase as measured by turbidity at OD_{600} with a HACH DR3000 spectrophotometer. Optimal temperature for growth was determined in triplicate 5 ml cultures in liquid sRB1 medium at pH 7.0 and 225 r.p.m. for temperatures of 23, 26, 28, 30, 32, 34, 35, 36 and 37 $^\circ$C. Additional temperatures were evaluated on solid sRB1 plates at 20 and 4 $^\circ$C. The effect of pH was tested in triplicate 5 ml tubes of sRB1 medium buffered to pH values of pH 5.0, 5.5, 6.0, 6.5 [buffered with 2-(*N*-morpholino)ethanesulfonic acid, 50 mM], 7.0, 7.5, 8.0 [4-(2-hydroxyethyl)−1-piperazineethanesulfonic acid, 50 mM] and 8.5, 9.0 [Tris/

HCl, 50 mM] and incubated at 225 r.p.m. and 30 $^\circ$C. Salinity effects were determined using triplicate 5 ml cultures of sRB1, pH 7.0, incubated at 30 $^\circ$C and 225 r.p.m., amended with either 0, 0.25, 0.5, 1, 2, 3, 4 or 5 % NaCl (w/v). The optimal temperature for growth was between 30 and 34 $^\circ$C, with obvious growth between 20 and 35 $^\circ$C. Strain Ca6^T grew between pH 6.5 and 7.5 with an optimum of pH 6.5. No growth was observed at salt concentrations >0.5 %, but strain Ca6^T grew equally well with either 0.25 % or no NaCl. Under optimal conditions in sRB1-pyruvate liquid media with constant shaking at 225 r.p.m., strain Ca6^T grew on pyruvate to maximum turbidity in approximately 3 days. Colonies on sRB1-agar plates with pyruvate as a carbon source developed over a period of 2 weeks when incubated at 30 $^\circ$C. Strain Ca6^T colonies on sRB1 plates with pyruvate were generally small (0.5–0.8 mm in diameter), circular, with a convex elevation and were pale yellow–white in colour.

The ability of Ca6^T to grow under low oxygen conditions was assessed using the GasPak 100 system containing a Gas-Pak EZ Anaerobe Container System sachet (BD Biosciences). Plates comprising sRB1-agar and pyruvate were inoculated and sealed in the system according to the manufacturer's directions. According to the manufacturer, the system achieves an atmosphere of <1 % oxygen and ≥13 % carbon dioxide within 2.5 h. Low-oxygen conditions were confirmed after 48 h by visual observation of an indicator strip soaked in 1 mM resazurin solution. Plates were incubated at 30 $^\circ$C and compared with concurrent aerobic controls. Ca6^T did not display growth under low oxygen conditions after 4 weeks. Unless otherwise noted, all subsequent tests of Ca6^T occurred at 30 $^\circ$C, pH 7.0 and 0 % salinity under aerobic conditions. Liquid cultures were additionally incubated with constant shaking at 225 r.p.m.

The cellular morphology of strain Ca6^T was investigated using transmission and scanning electron microscopy (TEM, SEM). For the preparation of samples, cells were grown in sRB1 with either pyrene or pyruvate. Specimens were observed and images taken using a Zeiss Supra 25 FESEM operating at 5 kV, 5 mm working distance and 10 µm aperture (Carl Zeiss SMT) or a LEO EM910 transmission electron microscope operating at 80kV (Carl Zeiss Microscopy). Meancell sizes were determined from SEM digital micrographs of well-distinguished cells. The cellular morphology of strain Ca6^T was rod-shaped with a mean size of 0.96±0.19 µm by 0.25±0.02 µm (*n*=100 cells). Many cells were observed to be curved (Fig. 1), and in such instances length was measured using the linear distance from end-to-end of the cell. The outer membrane appeared wrinkled and uneven, and potential outer membrane vesicles were evident on some (but not all) cells examined after growth on either pyrene or pyruvate as a carbon source (Fig. 1c, d). Cells grown on pyruvate appeared more often individually or in pairs or small clusters, while cells grown on pyrene were generally densely clumped, presumably around pyrene crystals, with larger amounts of extracellular material
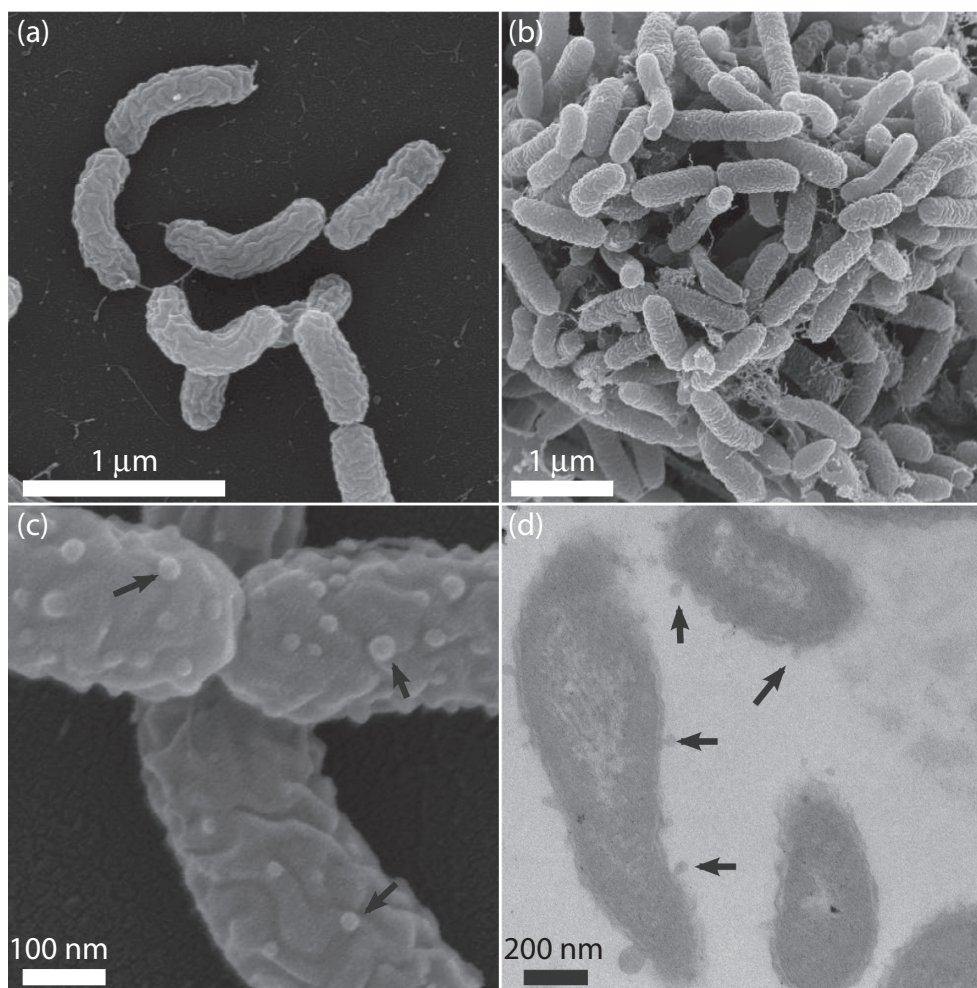
Fig. 1. Scanning electron micrographs (a, b, c) and transmission electron micrograph (d) of strain Ca6^T grown on pyruvate (a, c) or pyrene (b, d). Bars, 1 µm (a, b); 100 nm (c); 200 nm (d). Arrows indicate some potential outer membrane vesicles in the micrographs (c, d).

apparent (Fig. 1a, b). Electron-transparent inclusions were evident in some pyrene-grown cells examined by TEM, which were absent in cells grown on pyruvate (not shown). Gram staining performed using the standard reaction and visualized by light microscopy indicated strain Ca6^T was Gram-type-negative.

Cellular motility was tested using sRB1-agar stab-tubes with agar added at 0.3 % (w/v) and pyruvate as the carbon source. No outward motility from the stab was observed. No flagella were observed during microscopy, and few genes for flagellar synthesis were indicated in the annotated genome [14].

Metabolism of a variety of carbon substrates was tested using the Biolog GN2 Microplate. For each plate, cells were grown in liquid sRB1 medium amended with pyruvate and washed three times in PBS (pH 7.5) before being resuspended in Biolog GN/GP Inoculating Fluid as per the manufacturer's instructions. The microplates were incubated overnight at

30 °C and scored through visual examination in comparison with the no-carbon control. In triplicate samples, the only substrates that strain Ca6^T actively metabolized were formic acid, methyl pyruvate, monomethyl succinate and β-hydroxybutyric acid. The other 91 substrates, including a variety of sugars, other organic acids, nucleosides and amino acids were not metabolized.

Utilization of nitrogen sources was tested by substituting either 5 mM KNO$_3$ or NH$_4$Cl for NH$_4$NO$_3$ in sRB1 liquid media. Strain Ca6^T displayed growth on pyruvate with both nitrogen sources tested. Nitrate reduction was evaluated using a culture grown in sRB1 media with NH$_4$NO$_3$ substituted with 5 mM KNO$_3.$ No nitrate reduction to nitrite beyond what was required for assimilation and growth was observed. Starch hydrolysis and cellulase activity were evaluated by supplementing sRB1 plates with either starch (10 g l^{-1}) or cellulose (1 g l^{-1}) (adapted from Kasana *et al.* [15]). Inoculated plates were incubated for 2 weeks at 30 °C, after

which Gram's iodine was used to identify clear zones around colonies. Protease activity was tested by adding a skimmed milk solution aseptically (10 % skimmed milk powder dissolved in distilled water) to sRB1-agar after autoclaving. Plates were incubated at 30 °C for 1 month and monitored for zones of clearing. Gelatin hydrolysis was assessed using a nutrient gelatin stab method wherein powdered gelatin (120 g l^{-1}) was added to sRB1, gently heated to dissolve and aliquoted into 5 ml tubes. Gelatin tubes containing Ca6^T were incubated at 30 °C and monitored for liquefaction of the media. Ca6^T was negative for starch hydrolysis, cellulase, skimmed milk protease and gelatinase activities.

Lipase activity was assessed by adding tributyrin (98 %; Acros Organics) to sRB1-agar (1 %, v/v). Urease activity was determined using Stuart's urea broth, which was prepared by amending phosphate buffer with urea (20 g l^{-1}) and phenol red (10 mg l^{-1}) in addition to pyruvate, MgSO$_4$ and trace element solution. Urea tubes were monitored for 24 h for a colour change indicating urease production. Strain Ca6^T was positive for lipase activity and negative for urease activity.

Strain Ca6^T was tested for catalase activity by adding a 3 % hydrogen peroxide solution (v/v) to cells freshly scraped from the surface of an sRB1 plate. Oxidase activity was determined by adding a few drops of freshly-prepared 1 % N',N',N',N'-tetramethyl-p-phenylenediamine dihydrochloride (Acros Organics) to cells scraped from the same plate onto filter paper. Strain Ca6^T was catalase-negative and oxidase-positive.

Indole production was tested by supplementing sRB1 media with tryptone (10 g l^{-1}). After growth to turbidity, a few drops of Kovac's reagent were added to each tube. The lack of a colour change indicated no production of indole by strain Ca6^T. As indigo production from indole is indicative of activity by some ring-hydroxylating dioxygenases (RHD), enzymes involved in the initial step in the aerobic degradation of PAHs by bacteria [16, 17], the lack of indole production was anticipated. To further investigate this phenotype, indole was added as a small crystal to the lid of inverted Petri plates of sRB1-pyruvate medium 24 h after inoculation and incubated. A blue/purple colour developed by colonies on the plate due to indigo production confirmed the likely presence of at least one active RHD.

Growth of strain Ca6^T on select PAHs as sole sources of carbon and energy was tested in liquid sRB1 media amended with individual PAHs (final concentration 0.2 g l^{-1}). PAHs were added to tubes in a solvent (either acetone or dichloromethane), and the solvent was allowed to evaporate prior to adding the remaining media components. Tubes were additionally sonicated after autoclaving to help break up PAH crystals. Ca6^T cells were grown in sRB1-pyruvate and washed three times with reactor buffer prior to inoculation, and triplicate PAH-containing tubes were incubated at 30 °C at 225 r.p.m. for up to 19 days. As spectrophotometric

measurements of turbidity were not possible due to the presence of PAH crystals in the media, growth of Ca6^T on PAHs was defined by both protein accumulation and disappearance of the parent compound. Protein concentrations were calculated from aliquots of culture approximately every 48 h using a Pierce BCA Protein Assay according to manufacturer's instructions (ThermoFisher Scientific). Disappearance of each PAH was determined using a liquid-liquid extraction with an equal volume of n-hexanes and quantification using a HPLC system with fluorescence detection as previously described [12]. Extraction efficiency was determined from uninoculated tubes with PAH added. For all substrates except naphthalene, this method was able to quantify PAH concentration; naphthalene presumably volatilized from the tubes during either the incubation or extraction. Removal of pyrene (47 % of the initial mass) and phenanthrene (48 %) by Ca6^T was correlated with protein accumulation, indicating growth on those substrates. Incubation with benz[*a*]anthracene resulted in modest protein accumulation and removal of the PAH from the medium compared with phenanthrene and pyrene, and the protein concentration did not display a steady increase typical of exponential growth. This suggests that Ca6^T may be capable of weakly positive growth on this compound. Incubation with fluorene and anthracene resulted in PAH removal without protein accumulation, suggesting transformation but not growth on these substrates. Ca6^T was negative for growth on or transformation of chrysene, benzo[*a*]pyrene, fluoranthene, naphthalene and acenaphthene. Growth on the azaarene carbazole was determined by visual turbidity and protein accumulation over the course of the incubation, as the HPLC assay employed for quantifying PAHs was not suitable for this compound. Strain Ca6^T was positive for growth on carbazole.

Mineralization of the partially ^{14}C-labelled PAHs phenanthrene, fluoranthene, chrysene, benz[*a*]anthracene and benzo[*a*]pyrene was performed as previously described [18]. Ca6^T cells grown on sRB1-pyrene and washed three times with reactor buffer were used to inoculate the flasks. The extent of mineralization was measured after 24 and 48 h. Mineralization was assessed as a percentage of added PAH mineralized, and biotic samples were compared with acidified killed control replicates. Statistically significant mineralization occurred for phenanthrene (29 % mineralization, $P<0.01$) and chrysene (19 %, $P<0.05$), but did not occur for fluoranthene, benzo[*a*]pyrene or benz[*a*]anthracene. Differential results in PAH removal and mineralization for the PAHs chrysene and benz[*a*]anthracene may be attributable to several factors including growth of the inocula on aromatic versus non-aromatic carbon sources (potentially affecting gene expression), the low concentration of the ^{14}C-labelled compounds and the length of the incubations.

Growth on BTEX compounds (benzene, toluene, ethylbenzene and xylene) was investigated by creating an atmosphere in separate, tightly-sealed metal containers of either benzene (99 %), toluene (99.8 %), ethylbenzene (99.8 %) or a

mixture of xylenes (o-xylene 98.5 %, m- 99 %, p- 99 %, in equal volumes) by adding 0.5 ml of the chemical(s) to a piece of filter paper in a glass beaker. Inoculated sRB1-agar plates without additional carbon were incubated in these containers at room temperature for 1 month. Strain Ca6^T grew well on benzene, weakly on toluene, but did not grow on either ethylbenzene or mixed xylenes. Inoculated plates of sRB1-agar without amended carbon in normal atmosphere showed no growth over the same time period.

Cellular fatty acid profiling was performed using the MIDI Sherlock Microbial Identification System by Microbial ID on cells grown on sRB1 plates with pyruvate. The dominant fatty acids in Ca6^T were summed feature 3 ($C_{16:1}\omega7c$ or $C_{16:1}\omega6c$) (42.5 %) and $C_{16:0}$ (36.7 %). Ca6^T also contained $C_{17:0}$ cyclo (6.5 %), $C_{18:1}\omega7c$ (4.9 %), $C_{10:0}$ 3-OH (3.9 %) and $C_{12:0}$ (3.0 %). Trace amounts (<1 % each) of $C_{10:0}$, $C_{14:0}$, $C_{16:1}\omega5c$, $C_{17:1}\omega7c$, $C_{18:2}\omega6,9c$, $C_{18:0}$, $C_{19:0}$ cyclo $\omega8c$ and $C_{19:0}$ were also detected. Analysis of respiratory quinones and polar lipids was carried out by the Identification Service, DSMZ, Braunschweig, Germany. Major polar lipids in Ca6^T were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phospholipid, with minor amounts of aminolipid, glycolipid and lipid present (Fig. S1, available in the online Supplementary Material). Respiratory quinones present were Q7 (9 %), Q8 (80 %) and Q9 (11 %).

The genome of strain Ca6^T was determined as described previously [14] and was composed of a singular, circular chromosome of 2 934 611 bp with a DNA G+C content of 55.14 mol%. No plasmids were detected. The two copies of the 16S rRNA gene detected were identical, and the closest described relatives of Ca6^T were *Sulfuritalea hydrogenivorans* strain sk43H^T (93.6 % 16S rRNA gene similarity) [13], *Denitratisoma* sp. TSA61 (93.2 %) [19], *Georgfuchsia toluolica* G5G6^T (92.0 %) [20], *Sulfurisoma sediminicola* BSN1^T (91.8 %) [21], *Denitratisoma oestradiolicum* AcBE2-1^T (91.6 %) [22] and *Sterolibacterium denitrificans* ChoI-1S^T (91.5 %) [23]; these organisms are all members of genera within the betaproteobacterial family *Rhodocyclaceae* (Fig. 2).
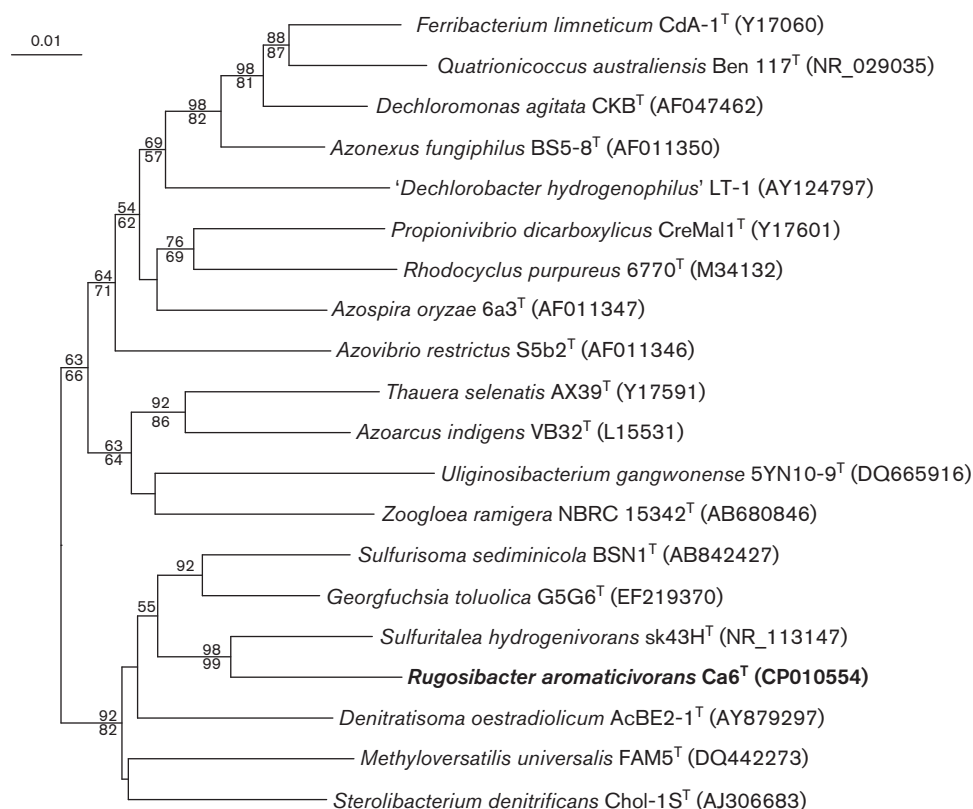

Fig. 2. 16S rRNA gene neighbour-joining phylogenetic tree of Ca6^T with type strains of genera from the family *Rhodocyclaceae*. Percentage bootstrap values of >50 % based on 1000 iterations are shown for the neighbour-joining [26] and maximum-parsimony algorithms [27] above and below nodes, respectively. The consensus neighbour-joining tree was reconstructed including all sequence information available and without considering positions with gaps. *Nitrosomonas europaea* strain ATCC 25978^T (GenBank accession HE862405) was included as an outgroup (not shown). GenBank accession numbers are included in parentheses to each strain. Bar, 0.01 substitutions per nucleotide position.

In addition to high 16S rRNA gene sequence divergence from described species of phylogenetically related genera, Ca6^T was also differentiated by its physiological characteristics (Table 1). Most species from closely related genera within the family *Rhodocyclaceae* were isolated from aquatic or anoxic environments, while Ca6^T was isolated from contaminated soil. Representatives from all closely related genera were also generally capable of facultative or anaerobic growth and nitrate reduction, while Ca6^T required an aerobic environment for growth under the growth conditions tested and was not able to reduce nitrate. None of the phylogenetically closest described genera to Ca6^T contain species reported to degrade PAHs. Among the genera examined, strain Ca6^T was also the only isolate reported to contain the respiratory quinones Q7 and Q9; all isolates for which such information is available, including Ca6^T, indicated Q8 as the major or sole respiratory quinone. Similarly, while species from all closely related genera (including strain Ca6^T) contained C$_{16:1}\omega7c$ (generally as part of a summed feature) and C$_{16:0}$ as major fatty acids, strain Ca6^T could be distinguished by the presence or absence of other, less abundant fatty acids. A significantly lower genomic DNA G+C content (mol%) further distinguished Ca6^T from strains of related genera. A complete genome comparison between Ca6^T and *Sulfuritalea hydrogenivorans* sk43H^T (the closest relative by 16S rRNA gene sequence identity) approximated a DNA–DNA hybridization of 19.00 % (16.8–21.4 %) based on a linear regression model [24]. The average nucleotide identity (ANI) between the two genomes was 76.69 % [25]. On the basis of genomic and physiological differences, we propose Ca6^T as the type strain of a novel species and genus, for which the name *Rugosibacter aromaticivorans* gen. nov., sp. nov. is proposed.

DESCRIPTION OF *RUGOSIBACTER* GEN. NOV.

Rugosibacter (Ru.go.si.bac′ter. L. adj. *rugosus* wrinkled; N. L. masc. n. *bacter* a rod; N. L. masc. n. *Rugosibacter* a wrinkled rod).

Cells are Gram-type-negative, non-motile and grow aerobically. Catalase-negative and oxidase-positive. Heterotrophic growth occurs on a limited number of organic acids. Predominant fatty acids are summed feature 3 (C$_{16:1}\omega7c$ or C$_{16:1}\omega6c$) and C$_{16:0}$. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phospholipid. The major respiratory quinone is Q8. Phylogenetically, the genus is a member of the family *Rhodocyclaceae* in the class *Betaproteobacteria*. The type species is *Rugosibacter aromaticivorans*.

DESCRIPTION OF *RUGOSIBACTER AROMATICIVORANS* SP. NOV.

Rugosibacter aromaticivorans [a.ro.ma.ti.ci.vo′rans. L. adj. *aromaticus* aromatic, fragrant; L. pres. part. *vorans*

Table 1. Differential characteristics of strain Ca6^T and closely related taxa within the family *Rhodocyclaceae*

Strains: 1, Ca6^T; 2, *Denitratisoma oestradiolicum* AcBE2-1^T [22]; 3, *Georgfuchsia toluolica* G5G6^T [20]; 4, species of the genus *Methyloversatilis* including *Methyloversatilis universalis* strains FAM5^T and Ehg5 [28], *Methyloversatilis discipulorum* strains FAM1^T, RZ18-153 and RZ94 [29], and *Methyloversatilis thermotolerans* 3t^T [30]; 5, *Sterolibacterium denitrificans* Chol-1S^T [23]; 6, *Sulfurisoma sediminicola* BSN1^T [21]; 7, *Sulfuritalea hydrogenivorans* sk34H^T [13]; 8, species of the genus *Rhodocyclus* including *Rhodocyclus purpureus* strain 'Ames' 6770^T [31, 32] and *Rhodocyclus tenuis* strain ATCC 25 093^T [31, 33, 34]. +, Positive; −, negative; var., variable results among strains; NR, not reported. DNA G+C content (mol%) values for the genus *Methyloversatilis* based on genomic data presented by Smalley *et al.* [29].

Characteristic	1	2	3	4	5	6	7	8
Isolation source(s)	Contaminated soil, USA	Activated sludge, Germany	Iron-reducing aquifer, Netherlands	Lake sediment, USA; hot springs, Russia	Anoxic reactor treating landfill leachate, Uruguay	Freshwater lake sediment, Japan	Freshwater lake, Japan	Swine waste lagoon, USA; freshwater pond, Germany
DNA G+C content (mol%)	55.1	61.4	NR	65.6–67	65.3	67	67	64.1–65.1
Diagnostic fatty acids								
C$_{8:0}$ 3-OH	−	+	−	−	+	−	−	−
C$_{10:0}$ 3-OH	+	−	−	var.	+	+	−	+
C$_{17:0}$ cyclo	+	−	+	var.	−	−	−	−
C$_{17:1}\omega7c$	+	−	−	−	−	−	−	−
C$_{19:0}$ cyclo $\omega8c$	+	−	−	−	−	−	−	−
Respiratory quinones	Q8, Q9, Q7	Q8	NR	Q8	Q8	NR	NR	Q8, MK8
Catalase	−	−	NR	+	+	+	−	+
Motility	−	+	+	−	+	+	NR	var.
Oxygen requirement	Aerobic	Facultative	Anaerobic	Facultative	Facultative	Facultative	Facultative	Facultative
Nitrate reduction	−	+	+	var.	+	+	+	NR

devouring; N.L. part. adj. *aromaticivorans* devouring aromatic (compounds)].

Cells are curved rods (0.77–1.15×0.23–$0.27\,\mu m$). On solid media, colonies are small, convex and circular with a yellow–white colour. Growth occurs aerobically between 20 and $35\,^{\circ}C$ (optimum 30–$34\,^{\circ}C$), at pH 6.5–7.5 (optimum pH 6.5) and with salinity $\leq 0.5\,\%$. Cells can grow on select monocyclic and polycyclic aromatic compounds as a sole source of carbon, and use both nitrate and ammonia as nitrogen sources. Cells are negative for gelatin hydrolysis, cellulase, skimmed milk protease, starch hydrolysis, nitrate reduction and urease activity, and positive for lipase activity. In addition to those listed in the genus description, fatty acids present in minor amounts are $C_{17:0}$ cyclo, $C_{18:0}\omega 7c$, $C_{10:0}$ 3-OH and $C_{12:0}$. Polar lipids and respiratory quinones are consistent with the genus description.

The type strain, Ca6^{T} (=ATCC TSD-59^{T}=DSM 103039^{T}), was isolated from PAH-contaminated soil from Charlotte, NC, USA. The genomic DNA G+C content of the type strain is 55.14 mol%.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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