Calvin University

Calvin Digital Commons

University Faculty Publications and Creative Works

University Faculty Scholarship

8-1-2016

Cephaloticoccus gen. Nov., a new genus of 'Verrucomicrobia' containing two novel species isolated from Cephalotes ant guts

Jonathan Y. Lin
Calvin University

Jacob A. Russell
Drexel University

Jon G. Sanders
Harvard University

John T. Wertz
Calvin University

Follow this and additional works at: https://digitalcommons.calvin.edu/calvin_facultypubs

Part of the Microbiology Commons

Recommended Citation

Lin, Jonathan Y.; Russell, Jacob A.; Sanders, Jon G.; and Wertz, John T., "Cephaloticoccus gen. Nov., a new genus of 'Verrucomicrobia' containing two novel species isolated from Cephalotes ant guts" (2016). *University Faculty Publications and Creative Works*. 240.
https://digitalcommons.calvin.edu/calvin_facultypubs/240

This Article is brought to you for free and open access by the University Faculty Scholarship at Calvin Digital Commons. It has been accepted for inclusion in University Faculty Publications and Creative Works by an authorized administrator of Calvin Digital Commons. For more information, please contact digitalcommons@calvin.edu.

International Journal of Systematic and Evolutionary Microbiology (2016), 66, 2869–2875 DOI 10.1099/ijsem.0.001068

Ventosimonas gracilis gen. nov., sp. nov., a member of the *Gammaproteobacteria* isolated from *Cephalotes varians* ant guts representing a new family, *Ventosimonadaceae* fam. nov., within the order '*Pseudomonadales*'

Jonathan Y. Lin, William J. Hobson and John T. Wertz

Department of Biology, Calvin College, Grand Rapids, MI, USA

Correspondence
John T. Wertz
jwertz59@calvin.edu

Cephalotes 'turtle' ants are known to harbor a core group of gut symbionts, including members belonging to the *Gammaproteobacteria*. Here, we describe the cultivation and characterization of strain CV58^T, a novel member of the *Gammaproteobacteria* order *Pseudomonadales* isolated from the guts of the ant *Cephalotes varians*. Strain CV58^T was rod-shaped, Gram-stain-negative, non-motile and formed pale-yellow colonies on trypticase soy agar. Optimum growth occurred under an atmosphere of 4–20 % (v/v) O_2. Growth was possible for strain CV58^{T}at NaCl concentrations of 0–1.5 % (w/v), temperatures of 23–40 °C, and pH values of 5.5–8.5. The G+C content of the genomic DNA was 54.9 mol% and the major fatty acids were $C_{18:1}\omega7c$, $C_{16:0}$, $C_{16:1}\omega7c/C_{16:1}\omega6c$, $C_{12:0}$ and $C_{12:0}$3OH. The only respiratory quinone detected was ubiquinone–9 (Q–9) and the major polar lipids were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Based on phylogenetic analysis of the 16S rRNA gene sequence, strain CV58^T shared an 88.3 % nucleotide identity with its closest cultivated neighbor, *Pseudomonas putida* R43. We believe that this, combined with the housekeeping gene phylogeny, differences in phenotypic characteristics and cellular fatty acid compositions of other cultivated members indicates that strain CV58^T represents a novel species occupying a novel genus and family within the order *Pseudomonadales*. Thus, we propose the name *Ventosimonadaceae* fam nov., followed by *Ventosimonas gracilis* gen. nov., sp. nov., to classify strain CV58^T (=NCIMB 15011^T =DSM 100910^T).

Turtle ants (genus *Cephalotes*) represent a highly diverse and species-rich neotropical clade (Price *et al.*, 2014). Studies using PCR amplification and DNA sequencing techniques have revealed that *Cephalotes* ants harbor a core gut microbiota (Anderson *et al.*, 2012; Hu *et al.*, 2014; Kautz *et al.*, 2013; Russell *et al.*, 2009). Bacteria in this core microbiota consistently include members of the *Burkholderiales*, *Rhizobiales*, *Xanthomonadales*, *Opitutales* and *Pseudomonadales* phylotypes (Anderson *et al.*, 2012; Hu *et al.*, 2014; Russell *et al.*, 2009), signifying their importance as stable, autochthonous members of the gut community (Hu *et al.*, 2014; Sanders *et al.*, 2014). These symbionts are likely to have coevolved with their hosts over their evolutionary history (Sanders *et al.*, 2014), and, as shown for symbionts in the termite (Brune & Ohkuma, 2011; Wertz *et al.*, 2012; Wertz & Breznak, 2007b) and honeybee gut (Engel *et al.*, 2012; Engel & Moran, 2013; Kwong & Moran, 2013), likely confer beneficial functions to host nutrition and disease resistance (Dillon & Dillon, 2004).

To date, the analysis and characterization of the *Cephalotes* gut community have largely been limited to culture-independent 16S rRNA gene and metagenomic studies (i.e. Kautz *et al.*, 2013). However, *in vitro* studies of these gut symbionts have not yet been carried out. Thus, the cultivation and physiological characterization of these bacteria will help to elucidate their contributory roles to host nutrition, defense and evolution. In this study, we describe the cultivation and physiological characterization of strain CV58^T, a

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CV58^T is KT258896. The accession numbers for the housekeeping sequences of strain CV58^T are KXU36114 (RpoB), KXU38534 (RadA), KXU38604 (UvrB), KXU34835 (UvrC), and KXU37547 (GyrB). The whole genome shotgun project for strain CV58^T is deposited under the accession number LSZO00000000. The version described in this paper is version LSZO01000000.

Two supplementary figures are available with the online Supplementary Material.

novel gammaproteobacterium isolated from *Cephalotes varians* ant guts representing a novel family within the order *Pseudomonadales*. We propose the taxa *Ventosimonadaceae* fam. nov., followed by *Ventosimonas gracilis* gen. nov., sp. nov., to classify strain CV58^T.

Strain CV58^T was isolated from the guts of *Cephalotes varians* ants on trypticase soy agar (TSA; Difco BD). Wild *C. varians* ants were collected from hollow mangrove twigs from Crocodile Lake National Wildlife Refuge, Key Largo, FL, USA (25° 17.55′ N, 80° 18.33′ W) in October 2012. The gasters (terminal abdominal segments containing most internal organs) of 12–15 ants were removed using sterile forceps and homogenized in a glass tissue homogenizer in sterile trypticase soy broth (TSB; Difco BD). Afterwards, the homogenate was serially diluted and spread onto TSA plates. The plates were incubated in air or in atmosphere-controlled glove boxes (Coy Labs) under hypoxic (2% O_2, 5% CO_2, 93% N_2 (v/v)) or anoxic (5% H_2, 5% CO_2, 95% N_2 (v/v)) conditions at room temperature for approximately one month. Resulting colonies were isolated, purified and then identified by 16S rRNA gene sequencing using the universal primers 63F (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and 1389R (5′-ACG GGC GGT GTG TAC AAG-3′) (Baker *et al.*, 2003). Strain CV58^T was isolated from plates maintained under hypoxia, but was routinely grown on TSA plates or in TSB shaken at approximately 200 r.p.m at 28–30 °C under atmospheric air (21% O_2) supplemented with 1% CO_2. Additional testing showed that strain CV58^T could grow on brain heart infusion agar (Difco BD), but not on nutrient agar (Difco BD) or R2A agar (EMD Millipore). Frozen stocks were prepared by suspending bacterial cultures in TSB supplemented with 20% (v/v) glycerol and storing them in rubber-sealed cryovials (Simport) at −80 °C.

A number of growth experiments and biochemical analyses were completed to characterize strain CV58^T. Unless otherwise stated, all experiments were performed with 3–5 replicates. To determine the optimum O_2 and CO_2 concentrations for growth, cultures of strain CV58^T were incubated in stoppered anaerobic culture tubes (Chemglass) containing 5 ml TSB and growth was monitored by spectrophotometry at a wavelength of 600 nm (Wertz & Breznak, 2007b). The TSB media was deoxygenated by sparging the liquid with 100% N_2. Under a starting headspace of 100% N_2, culture tubes were injected with 100% CO_2 or atmospheric air (21% O_2). After injection, the overpressure was released to obtain final headspace concentrations of 0% or 1% CO_2 (v/v) and 0–20% O_2 (v/v). To test if the strain could grow under anoxic conditions by fermentation or anaerobic respiration, tubes containing 0% O_2 with 0 or 1% CO_2 were injected with a reducing solution of 1 mM (final concentration) dithiothreitol (DTT) and cysteine or 0.05% (w/v final concentration) cysteine · HCl (Breznak & Costilow, 2007). Several of these tubes were also supplemented with 10 mM (final concentration) sodium nitrate, sodium nitrite, or sodium fumarate to test for the usage of alternative electron acceptors (Wertz & Breznak, 2007a). An increase in turbidity by at least 50% of the optical density (OD_{600}) measured at the time of inoculation was considered evidence for growth.

Strain CV58^T grew under atmospheres containing 1–20% O_2 and did not require supplemental CO_2 (1%) for growth (Table 1). The generation time for the strain decreased as the O_2 concentration increased (Table 1). Growth for strain CV58^T at 1 and 2% O_2 was significantly slower compared to growth at 4–20% O_2 ($P<0.05$; Student's *t* test, all groups). No significant differences were observed in the generation times between O_2 concentrations from 4 to 20% O_2 ($P>0.05$; Student's *t* test, all groups). Thus, optimum growth for strain CV58^T occurred under an atmosphere of 4–20% O_2. Strain CV58^T did not grow in the absence of O_2 in any of the conditions tested (Table 1), suggesting an inability to grow by fermentation or anaerobic respiration under reducing conditions using any endogenous or alternative electron acceptors (nitrate, nitrite or fumarate) added to the medium. In addition, no alternative electron acceptors (nitrate reductase, nitrite reductase etc.) were detected in the genome, suggesting that strain CV58^T is likely an obligate aerobe. However, our *in vitro* tests for its ability to grow by anaerobic respiration were not exhaustive.

The NaCl concentration for growth was determined by incubating the bacteria in TSB media containing 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 or 10 % (w/v) NaCl. Growth in the absence of NaCl was tested by incubating the bacteria on media containing all the components of TSA [1.5% (w/v) peptone, 0.5% (w/v) tryptone, and 1.5% (w/v) agar], but without NaCl. Optimum growth occurred at 0.5% (w/v) NaCl, although growth was possible for strain CV58^T in 0–1.5% (w/v) NaCl and was inhibited above 1.5% (w/v) NaCl. The pH tolerance was assessed by incubating

Table 1. Effect of headspace O_2 and CO_2 concentrations on the generation time and final optical density (OD_{600}) of *Ventosimonas gracilis* CV58^T

Growth was measured by spectrophotometry at a wavelength of 600 nm in stoppered anaerobic culture tubes without replacement of the atmosphere. Values are expressed as mean±SD and were obtained from 3–4 replicates. NG, no growth.

O_2 (%)	CO_2 (%)	Mean±SD	
		Generation time (h)	Final OD_{600}
0	0	NG	–
0	1	NG	–
1	1	8.5±0.6	0.085±0.017
2	1	7.0±0.7	0.14±0.01
4	1	5.3±1.1	0.26±0.02
8	1	5.4±0.4	0.44±0.04
12	1	5.2±0.4	0.62±0.04
16	1	4.7±0.2	0.98±0.02
20	1	4.5±0.4	1.02±0.14
20	0	4.9±0.2	1.01±0.14

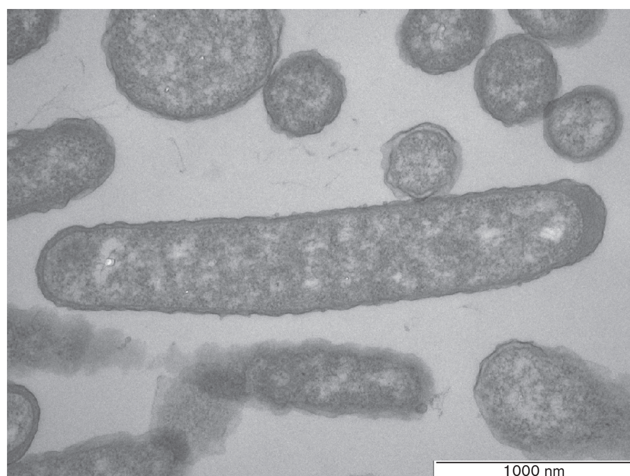

Fig. 1. Transmission electron micrograph of cells of *Ventosimonas gracilis* CV58^T. Bar, 1.0 μm.

the bacteria in stoppered anaerobic culture tubes with 5 ml TSB at pH values of 4.0–9.0 with 0.5 pH unit increments. The final headspace concentration was maintained at 20% O_2 and 1% CO_2, and the pH of the medium was buffered using 0.1 M citric acid (pH 4.0–4.5), 0.1 M succinate (pH 5.0–5.5), 0.1 M MOPS (pH 6.0–7.5), or 0.1 M Tris-HCl (pH 8.0–9.0). Growth was measured by spectrophotometry as before. Growth was possible for strain CV58^T within a pH range of 5.5–8.8, while optimum growth occurred between a pH range of 6.0–7.0.

The temperature range for growth was determined by incubating the bacteria in stoppered anaerobic culture tubes containing 5 ml TSB under headspace concentrations of 20% O_2 and 1% CO_2. Tubes inoculated with bacteria were incubated in temperature-controlled shakers (Benchmark) set at 23, 37, 40, 45 or 47 °C. Growth was measured by spectrophotometry as before. Strain CV58^T could grow at 23–40 °C and optimum growth occurred at 37 °C.

Substrate usage was determined by using GN2 microplates (Biolog) and the API 20NE system (bioMérieux). GEN III microplates (Biolog) were used to characterize antibiotic susceptibility and the usage of additional carbon sources. Enzyme activity was analyzed by using the API ZYM system (bioMérieux). All substrate and enzyme assays were performed in duplicate, according to the manufacturers' instructions. From GN2 microplates, strain CV58^T was able to use pyruvic acid methyl acid, succinic acid methyl ester, *cis*-aconitic acid, citric acid, α-ketobutyric acid, α-ketoglutaric acid, propionic acid, succinamic acid, L-alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, L-ornithine, L-proline, D-serine, L-serine, γ-aminobutyric acid and putrescine. From GEN III microplates, strain CV58^T was able to use L-arginine, glucuronamide, methyl pyruvate and acetoacetic acid. Strain CV58^T grew in the presence of the antibiotics rifamycin SV, troleandomycin, lincomycin, vancomycin and aztreonam. Examples of substrates that strain CV58^T could not use included adonitol, D-arabitol, D-fructose, D-galactose, D-glucose, D-mannose, sucrose, D-cellobiose, D-raffinose, D-mellibiose, L-fucose, α-D-lactose, lactulose, xylitol, pectin, L-leucine and D, L-carnitine.

API ZYM results were positive for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Strain CV58^T was negative for alkaline phosphatase, lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Using the API 20 NE system, strain CV58^T only tested positive for the assimilation of citrate.

Catalase activity was determined by placing cells on a microscope slide and monitoring for gas accumulation beneath a coverslip after the addition of 3% (v/v) H_2O_2. The presence

Table 2. Cellular fatty acid composition of *Ventosimonas gracilis* CV58^T compared to other cultivated representatives from the order *Pseudomonadales*

Strains: 1, *Ventosimonas gracilis* CV58^T; 2, *Pseudomonas aeruginosa* ATCC 27853; 3, *Pseudomonas fluorescens* ATCC 1352; 4, *Pseudomonas arsenicoxydans* VC-1^T (data from Campos *et al.*, 2010). Results are presented as percentages of total fatty acids. TR, trace (<0.1 %); ND, not detected.

Fatty Acid	1	2	3	4
$C_{10:0}$	0.7	0.2	0.2	0.3
$C_{10:0}$ 3OH	0.3	2.5	3.1	3.0
$C_{12:0}$	10.3	3.6	3.1	5.0
$C_{12:0}$ 2OH	ND	4.2	3.8	3.8
$C_{12:0}$ 3OH	9.1	2.2	3.4	4.6
$C_{13:0}$	ND	TR	TR	ND
$C_{14:0}$	2.4	0.9	0.3	0.5
$C_{14:0}$ 3OH	ND	ND	0.2	ND
$C_{15:0}$	0.1	0.4	TR	ND
$C_{16:0}$	18.4	21.2	25.1	27.9
$C_{16:0}$ 3OH	1.7	ND	ND	ND
$C_{16:1}\omega5c$	ND	ND	0.1	ND
$C_{16:1}\omega7c$/iso-$C_{15:0}$2OH	ND	ND	ND	21.2
$C_{16:1}\omega7c$/$C_{16:1}\omega6c$	11.1	23.7	31.8	ND
$C_{17:0}$	ND	0.1	0.1	ND
$C_{17:0}$ cyclopropane	ND	0.2	3.2	12.2
$C_{17:1}\omega6c$	ND	Tr	ND	ND
$C_{17:1}\omega8c$	ND	0.1	0.1	ND
$C_{18:0}$	0.1	0.3	0.8	0.8
$C_{18:1}\omega5c$	ND	ND	TR	ND
$C_{18:1}\omega7c$	45.3	39.5	24.3	17.1
$C_{18:1}\omega7c$ 11-methyl	ND	ND	0.1	ND
$C_{18:1}\omega9c$	ND	ND	ND	1.7
$C_{19:0}$ cyclo $\omega8c$	ND	0.6	0.1	ND
$C_{19:0}$ cyclo $\omega9c$	ND	ND	ND	0.8

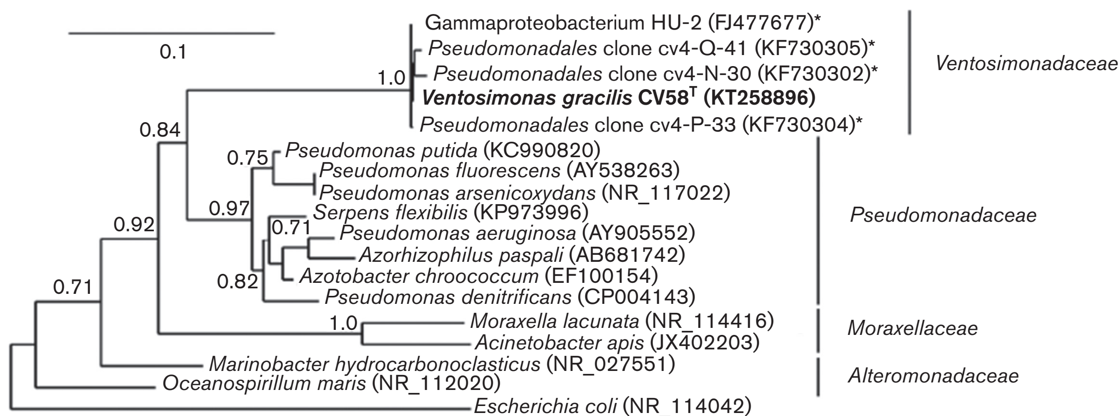

Fig. 2. Maximum likelihood-based 16S rRNA gene phylogeny of *Ventosimonas gracilis* CV58^T. The phylogeny was based on 1213 shared nucleotide positions. * =16S rRNA gene clones from *C.varians* guts. Branch point support is indicated by the numbers at branch nodes. Other members of *Gammaproteobacteria* are given as reference species. Genbank accession numbers are shown in brackets. Bar, 0.1 changes per nucleotide.

of cytochrome c oxidase was indicated the blue color of cells after the addition of oxidase diagnostic reagent (Becton Dickinson). Urease activity was tested by monitoring cells for growth and media color change in urea broth (Difco BD) supplemented with TSB and trace mineral supplement (ATCC). Motility was tested by inoculating the bacteria in sulfide-indole-motility medium (SIM; EMD Millipore). Strain CV58^T was non-motile, positive for oxidase and negative for catalase and urease.

For transmission electron microscopy, the bacteria were fixed with 2.5 % glutaraldehyde in 0.1 m. cacodylate solution and sent to the Michigan State University Center for Advanced Microscopy (East Lansing, MI, USA). Cells were post-fixed in 1% osmium tetroxide in 0.1 m phosphate buffer for 1 h, dehydrated using a 30–95% (v/v) acetone graded series, embedded in Poly/Bed 812 (Polysciences) and cut into 70 nm ultrathin sections. The sections were then stained with uranyl acetate, lead citrate, and examined under a Jeol 100 CXII transmission electron microscope. Cells of strain CV58^T were rod-shaped and ranged from 2.5–4.5 µm in length and 0.4–0.5 µm in width (Fig. 1). Strain CV58^T appeared to have a Gram-stain-negative type outer membrane and thin cell wall. Its Gram-stain-negative status was confirmed by Gram-staining followed by light microscopy.

To determine the cellular fatty acid composition, cultures were grown on TSA plates at 28 °C for 7 days under atmospheric air (21 % O_2) supplemented with 1 % CO_2. Cells were pelleted in TSB containing 20% (v/v) glycerol and frozen in rubber-sealed cryovials at −80 °C. Frozen cells were sent over in dry ice to MIDI labs. (Newark, DE, USA) for analysis using the Sherlock Microbial ID system (MIS). The analysis of cellular respiratory quinones and polar lipid composition was carried out by the identification service of the DSMZ (Braunschweig, Germany), according to Tindall *et al.* (2007). As shown in Table 2, the major fatty acids of strain CV58^T were $C_{18:1}\omega7c$ (45.3 %), $C_{16:0}$ (18.4 %), $C_{16:1}\omega7c$/$C_{16:1}\omega6c$ (11.1 %), $C_{12:0}$ (10.3 %) and $C_{12:0}$3OH (9.1 %). The only respiratory quinone detected was ubiquinone–9 (Q–9) and the major polar lipids were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. An unidentified glycolipid, aminolipid, and phospholipid were also present (Fig. S1, available in the online Supplementary Material).

DNA was extracted as described by Matson *et al.* (2007) and the genome of the strain was sequenced using a HiSeq 2500 machine (Illumina) at the Harvard University FAS Center for Systems Biology core facility (Cambridge, MA, USA). Raw sequences were trimmed for quality and adapter read-through using Trimmomatic (Bolger *et al.*, 2014) and preliminary assemblies were carried out using Velvet (Zerbino & Birney, 2008). The G+C content of the genomic DNA of strain CV58^T was 54.9 mol%. The isolate had a single copy of the 16S rRNA gene, the full-length sequence of which was obtained from the genome and used in phylogenetic analyses. For phylogenetic analysis, the sequence alignment was carried out using SINA (Pruesse *et al.*, 2012) and the maximum-likelihood-based tree was constructed with PhyML (Guindon *et al.*, 2005) using the phylogeny.fr program (Dereeper *et al.*, 2008). The analysis revealed that strain CV58^T formed a monophyletic group within the *Gammaproteobacteria* order *Pseudomonadales* (Fig. 2) with other 16S rRNA gene clones obtained from *Cephalotes varians* guts (Hu *et al.*, 2014). Based on the analysis of the 16S rRNA gene sequence, strain CV58^T shared a sequence similarity of 88.3 % with its closest neighbor with a validly published name, *Pseudomonas putida* strain R43 (Skerman *et al.*, 1989). Consistent with the 16S rRNA gene tree, further analysis using the concatenated-deduced amino acid

Table 3. Phenotypic Characteristics of *Ventosimonas gracilis* CV58[T] compared to other cultivated representatives from the order *Pseudomonadales*

Strains: 1, *Ventosimonas gracilis* CV58[T]; 2, *Pseudomonas aeruginosa* ATCC 27853 (G+C content from Fang *et al*., 2012); 3, *Pseudomonas arsenicoxydans* VC-1[T] (data from Campos *et al*., 2010); 4, *Acinetobacter apis* HYN18[T] (data from Kim *et al*., 2014). Substrate usage and enzyme activity were determined using GN2 and GEN III microplates and API 20 NE strips. NR, not reported.

Characteristic	1	2	3	4
Isolation source	Ant gut	Human blood	Sediment	Honeybee gut
Cell shape	Rods	Rods	Rods	Rods
Cell length (µm)	2.5–4.5	1.0–5.0	5.5	1.8
Cell width (µm)	0.4–0.5	0.5–1.0	0.5–1.0	0.6
Gram stain	Negative	Negative	Negative	Negative
Colony Colour	Pale yellow	Pale yellow	NR	Cream-white
Metabolism	Obligate aerobe	Obligate aerobe	Obligate aerobe	Obligate aerobe
Motility	–	+	+	–
Catalase	–	+	+	+
Oxidase	+	–	+	–
Urease	–	–	–	–
Temperature range (°C)	23–40	23–40	4–37	15–30
pH range	5.5–8.5	4.5–9.0	6.5–10.0	6.0–8.0
NaCl range (%)	0–1.5	0–8.0	0–2.0	0–2.0
DNA G+C content (mol%)	54.9	66.2	NR	40.6
D-Glucose	–	+	+	+
L-Arabinose	–	–	+	+
D-Mannose	–	–	+	+
D-Mannitol	–	+	+	+

sequences of five housekeeping genes (RpoB, COG0085; RadA, COG2255; UvrB, COG0556; UvrC, COG0322; GyrB, COG0187) also placed strain CV58[T] in its own monophyletic group within the *Pseudomonadales* with strong bootstrap values (Fig. S2). The average amino acid identity between strain CV58[T] and the members of *Pseudomonadaceae* was 67.9±0.9 %, whereas the average sequence identity of the housekeeping genes for the members within the family *Pseudomonadaceae* was 88.3±2.2 %.

To compare the phenotypic characteristics of strain CV58[T] to its cultivated neighbors, reference strains of *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas fluorescens* ATCC 1352 were grown on TSA under the same conditions and analyzed for biochemical and cellular fatty acid properties as mentioned before. Strain CV58[T] showed distinct phenotypic features from other cultivated members from the order *Pseudomonadales* (summarized in Table 3). Furthermore, strain CV58[T] also had a distinct cellular fatty acid profile (e.g. $C_{12:0}$ Table 2), a 16S rRNA sequence similarity of 88.3 % with its closest cultivated neighbor, and a significantly lower average amino acid sequence identity of several housekeeping genes compared to the members of the *Pseudomonadaceae*. We believe that these differences warrant the classification of this isolate as a new species occupying a novel genus and family. Therefore, we propose the taxa *Ventosimonadaceae* fam. nov. and *Ventosimonas gracilis* gen. nov., sp. nov., to classify strain CV58[T].

Description of *Ventosimonas* gen. nov.

Ventosimonas (Ven.to.si.mo′nas. L. adj. *ventosus* capricious; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Ventosimonas* a capricious single cell).

Cells are Gram-negative, rod-shaped, non-motile, and colonies on TSA are pale-yellow in colour. Metabolism is likely obligate aerobic. The type species of the genus is *Ventosimonas gracilis*.

Description of *Ventosimonas gracilis* sp. nov.

Ventosimonas gracilis (gra′ci.lis. L. fem. adj. *gracilis* slender, elongated; *gracilis* meant to denote slender, elongated cells).

Cells are rod-shaped (2.5–4.5 µm length, 0.4–0.5 µm width), Gram-negative, non-motile, and colonies on TSA are pale yellow in colour. Metabolism is likely obligate aerobic. Growth is possible on brain heart infusion agar and on solid and liquid trypticase soy medium at pH values between 5.5–8.5, NaCl concentrations between 0–1.5 %, temperatures between 23–40 °C, and under atmospheres of 1–20 % O_2 with 0 or 1 % CO_2. Optimum growth occurs at pH 6.0–7.0, 0.5 % NaCl, 37 °C, and under atmospheric conditions of 4–20 % O_2. The major fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$, $C_{16:1}\omega7c/C_{16:1}\omega6c$, $C_{12:0}$ and $C_{12:0}$OH. The only respiratory quinone detected is ubiquinone–9 (Q–9) and

the major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Cells are negative for catalase and urease and positive for oxidase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase. Substrates used include methyl pyruvate, pyruvic acid methyl acid, succinic acid methyl ester, cis-aconitic acid, citric acid, α-ketobutyric acid, α-ketoglutaric acid, propionic acid, succinamic acid, acetoacetic acid, L-alaninamide, glucuronamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-arginine, L-aspartic acid, L-glutamic acid, L-ornithine, L-proline, D-serine, L-serine, γ-aminobutyric acid, and putrescine. Substrates not used include adonitol, D-arabitol, D-fructose, D-galactose, D-glucose, D-mannose, sucrose, D-cellobiose, D-raffinose, D-mellibiose, L-fucose, α-D-lactose, lactulose, xylitol, pectin, L-leucine, and D, L-carnitine. Cells are resistant to rifamycin SV, troleandomycin, lincomycin, vancomycin, and aztreonam.

The type strain, CV58^{T} (=NCIMB 15011^{T} =DSM 100910^{T}), was isolated from the gut of the arboreal ant *Cephalotes varians*, collected from Key Largo, FL, USA. The G+C content of the genomic DNA of the type strain is 54.9 mol%.

Description of *Ventosimonadaceae* fam. nov.

Ventosimonadaceae (Ven.to.si.mo.na.da.ce′ae. N.L. fem. n. *Ventosimonas* a bacterial genus; *–aceae* ending to denote a family; N.L. fem. pl. n.; *Ventosimonadaceae* the *Ventosimonas* family)

The description is the same as for the proposed genus *Ventosimonas*. The type genus is the genus *Ventosimonas*.

Acknowledgements

We are grateful to Yi Hu, Piotr Łukasik, Jacob Russell, and Jon Sanders for the provision of *C. varians* ants, genome sequencing, and advice on cell metabolic function. We also thank Alanna Van Huizen for technical assistance, Alicia Withrow for transmission electron microscopy, and David Noe for advice on nomenclature and construction of Latinized names. This research was supported by a grant from the National Science Foundation (DEB-1442156 to J. T. W.) and a Calvin College Harvey Rozema Fellowship (to J. Y. L.).

References

Anderson, K. E., Russell, J. A., Moreau, C. S., Kautz, S., Sullam, K. E., Hu, Y., Basinger, U., Mott, B. M., Buck, N. & Wheeler, D. E. (2012). Highly similar microbial communities are shared among related and trophically similar ant species. *Mol Ecol* **21**, 2282–2296.

Baker, G. C., Smith, J. J. & Cowan, D. A. (2003). Review and re-analysis of domain-specific 16S primers. *J Microbiol Methods* **55**, 541–555.

Bolger, A. M., Lohse, M. & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120.

Breznak, J. A. & Costilow, R. N. (2007). Physicochemical factors in growth. In *Methods Gen Mol Microbiol*, 3 Edn, pp. 309–329. Edited by G. A. Marzluf, C. A. Reddy, T. J. Beveridge, T. M. Schmidt, L. R. Snyder & J. A. Breznak. Washington: American Society of Microbiology.

Brune, A. & Ohkuma, M. (2011). Role of the termite gut microbiota in symbiotic digestion. In *Biol Termit Mod Synth*, pp. 439–475. Edited by D. E. Bignell, Y. Roisin & N. Lo. Netherlands: Springer.

Campos, V. L., Valenzuela, C., Yarza, P., Kämpfer, P., Vidal, R., Zaror, C., Mondaca, M. A., Lopez-Lopez, A. & Rosselló-Móra, R. (2010). *Pseudomonas arsenicoxydans* sp nov., an arsenite-oxidizing strain isolated from the Atacama desert. *Syst Appl Microbiol* **33**, 193–197.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V. & other authors (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res* **36**, W465–W469.

Dillon, R. J. & Dillon, V. M. (2004). The gut bacteria of insects: nonpathogenic interactions. *Annu Rev Entomol* **49**, 71–92.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**, 1792–1797.

Engel, P., Martinson, V. G. & Moran, N. A. (2012). Functional diversity within the simple gut microbiota of the honey bee. *Proc Natl Acad Sci U S A* **109**, 11002–11007.

Engel, P. & Moran, N. A. (2013). Functional and evolutionary insights into the simple yet specific gut microbiota of the honey bee from metagenomic analysis. *Gut Microbes* **4**, 60–65.

Fang, X., Fang, Z., Zhao, J., Zou, Y., Li, T., Wang, J., Guo, Y., Chang, D., Su, L. & other authors (2012). Draft genome sequence of *Pseudomonas aeruginosa* strain ATCC 27853. *J Bacteriol* **194**, 3755.

Guindon, S., Lethiec, F., Duroux, P. & Gascuel, O. (2005). PHYML Online–a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res* **33**, W557–W559.

Hu, Y., Łukasik, P., Moreau, C. S. & Russell, J. A. (2014). Correlates of gut community composition across an ant species (*Cephalotes varians*) elucidate causes and consequences of symbiotic variability. *Mol Ecol* **23**, 1284–1300.

Kautz, S., Rubin, B. E., Russell, J. A. & Moreau, C. S. (2013). Surveying the microbiome of ants: comparing 454 pyrosequencing with traditional methods to uncover bacterial diversity. *Appl Environ Microbiol* **79**, 525–534.

Kim, P. S., Shin, N. R., Kim, J. Y., Yun, J. H., Hyun, D. W. & Bae, J. W. (2014). *Acinetobacter apis* sp. nov., isolated from the intestinal tract of a honey bee, *Apis mellifera*. *J Microbiol* **52**, 639–645.

Kwong, W. K. & Moran, N. A. (2013). Cultivation and characterization of the gut symbionts of honey bees and bumble bees: description of *Snodgrassella alvi* gen. nov., sp. nov., a member of the family *Neisseriaceae* of the *Betaproteobacteria*, and *Gilliamella apicola* gen. nov., sp. nov., a member of *Orbaceae* fam. nov., *Orbales* ord. nov., a sister taxon to the order '*Enterobacteriales*' of the Gammaproteobacteria. *Int J Syst Evol Microbiol* **63**, 2008–2018.

Matson, E., Ottesen, E. & Leadbetter, J. (2007). Extracting DNA from the gut microbes of the termite (*Zootermopsis nevadensis*). *J Vis Exp* **4**, e195.

Price, S. L., Powell, S., Kronauer, D. J., Tran, L. A., Pierce, N. E. & Wayne, R. K. (2014). Renewed diversification is associated with new ecological opportunity in the Neotropical turtle ants. *J Evol Biol* **27**, 242–258.

Pruesse, E., Peplies, J. & Glöckner, F. O. (2012). SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* **28**, 1823–1829.

Russell, J. A., Moreau, C. S., Goldman-Huertas, B., Fujiwara, M., Lohman, D. J. & Pierce, N. E. (2009). Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. *Proc Natl Acad Sci* **106**, 21236–21241.

Sanders, J. G., Powell, S., Kronauer, D. J., Vasconcelos, H. L., Frederickson, M. E. & Pierce, N. E. (2014). Stability and phylogenetic

correlation in gut microbiota: lessons from ants and apes. *Mol Ecol* **23**, 1268–1283.

Skerman, V. B. D., McGowan, V. & Sneath, P. H. A. (1989). *Approved Lists of Bacterial Names (Amended)*. Washington (DC): ASM Press.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* **30**, 2725–2729.

Tindall, B. J., Sikorski, J., Smibert, R. A. & Krieg, N. R. (2007). Phenotypic characterization and the principles of comparative systematics. In *Methods Gen Mol Microbiol Third Ed*, pp. 330–393. Edited by G. A. Marzluf, C. A. Reddy, T. J. Beveridge, T. M. Schmidt, L. R. Snyder & J. A. Breznak. Washington: American Society of Microbiology.

Wertz, J. T. & Breznak, J. A. (2007a). *Stenoxybacter acetivorans* gen nov., sp. nov., an acetate-oxidizing Obligate microaerophile among diverse O_2-consuming bacteria from termite guts. *Appl Environ Microbiol* **73**, 6819–6828.

Wertz, J. T. & Breznak, J. A. (2007b). Physiological ecology of *Stenoxybacter acetivorans*, an obligate microaerophile in termite guts. *Appl Environ Microbiol* **73**, 6829–6841.

Wertz, J. T., Kim, E., Breznak, J. A., Schmidt, T. M. & Rodrigues, J. L. M. (2012). Genomic and physiological characterization of the *Verrucomicrobia* isolate *Diplosphaera colitermitum* gen. nov., sp. nov., reveals microaerophily and nitrogen fixationgenes. *Appl Environ Microbiol* **78**, 1544–1555.

Zerbino, D. R. & Birney, E. (2008). Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* **18**, 821–829.