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TAXONOMIC DESCRIPTION

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Oleiharenicola alkalitolerans gen. nov., sp. nov., a new member of the phylum *Verrucomicrobia* isolated from an oilsands tailings pond

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Abstract

A novel member of the phylum *Verrucomicrobia* was isolated from an oilsands tailings pond in Alberta, Canada. Cells of isolate NVT^T are Gram-negative, strictly aerobic, non-pigmented, non-motile cocci to diplococci 0.5–1.0 µm in diameter. The bacterium is neutrophilic (optimum pH 6.0–8.0) but alkalitolerant, capable of growth between pH 5.5 and 11.0. The temperature range for growth is 15–40 °C (optimum 25–37 °C). Carbon and energy sources include sugars and organic acids. Nitrogen sources include nitrate, urea, L-glycine, L-alanine, L-proline and L-serine. Does not fix atmospheric nitrogen. Does not require NaCl and is inhibited at NaCl concentrations above 3.0 % (w/v). The DNA G+C content of strain NVT^T, based on a draft genome sequence, is 66.1 mol%. MK-6 and MK-7 are the major respiratory quinones. Major cellular fatty acids are anteiso-$C_{15:0}$ and iso-$C_{15:0}$. Phylogenetic analysis of 16S rRNA gene sequences revealed that the strain belongs to the family *Opitutaceae* of the phylum *Verrucomicrobia*. The most closely related validated species is *Opitutus terrae* (93.7 % 16S rRNA gene sequence identity to its type strain PB90-1^T). Based on genotypic, phenotypic and chemotaxonomic characteristics, it was concluded that this strain represents a novel genus and species, for which the name *Oleiharenicola alkalitolerans* gen. nov., sp. nov. is proposed. The type strain of this novel species is NVT^T (=ATCC BAA-2697^T;=DSM 29249^T).

The phylum *Verrucomicrobia* [1] contains diverse microorganisms that utilize various carbon and energy sources. Cultivation-independent approaches have revealed a broad distribution of this phylum in various niches. Members of this phylum have been detected in open oceans and sediments [2], geothermal fields [3, 4], hot springs [5], soils [6], fresh waters [7, 8] and termite guts [9], with wide ranges of temperature, O_2 levels, depth, salinity and nutrient availabilities. However, only a handful of representatives have been successfully cultivated, and, physiologically, still little is understood about the phylum *Verrucomicrobia*.

At the time of writing, three classes of *Verrucomicrobia* have been formally defined: *Verrucomicrobiae, Spartobacteria* and *Opitutae* [10]. The class *Opitutae*, whose members have mostly been cultivated from marine environments, is one of the most commonly detected lineages of the phylum [10, 11]. Molecular studies have detected the class *Opitutae* in diverse habitats including Arctic sea ice and seawater, shrimp and tilapia culture ponds, and diverse surface soils in Antarctica, Europe and the Americas [12–15]. Altogether, there are 11 validated species in the class *Opitutae* (Fig. 1).

'*Didymococcus colitermitum*' [16] (formerly '*Diplosphaera colitermitum*' [17]) and three species within the genus '*Lacunisphaera*' [18] have been recently cultivated and are reported to belong to the family *Opitutaceae*, but have not yet been validated. Currently, the validated members of the family are limited to *Alterococcus agarolyticus*, which is the type species for the genus *Alterococcus* [19], *Opitutus terrae*, which is the type species for the genus *Opitutus* [6], and two species in the genus *Cephaloticoccus* [20]: *Cephaloticoccus primus* and *Cephaloticoccus capnophilus*, with the former being the type species for the genus.

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Keywords: oilsands tailings ponds; alkalitolerant bacterium; *Verrucomicrobia*; *Opitutaceae*; *Oleiharenicola alkalitolerans*.
Abbreviations: DDH, DNA-DNA hybridization; DMDS, dimethyl disulfide; GC-MS, gas chromatography–mass spectrometry; GGDC, Genome-to-Genome Distance Calculator; OD, optical density.
The IMG/JGI genome submission ID of *Oleiharenicola alkalitolerans* NVT^T is 43214. The GOLD Analysis Project ID for the genome is Ga0049946.
One supplementary figure is available with the online version of this article.

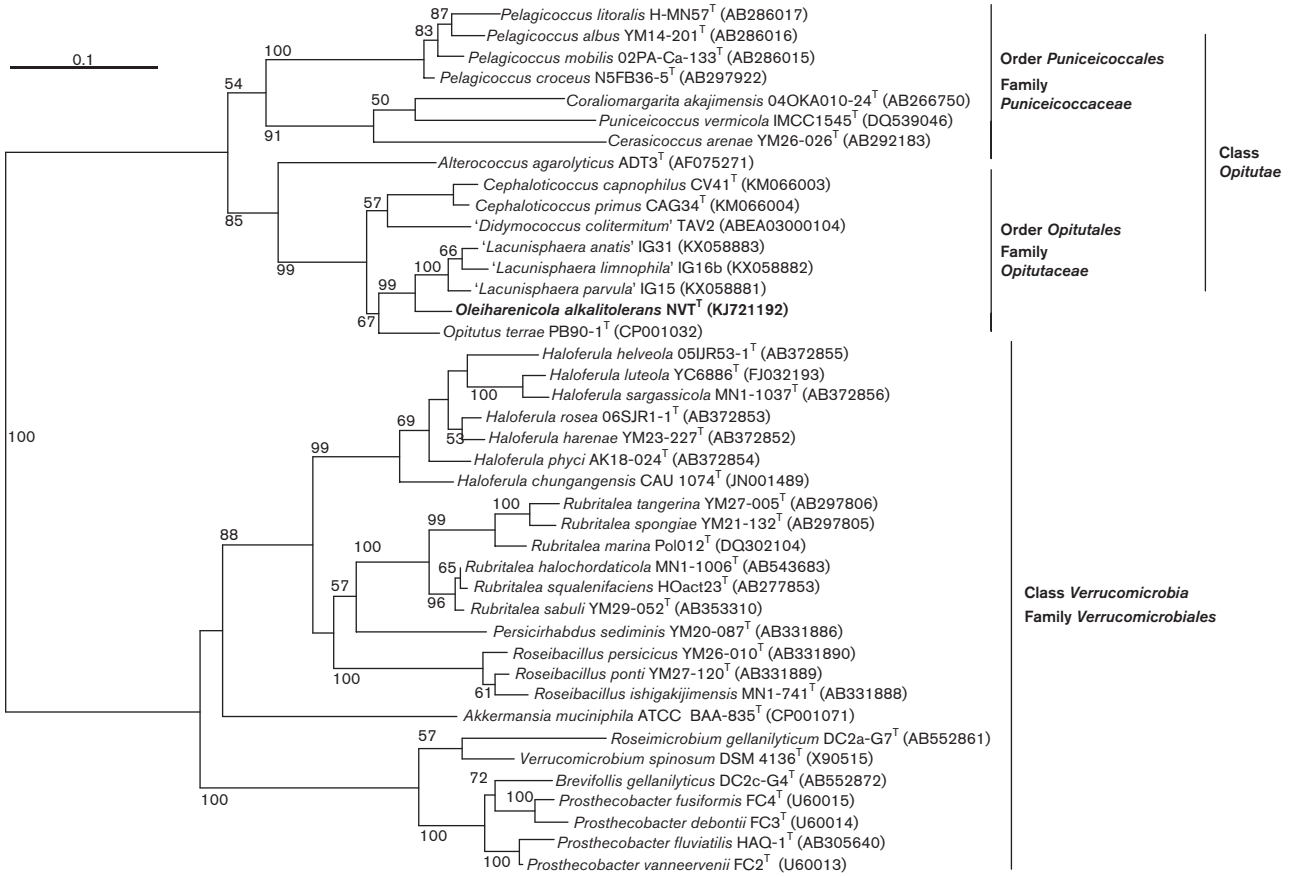

Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain NVT^T within the *Verrucomicrobia* phylum. Numbers at branch nodes are bootstrap values based on 1000 replications. The accession numbers of the 16S rRNA gene sequences of the representative strains in the tree are indicated in brackets. The tree was aligned via ARB [46] and reconstructed via SeaView Phylogeny [49]. Bar, 0.1 substitutions per site.

Alterococcus agarolyticus ADT3^T is a halophilic thermophile isolated from hot springs in Taiwan [19]. Five isolated strains belonging to the genus *Alterococcus* were all able to produce extracellular agarase on agar medium, and were capable of both aerobic growth and anaerobic growth by fermenting glucose to various organic acids [19]. *Opitutus terrae* PB90-1^T is an obligately anaerobic bacterium commonly found in rice paddy soils [6, 21]. The strain is a chemoheterotroph that grows on mono-, di- and polysaccharides, with propionate and acetate as the major fermentation products [6]. *Cephaloticoccus primus* CAG34^T and *Cephaloticoccus capnophilus* CV41^T are considered likely to be obligately aerobic and were isolated from the gut of *Cephalotes* ants [20]. Both species were capable of growth under atmospheres ranging from 1–20 % O_2 and up to 5 % CO_2 with the latter strain requiring CO_2 for growth.

In this study, a novel mesophilic bacterium belonging to the family *Opitutaceae* was isolated from an oilsands tailings pond located in Fort McMurray, Alberta, Canada. The tailings water sample was collected in June 2012 from 0 to 10 cm below the surface and about 15 m from the shore. Tailings ponds contain process-affected waters and fluid fine tailings from the industrial oilsands extraction processes [22, 23]. The sample used in this study was alkaline (pH 8), 20 °C, contained 2770±12.4 mg l^{-1} total major ions, 9.9±0.1 mg N l^{-1} total nitrogen and 40±1.0 mg C l^{-1} dissolved organic carbon [23]. In 2012, a sequencing analysis of 16S rRNA gene amplicons from the water sample found that an operational taxonomic unit from the family *Opitutaceae* was quite abundant, accounting for 0.66 % of all reads in the tailings pond surface water (24 out of 3636) [23].

To isolate the bacterium, sample water was diluted 1 : 10 with deionized water and 100 µl aliquots were streaked on plates of the mineral salts medium 10. Medium 10 was prepared as described previously [24], except that agar was substituted with 1.5 % phytagel and 1 % $MgSO_4$ (Sigma Aldrich), and deionized water was substituted with filtered sample water to mimic the native environment and provide potential energy sources. The sample water was filtered through a 0.2 µm filter (Pall Life Sciences). We named this modified medium as M10-T. Plates were inoculated with a drop of sample, streaked, and incubated at 20–25 °C under

air. Colonies growing on M10-T were picked and streaked to new M10-T plates. This was repeated several times until purified strain NVT^T was obtained.

Strain NVT^T was initially found to grow well on medium M10-T plates together with an *Ancylobacter* species (identified via colony PCR of 16S rRNA genes as having 100 % sequence similarity to *Ancylobacter polymorphus* DSM 2457^T). Members of the genus *Ancylobacter* are abundant in nature, especially in aquatic environments such as lowland marshes, shallow waters and aerobic wastewater treatment ponds [25–29]. A pure culture of strain NVT^T initially grew much more slowly than in mixed culture. It was hypothesized that the companion bacterium provided growth factors not present in the mineral medium. Therefore, growth was tested in medium supplemented with cell extract of the companion bacterium, as well as on several other complex media.

The optimal complex medium was a diluted 20 % strength R2A medium [30], adjusted to pH 8 with NaOH, and supplemented with an *Ancylobacter* species cell-free extract prepared based on a previously described method [31]. To prepare the extract, *Ancylobacter* species culture was grown on the same 20 % R2A medium and harvested in its exponential growth phase. The culture was centrifuged (Beckman Coulter) to separate the cell pellet (C1) and supernatant (S1). C1 was suspended in 500 ml of 20 % strength R2A medium, sonicated, and centrifuged (Beckman Coulter) at 4 °C to separate C1 from its second supernatant (S2). Supernatants (S1 and S2) were filtered with a 0.2 µm filter (Pall Life Sciences). S1, S2 and 1.5 % phytagel (Sigma Aldrich) were combined to make plates of R2A–Ancylo medium and C1 was discarded. This method increased the cell growth of strain NVT^T so that visible growth occurred in 2–3 days, instead of 5–7 days of incubation in liquid medium. However, after several transfers, cells grown on the *Ancylobacter* species extract medium could then be grown subsequently on 20 % R2A medium with a similar enhanced growth rate. Therefore, strain NVT^T was subsequently cultured routinely at 20 °C under aerobic conditions on 20 % strength R2A (pH 8.5) plates without the laborious addition of cell extract. Except where noted, this medium was used for all subsequent physiological tests. On R2A plates after 2–3 days, colonies were pale white, smooth, moist, raised, opaque and circular with smooth margins, measuring 0.5–4 mm in diameter.

Gram staining was performed according to the protocol provided by the Gram Stain Kit No. 212539 (Becton Dickinson). Cell morphology was observed via light microscopy (BX51; Olympus Life Science Solutions) using cells from exponentially growing culture in 20 % R2A broth (pH 8.5, 20 °C). Bacterial flagella staining was performed as described previously [32]. A motility test was done as described previously [33] except that R2A was used as a medium and agar was substituted with 0.4 % phytagel. *Escherichia coli* K-12 strain W3110^T was used as a positive control. Cells of strain NVT^T were Gram-negative, coccoid in shape, and ranged from 0.5 to 1.0 µm in diameter. Electron microscopy (see Fig. S1, available in the online version of this article) showed a Gram-negative cell architecture with a large periplasmic space, a visible nucleoid and internal vesicular structures. Flagella were not observed. Cells were not motile after 2–5 days of incubation at 20 or 37 °C.

To determine the temperature range for growth, isolate NVT^T was grown at 10–50 °C (at intervals of 5 °C) and growth was observed for 2 weeks. Growth at pH 4.0–12.0 (at intervals of 0.5 pH units) was evaluated for 7 d at 20 °C in medium adjusted with 10 % NaOH (Sigma Aldrich) and 10 M H_2SO_4 (Sigma Aldrich). pH was measured with an AB15 pH meter (Fisher Scientific) before and after medium autoclaving (with only minor pH change of <0.2 units). Growth in the presence of 0–4 % (w/v) NaCl (at intervals of 0.5 % NaCl) was investigated for 2 weeks. Anaerobic growth via fermentation was tested under N_2 and N_2/CO_2 (80 : 20, v/v) atmospheres, using resazurin sodium salt (1 mg per litre; Sigma Aldrich) as a redox indicator. Growth was observed for 2 weeks. Growth of strain NVT^T was determined by monitoring the optical density at 600 nm (OD_{600}) using an Ultrospec 10 Cell Density Meter (Amersham Biosciences) daily.

Cells of strain NVT^T were able to grow at 15–40 °C (optimum 25–37 °C). No growth was observed at 10 or 45 °C. Growth was observed from pH 5.5–11 (optimum pH 6.0–8.0). No growth was observed at initial pH 5.0 or pH 11.5. Cells could tolerate up to 3 % of NaCl (w/v). The doubling time on 20 % R2A liquid medium at pH 7 was 0.6 h based on OD_{600} measurements of its growth in 20 °C. No growth was observed under anaerobic conditions.

Catalase and oxidase activities were tested using 3 % (v/v) H_2O_2 and an oxidase reagent dropper (Becton Dickinson), respectively. The catalase test was positive while the oxidase test was negative. Other enzymatic tests were determined using API 20NE and API ZYM kits (bioMérieux) according to the manufacturer's instructions and reported in the species description. The substrate range for growth was tested with the API 50CH kit (bioMérieux). The strain grew on only a few monosaccharides and sugar alcohols, as well as ferric acetate. Detailed results are listed in the species description. In addition, growth on catechol was tested by growing strain NVT^T in 0.25 % (w/v) of dissolved catechol (99 %, Sigma Aldrich) in medium 10 (without carbon sources). Cell growth was negative on catechol.

The ability of NVT^T cells to grow aerobically on a variety of nitrogen sources was investigated by growing them in a nitrogen-free medium (medium 10 without NH_4Cl) with 0.1 % glucose as a carbon source. Cells were grown in 20 ml medium in 100 ml serum bottles containing 10 mM of sodium nitrite, sodium nitrate, urea, aspartic acid, L-valine, L-glycine, L-alanine, L-proline, L-serine or no added fixed nitrogen. All chemicals were purchased from Sigma-Aldrich. Cells were able to use sodium nitrate, urea, L-glycine, L-alanine, L-proline and L-serine, but not L-valine and N_2 gas as nitrogen sources.

Nitrite and nitrate reduction were tested by growing the cells anaerobically in nitrogen-free R2A medium amended with 10 mM sodium nitrite or 10 mM sodium nitrate, in 100 ml serum bottles capped with butyl rubber stoppers and flushed well with N_2 gas. No growth was observed in either test, indicating that strain NVT^T was unable to grow by anaerobic respiration with nitrite or nitrate, nor via fermentation.

For analysis of cellular fatty acids and respiratory quinones, cells were harvested during the late-exponential growth phase (4 days). Cell culture was centrifuged and the pellet was freeze-dried overnight. Analysis of cellular fatty acids after alkaline hydrolysis of cell material was carried out as described previously [34], and the fatty acids were identified as their methyl ester derivatives with gas chromatography–mass spectrometry (GC–MS). Double-bond positions were also determined with GC–MS after dimethyl disulfide (DMDS) derivatization. The major cellular fatty acids for this strain were iso $C_{15:0}$ and anteiso $C_{15:0}$ (Table 1). Isoprenoid quinones were extracted from lyophilized cells according to the method of Collins *et al.* [35] and the profile was analysed by high-performance liquid chromatography (Shimadzu LC 20A; Shimadzu) [36]. The predominant respiratory quinones detected in strain NVT^T were menaquinone MK-7 and MK-6 (80 : 12), which are common among isolated *Verrucomicrobia* species [37, 38].

A draft genome of strain NVT^T was generated to support genomic characterization. The G+C content, 16S rRNA gene sequence and information about the encoded enzyme pathways were determined from the draft genome. Genomic DNA was extracted using the FastDNA SPIN kit (MP Biomedical). A paired-end library was sequenced in 1/10 of a lane of a HiSeq2000 Illumina platform at McGill University and Genome Québec Innovation Centre following the centre guidelines (http://gqinnovationcenter.com/index.aspx). Raw reads were passed through an in-house quality control program as described previously [23]. The draft genome was assembled via Velvet Software version 1.1 [39] using different k-mer size values (51, 55, 59, 63, 67, 71, 75, 79, 83, 87, 91, 95 and 99). The 95 k-mer assembly was selected as the highest quality for the further characterization producing a draft genome of 4.72 Mbp. Annotation was performed by submission to the IMG platform of the Joint Genome Institute website (http://www.jgi.doe.gov/) [40, 41].

Table 1. Major (>1 % of total) cellular fatty acids after alkaline hydrolysis of cell material of strain NVT^T

Values are the average of three individual cultures.

Fatty acids	Total fatty acids (%)
Straight chain	
$nC_{14:0}$	1.4
$nC_{16:0}$	8.4
$nC_{16:1}$ Δ11	9.4
Branched chain	
Iso-$C_{13:0}$	5.7
Anteiso-$C_{13:0}$	2.9
Iso-$C_{14:0}$	9.2
Iso-$C_{15:0}$	17.1
Iso-$C_{15:1}$ Δ4	2.5
Anteiso-$C_{15:0}$	28.4
Iso-$C_{16:0}$	4.1
Iso-$C_{16:1}$ Δ4	2.0
β-Hydroxy	
βOH-C12	3.4
iso-βOH-C13	2.7

Around 4.5 % of the genes detected in the genome were classified into KEGG pathways for xenobiotics biodegradation and metabolism. Key genes included *cat*E (encoding catechol 2,3-dioxygenase) and *pra*C/*xyl*H (encoding 4-oxalocrotonate tautomerase). Both are involved in catechol degradation pathways. The PraC/XylH enzyme is known to oxidatively catabolize various aromatic hydrocarbons into the TCA cycle [42]. Strain NVT^T may feed on intermediate products from the degradation of aromatic compounds in the tailings pond, although the growth test on catechol was negative. Although the test for growth by denitrification was negative, strain NVT^T harbours putative *nrf*A and *nir*K genes, both encoding dissimilatory nitrite reductases, as well as a putative gene encoding *nos*Z (nitrous-oxide *reductase*). Genes for nitrogen fixation (*nif* genes) were not detected. The DNA G+C content of the genome sequence was 66.1 mol%.

The 1440 bp 16S rRNA gene sequence of strain NVT^T obtained from the genome was phylogenetically compared to the most related sequences via BLASTN [43] against the EZTaxon-e database [44] as well as the NCBI 16S rRNA gene sequence database. Sequence analyses suggested that strain NVT^T was phylogenetically related to members of the family *Opitutaceae*. The closest matches included '*Lacunisphaera parvula*' IG15 (95.6 %), '*Lacunisphaera limnophila*' IG16b (95.0 %) and '*Lacunisphaera anatis*' IG31 (95.8 %); none of which have been formerly validated. Among the type strains of validated strains names, *Opitutus terrae* PB90-1^T (93.7 %), *Cephaloticoccus primus* CAG34^T (92.7 %) and *Cephaloticoccus capnophilus* CV41^T (92.3 %) shared the greatest sequence identity to NVT^T. The median 16S rRNA gene sequence identity for divergence of genera is 96.4 % with a suggested threshold of 94.5 % proposed for defining a new genus [45]. Both of these values are higher than the 93.7 % identity of NVT^T to *Opitutus terrae,* which is the closest matching validated strain. A maximum-likelihood phylogenetic tree was generated to determine the phylogenetic position of strain NVT^T compared to other closely related *Verrucomicrobia* species (Fig. 1). Reference 16S rRNA gene sequences were obtained from the ARB SILVA database [46]. The sequence from strain NVT^T was aligned to the database using the SINA aligner [47]. The phylogenetic tree confirmed the BLASTN results showing that the closest relatives of strain NVT^T were *Opitutus terrae* PB90-1^T and the '*Lacunisphaera*' strains (Fig. 1). *In silico* DNA–DNA

hybridization (DDH) values comparing the draft genome of NVT^T to other *Opitutaceae* family members were inferred by using the online tool, the Genome-to-Genome Distance Calculator (GGDC) version 2.1 (http://ggdc.dsmz.de) [48]. Comparisons against all strains tested gave values <21 % (Table 2).

Chemotaxonomic characterization of *Opitutaceae* family members is generally very limited as, for example, quinone data are unavailable for other strains. However, strain NVT^T can still be differentiated from its closest validated relative *Opitutus terrae* PB90-1^T, as well as from other validated and not-yet-validated strains of the family (Table 2). The 16S rRNA gene identity and estimated DDH values between strain NVT^T and *Opititus terrae* PB90-1^T were well below the usual species or genus cutoff levels. Strain NVT^T is an obligate aerobe, differentiating it from the obligately anaerobic *Opitutus terrae* PB90-1^T, as well as from '*Didymococcus colitermitum*' TAV2 and *Alterococcus agarolyticus* ADT3^T. Strain NVT^T was capable of growth up to pH 11, making it the most alkalitolerant of all *Opitutaceae* strains described (Table 2). The '*Lacunisphaera*' strains, if validated, will probably prove to be the most closely related to strain NVT^T, but even based on presently available information these differ in pH tolerance, presence of oxidase and catalase, and substrate utilization patterns. Based on the results of the phylogenetic, biochemical and physiological analyses, strain NVT^T represents a novel genus within the family *Opitutaceae* and is proposed as the type strain of the

Table 2. Differential genetic and phenotypic characteristics of *Oleiharenicola alkalitolerans* NVT^T and the most closely related species. The GGDC indicates the estimated *in silico* DNA–DNA hybridization value of each strain against NVT^T

Strains: 1, *Oleiharenicola alkalitolerans* NVT^T; 2, *Opitutus terrae* PB90-1^T [6, 21]; 3, '*Didymococcus colitermitum*' TAV2 [16, 17]; 4, *Alterococcus agarolyticus* ADT3^T [19]; 5, *Cephaloticoccus primus* CAG34^T [20]; 6, *Cephaloticoccus capnophilus* CV41^T [20]; 7, '*Lacunisphaera parvula*' IG15 [18]; 8, '*Lacunisphaera limnophila*' IG16b [18]; 9, '*Lacunisphaera anatis*' IG31 [18]. +, Positive; W, weakly positive; –, negative; ND, no data available; NA, not applicable. Data for strain NVT^T were obtained from present research and other data were gathered from previously published sources as noted above. For growth temperature, pH, and NaCl levels, values in brackets are the known optimum conditions. All six strains are Gram-negative, with cocci and diplococci cell morphology, and sizes ranging from 0.5 to 1 µm in length.

Characteristic	1	2	3	4	5	6	7	8	9
Isolation source	Oilsands tailings pond	Rice paddy soil	Termite gut	Hot springs	Ant gut	Ant gut	Freshwater lake	Freshwater lake	Freshwater lake
% 16S rRNA gene identity	ref.	93.7	93.1	88.3	92.7	92.3	95.6	95.0	95.8
GGDC	ref.	19.1±2.3	20.0±2.4	NA	20.4±2.4	20.4±2.4	NA	20.9±2.5	NA
Aerobe/anaerobe	Obligate aerobe	Obligate anaerobe	Microaerophile	Facultative anaerobe	Obligate aerobe	Obligate aerobe	Obligate aerobe	Obligate aerobe	Obligate aerobe
Motility	–	+	–	+	–	–	+	+	+
Colony colour	White	Colourless	Colourless	White	Cream	Cream	Cream	Cream	Cream
Growth temperature (°C)	15–40 (25–37)	10–37	15–35 (30)	38–58 (48)	23–37 (37)	23–37 (37)	12–38 (33)	13–36 (32)	15–36 (30)
Growth pH	5.5–11 (6-8)	5.5–9 (7.5–8)	5.5–7.5 (7)	7–8.5	6.9–7.7 (7)	6.9–7.3 (7)	6–9 (7.5–8)	6–9 (7.5–8)	6–9 (7.5–8)
NaCl tolerance (%, w/v)	3	3	1.5	1–3.5 (2)	0.5–1.5 (1)	0.5–1.5 (1)	ND	ND	ND
G+C content (mol%)	66.1	65.3	60.5	65.8	60.7	60.5	65.9	66.5	67.2
N_2 fixation/*nif* genes	–	–	+	ND	–	–	ND	–	ND
Nitrate reduction:	–	+	ND	ND	–	–	ND	ND	ND
Catalase	+	–	–	+	–	–	–	–	–
Oxidase	–	–	–	+	–	–	+	+	+
Assimilation tests:									
Arabinose	W	+	–	–	–	–	–	–	–
Mannose	–	+	–	W	+	+	+	+	–
Mannitol	+	+	–	–	+	+	–	–	–
Maltose	–	+	+	ND	+	+	+	+	–
Sucrose	–	+	–	+	+	+	+	+	–
Lactose	–	+	ND	+	+	+	+	+	–
Xylose	+	–	–	+	ND	ND	ND	ND	ND
Melibiose	–	+	ND	–	+	–	+	+	–
Fructose	+	+	–	ND	+	+	+	+	+
Cellobiose	–	+	+	+	+	+	+	+	–
Major fatty acids (%)	Anteiso-$C_{15:0}$ (28.4) iso-$C_{15:0}$ (17.1)	ND	Anteiso-$C_{15:0}$ (33.0) iso-$C_{15:0}$ (21)	Anteiso-$C_{15:0}$ (51.5) iso-$C_{15:0}$ (2.1)	Anteiso-$C_{15:0}$ (55.4) iso-$C_{15:0}$ (2.7)	Anteiso-$C_{15:0}$ (58.0) iso-$C_{15:0}$ (1.3)	Anteiso-$C_{15:0}$ (-) iso-$C_{15:0}$ (33.3)	Anteiso-$C_{15:0}$ (12.1) iso-$C_{15:0}$ (48.6)	Anteiso-$C_{15:0}$ (10.6) iso-$C_{15:0}$ (9.06)

new genus and species, *Oleiharenicola alkalitolerans* gen. nov., sp. nov.

DESCRIPTION OF *OLEIHARENICOLA* GEN. NOV.

*Oleiharenicola (*O.le.i.ha.re.ni'co.la. L. n. *oleum*, oil; L. n. *harena*, sand; L. suffix *-cola,* inhabitant, dweller; N.L. masc. n. *Oleiharenicola* an inhabitant of oil sand, referring to the oilsands process-affected water, from where the type strain was isolated).

Non-spore-forming, non-motile, mesophilic and strictly aerobic chemoorganoheterotrophic bacteria. Gram-negative cocci or diplococci (0.5–1.5 µm) that occur mainly as single cells or pairs. No flagella are observed. Belongs to the family *Opitutaceae*, closest neighbour is the genus *Opitutus*. Menaquinones MK-7 and MK-6 are major respiratory quinones. The major cellular fatty acids are iso-$C_{15:0}$ and anteiso-$C_{15:0}$. The type species of the genus is *Oleiharenicola alkalitolerans* sp. nov.

DESCRIPTION OF *OLEIHARENICOLA ALKALITOLERANS* SP. NOV.

Oleiharenicola alkalitolerans [al.ka.li.to'le.rans. N.L. n. *alkali* (from Arab. al qali) alkali, L. part. pres. *tolerans*, tolerating. N.L. part. adj. *alkalitolerans,* referring to an alkali-tolerating micro-organism].

Colonies grown on 20 % R2A plates are pale white, smooth, moist, raised, opaque and circular with smooth margins, measuring around 0.5–4 mm in diameter. Able to grow at 15–40 °C (optimum 25–37 °C) and pH 5.5–11 (optimum pH 6.0–8.0). Can tolerate up to 3 % NaCl (w/v) but does not require NaCl to grow. Doubling time under optimum conditions is 0.6 h. Catalase-positive and oxidase-negative. Negative for growth on catechol, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis and assimilation of mannose, n-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Cells are positive for urease, β-glucosidase hydrolysis (aesculin), protease hydrolysis (gelatin), β-galactosidase, and glucose, arabinose, mannitol and malate assimilation. The strain is able to metabolize the following carbon sources: of D-galactose, D-glucose, D-fructose, D-mannitol, D-sorbitol, aesculin, ferric acetate, xylitol, D-xylose, D-fucose and D-arabitol. The cells fail to metabolize the following compounds: D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, gentiobiose, turanose, D-tagatose, L-fucose, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. For enzymatic activities, positive reactions are observed for alkaline phosphatase, esterase lipase (C8), trypsin, α-chymotrypsin, acid phosphatase and naphtol-AS-BI-phosphohydrolase, and negative for esterase (C4), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Able to use sodium nitrate, urea, L-glycine, L-alanine, L-proline and L-serine, but not L-valine and N_2 gas, as nitrogen sources. The type strain, NVT^T (=ATCC BAA-2697^T,=DSM 29249^T), was isolated from oilsands process-affected water in Alberta, Canada. The DNA G+C content of the type strain is 66.1 mol%.

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

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