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Alcanivorax dieselolei sp. nov., a novel alkane-degrading bacterium isolated from sea water and deep-sea sediment

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Two bacterial strains, B-5^T and NO1A, were isolated from the surface water of the Bohai Sea and deep-sea sediment of the east Pacific Ocean, respectively. Both strains were halophilic, aerobic, Gram-negative, non-spore-forming, catalase- and oxidase-positive motile rods. They grew on a restricted spectrum of organic compounds, including some organic acids and alkanes. On the basis of 16S rRNA gene sequence similarity, strains B-5^T and NO1A were shown to belong to the *γ-Proteobacteria*. Highest similarity values were found with *Alcanivorax venustensis* (95·2 %), *Alcanivorax jadensis* (94·6 %) and *Alcanivorax borkumensis* (94·1 %). Principal fatty acids of both strains were $C_{16:0}$, $C_{16:1}\omega7c$ and $C_{18:1}\omega7c$. The chemotaxonomically characteristic fatty acid $C_{19:0}$ cyclo $\omega8c$ was also detected. On the basis of the above, together with results of physiological and biochemical tests, DNA–DNA hybridization, comparisons of 16S–23S internal transcribed spacer sequences and comparisons of the partial deduced amino acid sequence of alkane hydroxylase, both strains were affiliated to the genus *Alcanivorax* but were differentiated from recognized *Alcanivorax* species. Therefore, a novel species, *Alcanivorax dieselolei* sp. nov., represented by strains B-5^T and NO1A is proposed, with the type strain B-5^T (=DSM 16502^T=CGMCC 1.3690^T).

The genus *Alcanivorax* comprises three recognized species at present. The type species, *Alcanivorax borkumensis*, was first described in 1998 to accommodate Gram-negative, halophilic, aerobic *γ-Proteobacteria* that use aliphatic hydrocarbons as the sole source of carbon and energy (Yakimov *et al.*, 1998). A second species, *Alcanivorax venustensis*, was subsequently described and the misclassified species [*Fundibacter*] *jadensis* was assigned to *Alcanivorax jadensis* (Fernández-Martínez *et al.*, 2003). Since 1998, the isolation of *Alcanivorax* species, or detection of their 16S rRNA gene sequences, from diverse habitats worldwide has been reported increasingly. Thus they are regarded as cosmopolitan bacteria. This group of marine bacteria exclusively uses petroleum oil hydrocarbons as sources of carbon and energy, and has been used for bioremediative interventions in polluted marine and coastal systems. In addition, *Alcanivorax* species have obvious potential to produce biocatalysts in non-polluting industrial processes and to act as a biosensor for *in situ* monitoring of aromatic or aliphatic compounds (Golyshin *et al.*, 2003).

Strain B-5^T was isolated from oil-contaminated surface water of the Bohai Sea at the Yellow River dock of Shengli oilfield in November 2001; this dock had suffered a long period of crude oil pollution. A second strain, designated NO1A, was retrieved from a deep-sea sediment sample in the east Pacific Ocean. This was collected by a multi-core sampler from Pacific nodule region A station (7° 13′ 46″ N, 153° 52′ 19″ W, 5027 m water depth), a specific area with polymetallic nodules abundant on the sea bottom, during cruise DY105-11 of *DAYANG Number 1* in 2001. The sediment samples were loaded into sterile Falcon tubes aboard ship and stored at −20 °C until use.

The artificial sea water medium (ASM) used for enrichment contained (per litre of distilled water) 10 g diesel fuel, 24 g NaCl, 7·0 g $MgSO_4.7H_2O$, 1 g NH_4NO_3, 0·7 g KCl, 2·0 g

Abbreviations: AlkB (*alkB*), alkane hydroxylase; ITS, internally transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the nucleotide sequences reported in this study are AY683537 (*A. dieselolei* B-5^T, 16S rRNA gene), AY683538 (B-5^T, large ITS), AY683539 (B-5^T, small ITS), AY683540 (B-5^T, partial *alkB* gene), AY683531 (*A. dieselolei* NO1A, 16S rRNA gene), AY683532 (NO1A, large ITS), AY683533 (NO1A, small ITS), AY683534 (NO1A, partial *alkB* gene), AY683536 (*A. jadensis* T9^T, partial *alkB* gene) and AY683535 (*A. venustensis* ISO4^T, partial *alkB* gene).

Transmission electron micrographs of cells of strains B-5^T and NO1A and dendrograms showing the phylogenetic positions of the two strains plus recognized members of the genus *Alcanivorax* based on 16S rRNA, ITS and *alkB* gene sequences are available as supplementary figures in IJSEM Online, together with a table giving DNA–DNA relatedness values.

KH_2PO_4, 3·0 g Na_2HPO_4 and 10 ml trace element solution, pH 7·5. Trace element solution contained (per litre of distilled water) 2 mg $CaCl_2$, 50 mg $FeCl_3.6H_2O$, 0·5 mg $CuSO_4$, 0·5 mg $MnCl_2.4H_2O$ and 10 mg $ZnSO_4.7H_2O$. Strains B-5^T and NO1A were cultivated on HLB and SM1 media. HLB was modified from Luria–Bertani (LB) medium (Sambrook *et al.*, 1989), with the concentration of NaCl increased to 30 g l^{-1}. HLB was also used to check the presence of contaminants because both strains showed limited growth on this medium. SM1 (which was described by Yakimov *et al.*, 1998) was used for routine cultivation of the isolates and most phenotypic tests, when supplemented with 10 g n-alkanes l^{-1} or 10 g sodium citrate l^{-1} as the sole carbon source. All cultures were incubated at 28 °C and spun at 200 r.p.m. unless noted otherwise.

General cell morphology was studied under an Olympus inverted microscope using 3-day-old cultures of the strains grown on HLB agar. For electron microscopy, exponential-phase cells were harvested, subsequently suspended and absorbed on a Formvar–carbon-coated grid, then stained with phosphotungstic acid. Cells of both strains were Gram-negative, rod-shaped with lophotrichous flagella, and varied from 0·8 to 2·0 μm in length and from 0·3 to 0·7 μm in width (see Supplementary Fig. A in IJSEM Online). The optimal growth temperature was determined over the temperature range 4–55 °C. Sodium requirement was examined at 0, 0·5, 1, 5, 7, 10, 15 and 20 % (w/v) NaCl. The following physiological and biochemical properties were examined according to standard methods: glucose fermentation, denitrification, catalase and oxidase activities, gelatin liquefaction and Tweenase, agarase, gelatinase, amylase and arginine dihydrolase activities. Results are given in the species description.

Tests for use of various organic substrates as sole carbon sources at a concentration of 0·1 % (w/v) were performed in 5 ml SM1 medium. The strains were characterized using Biolog GN plates as described by Ivanova *et al.* (1998). These results are also given in the species description. The chain length range of n-alkanes oxidized by strain B-5^T was determined according to the method of Smits *et al.* (2002) except that rhamnolipids and dioctylphthalate were not used. Experiments were repeated at least three times. Tests of surface tension were performed after 7 days incubation on SM1 medium supplemented with n-alkanes using a Du Noüy ring tension meter (McInerney *et al.*, 1990). To test the ability of strain B-5^T to produce surface-active glucolipids, total lipid was extracted and fractionated using the method described by Yakimov *et al.* (1998). Glucolipids were further separated by TLC and analysed using electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (MSMS). Both strains were able to utilize various n-alkanes as the sole carbon source, ranging in chain length at least from C_5 to C_{36}, which was wider than that of *A. borkumensis* (C_6 to C_{20}; van Beilen *et al.*, 2004). Plentiful growth was observed with C_8 to C_{28} n-alkanes, while growth on C_5, C_6 and C_7 n-alkanes was weak, probably because of the high volatility of these compounds, such that they are only poorly available to cells, or because of their toxicity as cell membrane lipid solvents. For the long-chain n-alkanes, without adding any surfactants, both strains could grow slowly on C_{32} and C_{36}, although they were barely soluble in water. In addition, when strain B-5^T utilized C_{24} as the sole carbon source, the surface tension of the culture was reduced from 71·3 to 42·4 mN m^{-1} after 7 days cultivation. These results suggested that B-5^T produced a biosurfactant. However, ESI-MS and MSMS failed to detect the presence of glucolipid-like compounds (data not shown), which were the typical products of *A. borkumensis* (Yakimov *et al.*, 1998; Abraham *et al.*, 1998).

Antibacterial activities were assessed as described by Kobayashi *et al.* (2003). Strain B-5^T was sensitive to neomycin, kanamycin, amikacin and polymyxin B, but resistant to fortum, cefuroxime, cephradin, cefazolin, cefalexin, piperacillin, carbenicillin, ampicillin, oxacillin, penicillin, erythromycin, minomycin, vibramycin, tetracycline, gentamicin, cefobid, rocephin, vancomycin, ofloxacin, midecamycin, ciprofloxacin, norfloxacin, furazolidone, clindamycin, chloromycetin and co-trimoxazole. By contrast, strain NO1A was only sensitive to polymyxin B within the above antibiotics.

Cellular fatty acid analysis was carried out at the identification service laboratories of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). The fatty acid profiles of both strains are shown in Table 1. The three major components, fatty acids $C_{16:0}$, $C_{16:1}\omega 7c$ and $C_{18:1}\omega 7c$, found in both strains and recognized *Alcanivorax* species were also the principal fatty acids in members of the genera *Comamonas*, *Delftia* (Tamaoka *et al.*, 1987) and *Hydrogenophaga* (Willems *et al.*, 1989) as well as in species of the genera *Alicycliphilus* (Mechichi *et al.*, 2003) and *Oceanisphaera* (Romanenko *et al.*, 2003). No conclusions could thus be drawn from the major fatty acid profiles. The minor fatty acid profiles of the two strains were more similar to *A. venustensis* ISO4^T than to *A. borkumensis* SK2^T or *A. jadensis* T9^T. Fatty acid $C_{19:0}$ cyclo $\omega 8c$, which was suggested as a characteristic chemotaxonomic marker of *A. venustensis* (Fernández-Martínez *et al.*, 2003), was also detected in substantial amounts in strains B-5^T and NO1A.

For genotypic characterization, DNA was prepared according to the method of Ausubel *et al.* (1995). The G + C content of the DNA was determined by HPLC after digestion of the DNA with nuclease P1 (Tamaoka & Komagata, 1984; Mesbah & Whitman, 1989). The 16S rRNA gene was amplified by PCR using the following primers: 16SF (positions 8–27 of the *Escherichia coli* numbering; 5′-AGAGTTT-GATCCTGGCTCAG-3′) and 16SR (positions 1512–1493, 5′-ACGGCTACCTTGTTACGACT-3′). The 16S–23S internally transcribed spacer (ITS) regions of strains B-5^T and NO1A were amplified with primers as described by García-Martínez *et al.* (1996, 1999). To amplify the partial fragment of the putative alkane hydroxylase gene (*alkB*), highly degenerate primers, MonF (positions 401–419,

Table 1. Fatty acid compositions of strains B-5^T/NO1A, *A. borkumensis* SK2^T, *A. venustensis* ISO4^T and *A. jadensis* T9^T

Strains: 1, B-5^T/NO1A (this study); 2, *A. borkumensis* SK2^T (Yakimov *et al.*, 1998); 3, *A. venustensis* ISO4^T (Fernández-Martínez *et al.*, 2003); 4, *A. jadensis* T9^T (Bruns & Berthe-Corti, 1999).

Fatty acid	1	2	3	4
$C_{10:0}$	2·55/3·29	–	3·2	–
$C_{10:0}$ 3OH	–/0·16	–	–	–
$C_{11:0}$	0·09/–	–	–	–
$C_{12:0}$	8·89/7·9	–	5·1	5·2
$C_{12:0}$ 2OH	0·92/1·12	–	–	1·1
$C_{12:0}$ 3OH	2·91/7·18	–	10·7	4·9
$C_{14:0}$	0·58/0·51	1·1	1·4	1·7
$C_{15:1}\omega 6c$	–/–	–	1·1	–
$C_{15:0}$	–/–	–	2·0	–
anteiso-$C_{15:0}$	–/–	–	–	0·2
$C_{16:1}\omega 7c$	11·32*/11·87*	17·1†	15·4	13·5
$C_{16:0}$ N alcohol	–/1·25	–	–	–
$C_{16:0}$	32·12/27·0	31·5	20·2	23·4
$C_{17:1}\omega 8c$	–/–	–	0·5	–
$C_{17:0}$ cyclo	1·11/0·20	–	1·4	–
$C_{17:0}$	0·25/0·48	–	2·8	–
$C_{18:1}\omega 9c$	–/–	–	1·2	–
$C_{18:1}\omega 7c$	22·41/31·04	47·1‡	19·9	20·7
$C_{18:1}\omega 5c$	–/–	0·2	–	–
$C_{18:0}$	0·64/0·89	2·0	0·9	–
anteiso-$C_{18:1}\omega 7c$	–/–	–	1·2	–
$C_{19:0}$ cyclo $\omega 8c$	14·27/5·39	–	10·1	–
Unknown	1·92/1·49	0·9	1·5	29·2

*Sum of $C_{16:1}\omega 7c$/$C_{15:0}$ iso 2OH.
†Sum of $C_{16:1}\omega 7c$/$C_{16:1}\omega 9t$ (Yakimov *et al.*, 1998).
‡Sum of $C_{18:1}\omega 7c$/$C_{18:1}\omega 7t$/$C_{18:1}\omega 9t$/$C_{18:1}\omega 12c$ (Yakimov *et al.*, 1998).

Pseudomonas putida GPo1 *alkB* numbering; 5′-TCAAYA-CMGSNCAYGARCT-3′) and MonR (positions 820–801; 5′-CCGTARTGYTCNAYRTARTT-3′), were generated based on the conserved regions of several alkane hydroxylase gene sequences available in the GenBank database. The thermal cycles were taken in a T3 thermal cycler (Biometra). PCR products were purified and recovered by using a UNIQ-5 Column DNA Gel Extraction Kit (Sangon). Sequencing of the fragments was carried out on a model 377 automated DNA sequencer using a BigDye Terminators Cycle Sequencing Kit (Applied Biosystems). Sequence data were manually aligned with nucleotide sequences obtained from GenBank by using DNAMAN (version 5.1; Lynnon Biosoft). Alignments and phylogenetic analysis of ITS and *alkB* sequences were also carried out by using the DNAMAN program. Phylogenetic dendrograms of 16S rRNA gene sequences were constructed by three different algorithms: the neighbour-joining method (Saitou & Nei, 1987) using DNAMAN, and the maximum-likelihood (Felsenstein & Churchill, 1996) and maximum-parsimony (Fitch, 1971) methods using the PHYLIP package (version 3.6a2.1; Felsenstein, 2004). Bootstrapping analysis was used to evaluate the tree topology of the data obtained from the three algorithms based on 1000 resamplings.

Nearly full-length 16S rRNA gene sequences (1504 nt) of strains B-5^T and NO1A were determined. Sequence similarity between the two strains was 99·6 %. Their closest relatives were *A. venustensis* ISO4^T (95·2 %), *A. jadensis* T9^T (94·6 %) and *A. borkumensis* SK2^T (94·1 %). In all the three phylogenetic trees, strains B-5^T and NO1A were included in the *Alcanivorax* cluster, whose integrity was supported in 100 % of the trees generated. The topology of the phylogenetic tree, shown in Fig. 1 (a more complete tree is available as Supplementary Fig. B in IJSEM Online), was reconstructed by using the neighbour-joining method. In Fig. 1, both strains branched with *A. venustensis*, but this branching point was only recovered in 570 trees out of 1000 generated in the bootstrap analysis. Similarly, the bootstrap value of this branch point was 52 % for maximum-parsimony analysis and 58 % for maximum-likelihood analysis, which indicated that the branching pattern was not stable.

For ITS sequence comparison, PCR amplifications yielded two-band products in both strains. The lengths of the 16S–23S ITS sequences of strain B-5^T were 612 and

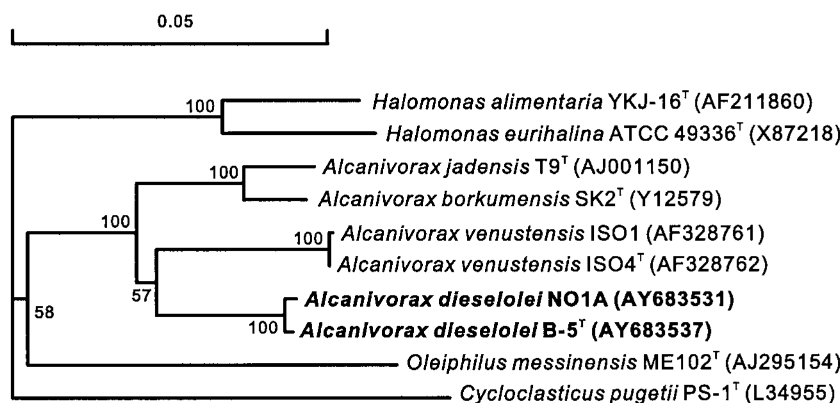

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strains B-5^T and NO1A and representatives of some other related taxa, based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0·05 nucleotide substitution rate (K_{nuc}) units.

322 nt, respectively. For strain NO1A, these were 585 and 322 nt, respectively. This indicated that strains B-5^T and NO1A carried at least two rRNA operons. The large ITS of each strain contained two tRNA genes (Ile, Ala) that were absent in the small ITS. These features were in good agreement with those of other *Alcanivorax* species. Sequence analysis of the ITS confirmed the same groupings as that for the 16S rRNA gene (see Supplementary Fig. C in IJSEM Online). The ITS sequences of both strains and recognized *Alcanivorax* species had a mean variation of $23{\cdot}22 \pm 14{\cdot}79$ %, which was still within the limit of a single and well-defined genus (García-Martínez & Rodríguez-Valera, 2000; Fernández-Martínez *et al.*, 2003).

In the alkane hydroxylase (AlkB) sequence comparisons, a 420 nt DNA fragment was obtained from strain B-5^T and encoded a polypeptide that showed 49·6 and 64·5 % similarity to the corresponding internal region of the *Pseudomonas putida* GPo1 AlkB and *Burkholderia cepacia* RR10 AlkB sequence, respectively. The amplified fragment contained two histidine motifs and a fourth motif (NYXEHYG[L/M]) conserved among the alkane hydroxylases (Shanklin *et al.*, 1994; Smits *et al.*, 1999). Similarly, the partial putative *alkB* genes were amplified from strain NO1A, *A. venustensis* ISO4^T and *A. jadensis* T9^T, but failed from *A. borkumensis* SK2^T (AlkB sequence data for *A. borkumensis* used in alignment were from van Beilen *et al.*, 2004). Alignment of deduced partial AlkB sequences was generated on the basis of these 420 nt internal gene fragments. The result showed that the AlkB sequences of strains B-5^T and NO1A were nearly identical and closely related to that of *A. venustensis* ISO4^T. The AlkB sequences of *A. jadensis* T9^T and *A. borkumensis* SK2^T formed a deep cluster and a separate group from the other taxa investigated (see Supplementary Fig. D in IJSEM Online). According to van Beilen *et al.* (2003), there was no clear linkage between the diversity of the *alkB* genes and phylogenetic lines. Nevertheless, when a particular genus, such as *Mycobacterium* or *Burkholderia*, was analysed independently, the phylogenetic tree of its partial AlkB was highly coincident with that of its 16S rRNA gene sequence (data not shown), as was the case for *Alcanivorax*.

DNA–DNA relatedness was determined using genomic DNA from the two strains and type strains of all *Alcanivorax* species using the method described by Coram & Rawlings (2002) and Tonjum *et al.* (1998). The genomic DNA of *Escherichia coli* DH5α was used as an outgroup sample. Each membrane contained salmon sperm DNA (Sigma) as a negative control. Quantification of hybridization signals was carried out on a White/ultraviolet transilluminator (UVP) using Grab-IT 2.51 and GelBase/GelBlot-Pro 3.00 (Synoptics). The results are shown in Supplementary Table A in IJSEM Online. Each value was the mean of at least two hybridization experiments. Strains B-5^T and NO1A showed high DNA–DNA relatedness with each other (92 %), but were distinct from the type strains of *Alcanivorax* species based on low levels of DNA–DNA relatedness (13–45 %). Notably, all strains had very low levels of DNA–DNA relatedness with *E. coli* DH5α ($\leqslant$7 %). DNA–DNA relatedness among *A. borkumensis* SK2^T, *A. jadensis* T9^T and *A. venustensis* ISO4^T fell in the range 17–49 %. A comparatively high level of DNA–DNA relatedness (36–45 %) was found between *A. venustensis* ISO4^T and the two novel strains.

According to the present results, strains B-5^T and NO1A shared high similarities in phenotypic and genotypic characteristics, such as 99·6 % 16S rRNA gene sequence similarity and 92 % DNA–DNA relatedness. Although isolated from two completely different marine habitats, the two strains should be classified as representing a single species. Phylogenetic analysis based on 16S rRNA gene sequence comparison showed that strains B-5^T and NO1A represented a novel species of the *γ-Proteobacteria*, branching within the clade of *Alcanivorax* and forming a distinct branch with *A. venustensis*. We initially considered that the two strains and *A. venustensis* should be classified in a new genus based on their close relationship in 16S rRNA, ITS and *alkB* gene sequence data and fatty acid composition. However, this hypothesis was precluded for the following reasons. Firstly, there were no distinct and decisive traits to differentiate them from other members of the genus *Alcanivorax*. Secondly, the two strains and recognized species of the genus *Alcanivorax* shared comparatively high DNA–DNA relatedness values (13–45 %). Thirdly, the branching point of the cluster consisting of strains B-5^T and NO1A and *A. venustensis* was statistically insignificant. Lastly, insufficient data regarding the fatty acids profiles of *A. borkumensis* and *A. jadensis*, as discussed by Fernández-Martínez *et al.* (2003), made it difficult to draw any definitive conclusions.

Thus, according to their phylogenetic relationships, DNA–DNA hybridization, ITS and *alkB* gene sequence comparisons and phenotypic traits, strains B-5^T and NO1A exhibited characteristics that defined the genus *Alcanivorax*. In addition, both strains have a number of distinct phenotypic features that allow them to be distinguished from other *Alcanivorax* species (Table 2), such as the ability to grow at 45 °C and to utilize citrate, succinate and long-chain n-alkanes. Therefore, we consider that the species represented by strains B-5^T and NO1A belongs to a novel species of the genus *Alcanivorax*, for which the name *Alcanivorax dieselolei* sp. nov. is proposed.

Description of *Alcanivorax dieselolei* sp. nov.

Alcanivorax dieselolei (die.sel.o'le.i. N.L. masc. n. *dieselius* latinized family name of Rudolf Diesel; L. gen. neut. n. *olei* of oil; N.L. gen. neut. n. *dieselolei* of diesel oil, the sole carbon source used in the isolation procedure of the two strains).

Cells are 0·8–2·0 μm long and 0·3–0·7 μm wide, motile, lophotrichous, non-spore-forming, Gram-negative rods. Colonies on HLB agar are characteristically small, translucent, non-pigmented and slightly raised in the centre, with

Table 2. Characteristics that differentiate *A. dieselolei* sp. nov. from other members of the genus *Alcanivorax*

Taxa: 1, *A. dieselolei* (this study); 2, *A. borkumensis* (Yakimov *et al.*, 1998); 3, *A. venustensis* ISO4[T] (Fernández-Martínez *et al.*, 2003); 4, *A. jadensis* (Bruns & Berthe-Corti, 1999). Data for utilization of organic acids for *A. dieselolei* were obtained from tests in SM1 medium. ND, No data; +, positive reaction or growth; −, no reaction or growth.

Characteristic	1	2	3	4
DNA G+C content (mol%)	62·1	53–54	66·4	63–64
Motility, flagella arrangement	+, Lophotrichous	−	+, Polar	−
NO_3^- reduction	+	+	−	+
Ionic requirements	Na^+	Complex (Na^+, Mg^{2+})	Complex	Na^+
Utilization of organic acids:				
Citrate	+	−	−	−
Succinate	+	−	−	−
β-Oxoglutarate	−	+	−	ND
γ-Hydroxybutyrate	+	−	+	ND
Optimal temperature (°C)	28–35	25–30	23–25	30
Growth at 4 °C	−	−	+	−
Growth at 45 °C	+	−	−	−
Maximal NaCl concentration (%)	15·0	12·5	20·0	15·0
Optimum NaCl concentration (%)	3·0–7·5	3·0–10·0	3·0–10·0	3·0

irregular, transparent and halo-like peripheries. Mesophilic. Growth temperature ranges from 15 to 45 °C (optimum 28 °C). NaCl is required for growth; cells grow in 1–15 % NaCl (optimum 3–7·5 %). Actively degrades Tween 80; catalase- and oxidase-positive, but negative for agarase, arginine dihydrolase, amylase and gelatinase. Nitrate is reduced to nitrite. Among the 95 carbon sources in the Biolog system, positive for Tweens 40 and 80, methyl pyruvate, mono-methyl succinate, acetic acid, citric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxy phenyl acetic acid, DL-lactic acid, propionic acid, bromo succinic acid and 2,3-butanediol. Negative for all carbohydrates using Biolog GN. Good growth occurs in SM1 medium with citrate, *p*-hydroxyphenylacetate, pyruvate, lactate or n-alkane as carbon source. Sensitive to neomycin, kanamycin, amikacin and polymyxin B. Cells are able to degrade n-alkanes with chain length C_5 to C_{36}. Cellular fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$, $C_{16:1}\omega7c$, $C_{19:0}$ cyclo $\omega8c$, $C_{12:0}$ and $C_{12:0}$ 3OH. G+C content of the DNA is 62·1–62·5 mol%. Table 2 shows characteristics used to distinguish strain B-5[T] from other members of the genus *Alcanivorax*.

The type strain, B-5[T] (=DSM 16502[T]=CGMCC 1.3690[T]), was isolated from an oil-contaminated sea water at the Yellow River dock of Shengli oilfield, Bohai Sea. Strain NO1A was isolated from a deep-sea sediment sample in the Pacific nodule region A station (5027 m water depth).

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