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Halomonas bachuensis sp. nov., Isolated from Gobi Soil

Zijun Xiao[1] · Jie Shen[1] · Zhuo Wang[1] · Fanghui Dong[1] · Jing-yi Zhao[1]

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Abstract

A novel aerobic bacterium designated DX6^T was isolated from a Gobi soil sample collected in Bachu County, China. Cells are Gram-stain-negative and rod-shaped and colonies are creamy, circular and smooth. The growth range of NaCl concentration was 1–15% (optimum 2–10%, *w*/*v*). Growth occurs at 10–45 °C (optimum 37 °C) and pH 5.0–10.0 (optimum pH 7.0–9.0). Phylogenetic analysis of the 16S rRNA gene sequences indicated that strain DX6^T formed a distinct lineage in the clade of genus *Halomonas* and is related to *Halomonas desiderata* DSM 9502^T (98.3%), *Halomonas kenyensis* AIR-2^T (97.7%), *Halomonas daqingensis* DQD2-30^T (97.6%), *Halomonas saliphila* LCB169^T (97.4%) and *Halomonas endophytica* MC28^T (96.2%). Analysis of the housekeeping genes *gryB* and *rpoD* and calculation of the average nucleotide identities and the digital DNA-DNA hybridization values between strain DX6^T and the related type *Halomonas* strains further revealed that strain DX6^T represented a distinct species. The main respiratory quinones of strain DX6^T were ubiquinone 9 (Q-9) and ubiquinone 8 (Q-8). The predominant cellular fatty acids were summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and $C_{16:0}$. The major polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unidentified phospholipids, an unidentified phosphatidylglycolipid, and four unidentified lipids. Based on the phenotypic, phylogenetic, chemotaxonomic and genomic features, strain DX6^T represents a novel species of the genus *Halomonas*. The name *Halomonas bachuensis* sp. nov. is proposed with strain DX6^T (= CCTCC AB 2020094^T = KCTC 82196^T) designated as the type strain.

Introduction

Halomonas is the type genus of the family of *Halomonadaceae*. Currently the genus includes more than 90 species with validly published names (https://lpsn.dsmz.de/genus/halomonas). A prominent feature of the genus *Halomonas* is halophily and/or halotolerance and the species of *Halomonas* typically occur in saline or hypersaline environments. From the variety of habitats and phenotypic heterogeneity, members of *Halomonas* may be regarded as ubiquitous, versatile chemoheterotrophs [1]. They are important candidates to make significant contributions to a wide array of biotechnological applications [2, 3]. In this study we determine the taxonomic position of a novel *Halomonas* isolate DX6^T.

✉ Zijun Xiao
zjxiao@upc.edu.cn

Jie Shen
shenjie@s.upc.edu.cn

Zhuo Wang
z17030395@s.upc.edu.cn

Fanghui Dong
z16030327@s.upc.edu.cn

Jing-yi Zhao
jyzhao@upc.edu.cn

[1] Center for Bioengineering and Biotechnology, College of Chemical Engineering, China University of Petroleum (East China), Qingdao 266580, China

Materials and Methods

Strains and Culture Conditions

The Gobi soil sample was collected from Bachu County, which locates on the northwest of the Taklimakan Desert, China (39°53′10″N, 78°27′8″E). The soil sample was suspended in 0.9% physiological saline solution, serially diluted and spread onto Luria-Bertani (LB) agar. After 2–3 days of incubation at 30 °C, individual colonies of distinct morphology were picked and purified. The isolates were routinely cultured on LB agar aerobically at 30 °C and maintained as glycerol suspensions (15%, *v*/v) at −80 °C. The 16S rRNA genes of all the isolates were sequenced and analyzed (as

described below) and a potential novel species closely related to *Halomonas desiderata* was further identified in this study. *H. desiderata* DSM 9502^T (= FB2^T) was obtained from the Leibniz Institute DSMZ and used as a reference strain.

Phylogenetic Analysis

PCR amplification of the 16S rRNA gene was performed using a bacterial universal primer set (27F and 1540R) [4]. The 16S rRNA gene was sequenced using the Sanger dideoxy sequencing method by TsingKe Biological Technology Company, Beijing, China. The isolate was identified using the EzBiocloud server on the basis of 16S rRNA sequence data [5]. Additionally, the GenBank database was used to search for *gyrB* and *rpoD* gene sequences in type species from the *Halomonas* genus and the sequence data of the two genes were download. Phylogenetic trees were constructed using the maximum composite likelihood model and the neighbor-joining statistical method (1000 bootstrap replicates) in the MEGA X program [6].

Genomic Analysis

Genomic DNA from strain DX6^T was extracted using TIANamp Bacteria DNA Kit from TIANGEN Biotech (Beijing) Co., Ltd. Genome sequencing of strain DX6^T and *H. desiderata* DSM 9502^T was performed using the Illumina HiSeq platform with paired-end reads of 2 × 150 bp by Personal Biotechnology Co., Ltd., Shanghai (China). Average nucleotide identity (ANI) values were calculated using Kostas Konstantinidis lab's online ANI calculator (http://enve-omics.ce.gatech.edu/ani/) [7]. DNA–DNA hybridization (DDH) experiments were carried out using the method described by De Ley et al. [8] with a Beckman DU 800 spectrophotometer. The digital DNA-DNA hybridization (dDDH) values between strain DX6^T and its closest type strains were determined using Genome-to-Genome Distance Calculator (GGDC) version 2.1 (https://ggdc.dsmz.de/ggdc.php#) and formula 2 was applied [9].

Phenotypic Analysis

Strain DX6^T was grown on LB agar medium at 37 °C for 12 h and cell morphology was examined and observed by transmission electron microscopy (JEM-1400; Jeol USA Inc., Peabody, MA) after uranyl acetate staining. Gram-staining was carried out by using the standard Gram reaction [10]. The growth of strain DX6^T at various NaCl concentrations was also investigated in LB medium supplemented with 0–20% of NaCl and incubated for 3–5 days. The temperature range for growth was determined in LB medium by incubating at 4, 10, 20, 25, 30, 35, 37, 43, 45, and 50 °C for 5 days. pH tolerance (pH 4.0–12.0, at intervals of 1 pH unit) was determined in LB medium pre-adjusted to various pH values by the addition of HCl or NaOH. Carbon source utilization was tested in a medium containing 2.5 g L^{-1} $(NH_4)_2SO_4$, 0.5 g L^{-1} $NaH_2PO_4{\cdot}2H_2O$, 0.5 g L^{-1} K_2HPO_4, 0.5 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.1 g L^{-1} $CaCl_2{\cdot}2H_2O$ and 1.0 g L^{-1} carbohydrate. Other physiological and biochemical properties were performed using the HBI Microbial Biochemical Identification Tubes from Hopebio-Technology Co., Ltd., Qingdao (China) according to the manufacturer's instructions. Antimicrobial susceptibility testing was performed according to the conventional Kirby–Bauer method [11].

Chemotaxonomic Analysis

Respiratory quinones were extracted and analyzed using reversed-phase HPLC (LC20AD system, Shimadzu) according to a previously described method [12]. Cellular fatty acid methyl esters were extracted, separated and identified according to the instructions of the Microbial Identification System (MIDI, Microbial ID). Polar lipids were extracted, separated using two-dimensional TLC and identified according to published procedures [13].

Results and Discussion

Phylogenetic Analysis

Comparative analysis of 16S rRNA gene sequence indicated that strain DX6^T (GenBank accession No. MT180568) was closely related to *H. desiderata* DSM 9502^T (X92417, 98.3%), *H. kenyensis* AIR-2^T (AY962237, 97.7%), *H. daqingensis* DQD2-30^T (EF121854, 97.6%), *H. saliphila* LCB169^T (KX008964, 97.4%) and *H. endophytica* MC28^T (MF850257, 96.2%). As shown in Fig. 1, strain DX6^T formed a distinct lineage in the clade of the genus *Halomonas.* Strain DX6^T showed 82.0–86.5% *gyrB* and 82.6–86.6% *rpoD* gene sequence similarity to the above five most closely related type strains of the *Halomonas* species. A neighbor-joining tree based on a concatenated alignment of 16S rRNA, *gyrB* and *rpoD* gene sequences was also reconstructed (Fig. 2) and the phylogenetic analysis also revealed that strain DX6^T clustered within the genus *Halomonas* and formed a distinct lineage.

Genomic Analysis

Draft genome sequencing of strain DX6^T (GenBank accession No. JAAQTO000000000) yielded a genome of 4,701,666 bp in length with 80 contigs (all >1049 bp, N50 was 106,214 bp). 4289 protein coding genes, 3 complete rRNA genes, 51 tRNA genes, and 4 ncRNAs genes were

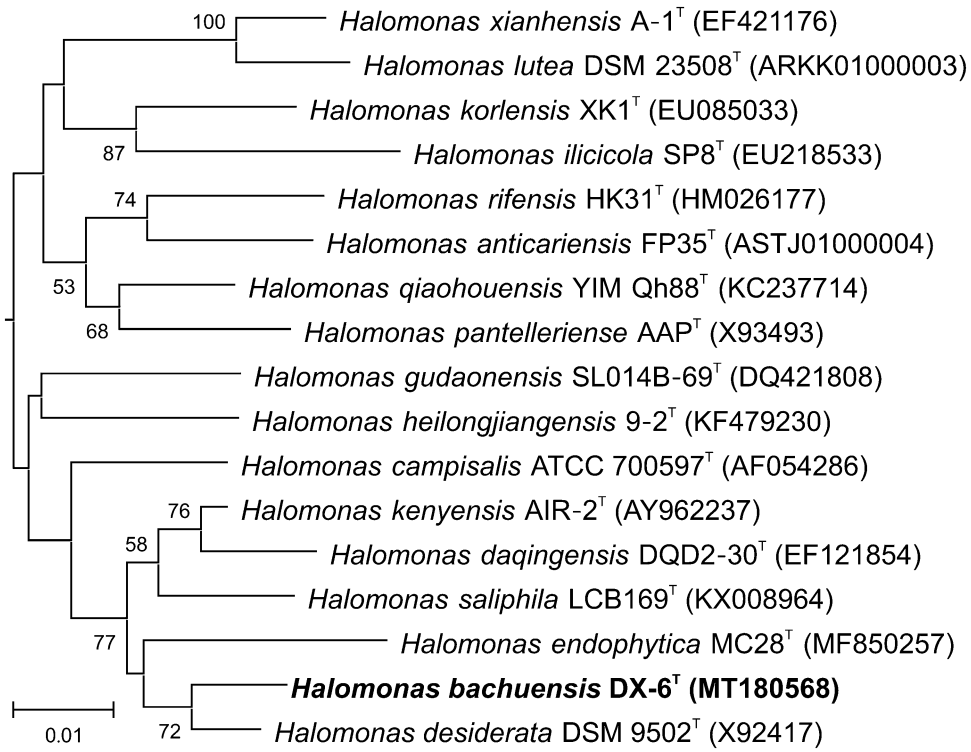

Fig. 1 Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain DX6^T with respect to species of the genus *Halomonas*. Accession numbers are given in parentheses. Numbers at branching points represent bootstrap values (%) from 1000 replicates; only values >50% are shown. Bar, 0.01 substitutions per nucleotide position

Fig. 2 Neighbor-joining tree based on concatenated 16S rRNA, *gyrB* and *rpoD* gene sequences, showing the position of DX6^T among members of the genus *Halomonas*. Numbers at branching points represent bootstrap values from 1000 replications; only values 50% are shown. Accession numbers are given in parentheses. Bar, 0.02 substitutions per nucleotide position

predicted. The 16S rRNA gene sequence extracted from the genome shared 99.9% similarity with the 16S rRNA gene sequenced by the Sanger dideoxy sequencing method. In contrast, the draft genome size of *H. desiderata* DSM 9502^T (GenBank accession No. JAAQTN000000000) was 4,886,954 bp with 106 contigs (all >1082 bp, N50 was 96,043 bp). 4405 protein coding genes, 3 complete rRNA genes, 54 tRNA genes, and 5 ncRNAs genes were predicted. The read size or the depth coverage of strain DX6^T and *H. desiderata* DSM 9502^T was 284x and 334x, respectively. The DDH value between DX6^T and *H. desiderata* DSM 9502^T was 35.4%. The ANI and the dDDH results between strain DX6^T and the five closely related *Halomonas* species (Table 1) were far below the 95% (ANI) and 70% (dDDH) threshold values suggested for the description of bacterial species [14, 15], indicating that strain DX6^T belongs to a novel species of the genus *Halomonas*.

Table 1 The dDDH and ANI values between strain DX6^T and its closest type species of the genus *Halomonas*

Member of the genus *Halomonas*	GenBank accession No.	Genome size (Mbp)	G+C content (mol%)	dDDH (%)	ANI (%)
DX6^T	JAAQTO000000000	4.70	63.6	–	–
H. desiderata DSM 9502^T	JAAQTN000000000	4.89	64.7	27.5	84.3
H. daqingensis DQD2-30^T	FNVC00000000	4.74	64.8	27.4	84.4
H. saliphila LCB169^T	PJRN00000000	4.34	64.1	27.2	84.8
H. endophytica MC28^T	PNRF00000000	4.98	62.1	22.6	81.0
H. kenyensis AIR-2^T	JACEFT000000000	4.42	63.8	26.0	83.4

Phenotypic Characterization

Strain DX6^T was Gram-stain-negative and cells were short rods (0.4–0.5×0.8–1.5 μm). Colonies were creamy, circular, smooth and 1–2 mm in diameter after 2 days of incubation at 30 °C on LB agar. Growth of strain DX6^T was observed at 10–45 °C (optimum 37 °C), pH 5.0–10.0 (optimum pH 7.0–9.0) and 1–15% (*w*/*v*) NaCl (optimum 2–10%). Antimicrobial susceptibility testing results showed that strain DX6^T was susceptible to (amounts per disc) gentamicin (10 μg), kanamycin (30 μg), rifampicin (5 μg), tetracycline (30 μg) and chloramphenicol (30 μg), resistant to carbenicillin (100 μg), penicillin (6 μg) and ampicillin (10 μg). Other detailed physiological and biochemical characteristics are summarized in the species description and a comparison of DX6^T and related type strains is given in Table 2.

Chemotaxonomic Characterization

Results of chemotaxonomic analyses revealed that Q-9 (78.5%) and Q-8 (21.5%) were the predominant respiratory quinones in strain DX6^T, which was consistent with the ubiquinone systems of members of the genus *Halomonas*. As shown in Table 3, strain DX6^T differed from related bacteria in the composition of cellular fatty acids. The major cellular fatty acids of strain DX6^T were summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and $C_{16:0}$. The predominant polar lipids found in cells of strain DX6^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unidentified phospholipids, an unidentified phosphatidylglycolipid, and four unidentified lipids.

Taxonomic Conclusion

Based on the distinct phylogenetic, phenotypic, biochemical, and chemotaxonomic characteristics, strain DX6^T represents a novel species within the genus *Halomonas*, for which the name *Halomonas bachuensis* sp. nov. is proposed.

Description of *Halomonas bachuensis* sp. nov.

Halomonas bachuensis (ba.chu.en'sis. N.L. fem. Adj. *bachuensis* pertaining to Bachu, north-western China, where the strain was isolated).

Cells are Gram-stain-negative, aerobic, rod-shaped, 0.4–0.5 μm wide and 0.8–1.5 μm long. Colonies are creamy, circular, smooth and 1–2 mm in diameter after 2 days of incubation at 30 °C on LB agar. Growth occurs at 10–45 °C (optimum 37 °C), pH 5.0–10.0 (optimum pH 7.0–9.0) and 1–15% (*w*/*v*) NaCl (optimum 2–10%). Positive for nitrate reduction, oxidase, D-glucose fermentation, methyl-red test, and hydrolysis of aesculin, ONPG, citric acid, gelatin. Negative for hydrolysis of myo-inositol and starch. D-galactose, D-glucose, D-mannose, maltose, D-ribose, D-xylose, L-rhamnose and sucrose are utilized as sole carbon and energy source but D-lactose, and D-arabinose are not utilized. Sensitive to (amounts per disc) gentamicin (10 μg), kanamycin (30 μg), rifampicin (5 μg), tetracycline (30 μg) and chloramphenicol (30 μg), but resistant to carbenicillin (100 μg), penicillin (6 μg) and ampicillin (10 μg). The respiratory quinone is Q-9 and Q-8. The predominant fatty acids are summed feature 8 (C18:1ω7c and/or C18:1ω6c), summed feature 3 (C16:1ω7c and/or C16:1ω6c) and C16:0. The polar lipid profile is composed of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unidentified phospholipids, an unidentified phosphatidylglycolipid, and four unidentified lipids. The genomic DNA G+C content is 63.6 mol%. The type strain is DX6^T (= CCTCC AB 2020094^T = KCTC 82196^T), isolated from a Gobi soil sample collected in Bachu County, Xinjiang province, China. The GenBank accession number for the 16S rRNA gene sequence of strain DX6^T is MT180568. The draft genome sequence accession numbers for strain DX6^T is JAAQTO000000000.

Table 2 Characteristics of strain DX6^T and the related *Halomonas* species. Strains: 1, DX6^T; 2, *H. desiderata* DSM 9502^T; 3, *H. daqingensis* DQD2-30^T [16]; 4, *H. saliphila* LCB169^T [17]; 5, *H. endophytica* MC28^T [3]; 6, *H. kenyensis* AIR-2^T [18]. Data for strain DX6^T and *H. desiderata* DSM 9502^T are from the present study. +, positive; −, negative; NA, no data available

Characteristic	1	2	3	4	5	6
Cell size, μm	0.4–0.5×0.8–1.5	0.4–0.6×1.0–2.6	0.7–0.8×1.0–1.2	0.3–0.5×1.0–1.6	0.5–0.8×1.0–1.5	0.5–0.7×1.5–2.5
Flagellation type	absent	peritrichous	peritrichous	peritrichous	peritrichous	peritrichous
NaCl for growth (w/v%)	1–15 (2–10)	0–18 (3–9)	1–15 (5.0–10.0)	0–17 (10–15)	0.5–6 (3.0)	0–13 (3–7)
pH for growth	5.0–10.0 (7.0–9.0)	7.0–11.0 (9.0–10.0)	8.0–10.0 (9.0)	6.0–10.0 (8.0)	6.0–9.0 (8.5)	7.5–10.6 (9.5)
Temperature for growth (°C)	10–45 (37)	10–45 (30)	10–50 (30)	10–52 (30)	10–45 (40)	10–55 (36–40)
Nitrate reduction	+	+	+	−	+	+
Oxidase	+	+	+	+	+	+
D-Glucose fermentation	+	+	−	−	+	−
Methyl-red test	+	−	−	−	−	NA
Hydrolysis of:						
Aesculin	+	−	NA	+	NA	NA
ONPG	+	−	−	NA	NA	NA
myo-Inositol	−	−	−	−	NA	NA
Citric acid	+	+	NA	NA	+	+
Starch	−	−	−	−	−	NA
Gelatin	+	−	−	+	−	NA
Growth on:						
D-Galactose	+	+	±	NA	+	−
D-Lactose	−	−	−	NA	NA	−
D-Glucose	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	±
Maltose	+	+	+	−	+	+
D-Arabinose	−	−	−	−	+	−
D-Ribose	+	+	−	+	+	±
D-Xylose	+	+	−	+	+	+
L-Rhamnose	+	−	NA	NA	NA	−
Sucrose	+	+	+	+	+	+
Respiratory quinones	Q-9, Q-8	Q-9	Q-9	Q-9	Q-9, Q-8	NA

Table 3 Cellular fatty acids compositions of strain DX6^T and other closely related species of the genus *Halomonas*. Strains: 1, DX6^T; 2, *H. desiderata* DSM 9502^T; 3, *H. saliphila* LCB169^T; 4, *H. daqingensis* DQD2-30^T; 5, *H. kenyensis* AIR-2^T; *H. endophytica* MC28^T. Values shown are percentages of total fatty acids; −, Percentage of fatty acids was less than 1.0% or not detected. Fatty acids representing to 10% or more of the total fatty acids are in bold

Fatty acid	1	2	3	4	5	6
C10:0	2.8	2.6	2.5	3.0	1.0	5.4
C12:0	2.3	–	3.1	–	–	–
C12:0 3-OH	6.2	9.8	**10.1**	**10.3**	7.9	3.6
C14:0	–	3.8	–	4.3	3.3	1.4
C14:0 cyclo	–	–	–	–	–	2.1
C16:0	**17.1**	**28.9**	**26.4**	**26.5**	**26.7**	**11.2**
Summed feature 3[a]	**21.7**	3.5	**10.8**	3.5	3.6	**18.3**
C16:1 ω9c	–	–	–	–	–	**16.1**
C17:0 cyclo	3.1	–	2.9	–	–	1.6
C17:1 ω9c	–	–	–	–	–	**10.6**
C18:0	–	4.9	3.8	5.1	8.7	–
Summed feature 8[a]	**39.6**	**43.4**	**31.1**	**42.7**	**47.0**	**22.7**
C19:0 cyclo ω8c	3.9	2.6	8.1	3.7	–	–

[a]Summed features represent the integration of two or three fatty acids which cannot be separated by the MIDI system. Summed feature 3 comprised C16:1ω7c and/or C16:1ω6c; summed feature 8 comprised C18:1ω7c and/or C18:1ω6c

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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