In the course of a study of extremely halophilic microorganisms from saline soils, Zvyagintseva & Tarasov (1987) proposed the species Halobacterium distributus, based on the features of four strains (designated 1mT, 4p, 13 and 32). This name was validly published and corrected to Halobacterium distributum (Zvyagintseva & Tarasov, 1989). The strain designated as the type strain of this species is strain 1mT, deposited as VKM B-1733T (Zvyagintseva & Tarasov, 1989). Two almost-simultaneous studies (Kamekura & Dyall-Smith, 1995; McGinity & Grant, 1995) showed that most species that were previously assigned to the genus Halobacterium were not related to Halobacterium salinarum, the type species of this genus, and they were placed in the novel genus Halorubrum (McGenity & Grant, 1995). On the basis of 16S rRNA gene sequence comparison, Halobacterium distributum was also included in the genus Halorubrum, as Halorubrum distributum (Oren & Ventosa, 1996).

Zvyagintseva et al. (1996) showed that the features of strain 1mT were different from those of the other three strains that were assigned to the species Halorubrum distributum and they proposed strain 4p (= VKM B-1739) as the new type strain of Halorubrum distributum. As this proposal was not in accordance with the rules of the International Code of Nomenclature of Bacteria (Lapage et al., 1992), Oren et al. (1997a) confirmed strain 1mT (= VKM B-1733T = JCM 9100T) as the type strain of Halorubrum distributum and suggested that an exhaustive study should be carried out in order to 'describe and validate a new species of Halorubrum and include a full phenotypic characterization', and to propose (preferably) strain 4p (= VKM B-1739) as its type strain.

In the present paper, we study in detail the type strain of Halorubrum distributum, a species that has not been well characterized phenotypically, as well as the other strains that were previously assigned to this species; we describe these as a novel species, for which we propose the name Halorubrum terrestre sp. nov.

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Halorubrum distributum (basonym, Halobacterium distributum) is an extremely halophilic, aerobic archaeon isolated from saline soils, which was described on the basis of phenotypic features of several strains. The designated type strain of the species (1mT = VKM B-1733T = JCM 9100T) was shown recently to differ from the other strains. In this study, Halorubrum distributum isolates have been characterized with regard to phenotypic features, polar lipid content, comparison of 16S rDNA gene sequences and DNA–DNA hybridization. On the basis of these data, a novel species that includes the other isolates is proposed, with the name Halorubrum terrestre sp. nov. The type strain of this novel species is 4p T (= VKM B-1739 T = JCM 10247T). The DNA G+C content of this novel species is 64·2–64·9 mol% (64·4 mol% for the type strain).
Table 1. DNA G+C contents and levels of DNA–DNA relatedness between Halorubrum distributionum VKM B-1733T, strain VKM B-1739 and other related halobacterial strains

Hybridization of strains VKM B-1733T and VKM B-1739 with the following strains was <20 %: Halobacterium salinarum DSM 3754T, Halococcus variabilis ATCC 29715T, Haloarcula argentinensis ATCC 700875T, Haloarcula hispanica ATCC 33960T, Haloarcula japonica JCM 7785T, Haloarcula marismortui ATCC 43049T, Haloarcula quadrata DSM 11927T, Halomicrobium mukohataei DSM 12286T, Halofexax volcanii NCMB 2012T, Halofexax denitrificans DSM 4425T, Halofexax gibbonsii ATCC 33995T, Halofexax mediterranei ATCC 33500T, Halofexax lucentens JCM 9276T, Halobaculum gomorrense DSM 9297T, Haloterrigena turkmenica JCM 9743, Natrialba asiatica JCM 9576T and Halococcus saccharolyticus ATCC 49257T.

<table>
<thead>
<tr>
<th>Source of unlabelled DNA</th>
<th>G+C content (mol%)</th>
<th>Relatedness (%) with 3H-labelled DNA from</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halorubrum distributionum VKM B-1733T</td>
<td>63-9</td>
<td>100</td>
</tr>
<tr>
<td>Strain VKM B-1739</td>
<td>64-4</td>
<td>63</td>
</tr>
<tr>
<td>Strain VKM B-1916</td>
<td>64-9</td>
<td>60</td>
</tr>
<tr>
<td>Strain VKM B-1954</td>
<td>64-6</td>
<td>65</td>
</tr>
<tr>
<td>Strain VKM B-2151</td>
<td>64-2</td>
<td>25</td>
</tr>
<tr>
<td>Halorubrum saccharovorum ATCC 29252T</td>
<td>64-3*</td>
<td>32</td>
</tr>
<tr>
<td>Halorubrum coriense JCM 9275T</td>
<td>66-3*</td>
<td>31</td>
</tr>
<tr>
<td>Halorubrum lacusprofundi DSM 5036T</td>
<td>65-5*</td>
<td>45</td>
</tr>
<tr>
<td>Halorubrum sodomense ATCC 33753T</td>
<td>66-0*</td>
<td>20</td>
</tr>
<tr>
<td>Halorubrum trapanicum NCIMB 13488T</td>
<td>64-3</td>
<td>33</td>
</tr>
</tbody>
</table>

*Data from Grant et al. (2001).

included in the species description. The methodology used has been described previously (Torreblanca et al., 1986). Unless otherwise indicated, tests were carried out in media that contained 25 % salts, at pH 7-5 and incubated at 37 °C.

Polar lipids were extracted with chloroform/methanol as described previously (Kamekura, 1993). One-dimensional TLC was done by using Merck HPTLC 10 × 20 cm plates, silica gel 60 (code 1.05641) or Aldrich 20 × 20 cm plates (code Z12271-8) in a solvent system that contained chloroform, methanol, acetic acid and water (85:22:5:10:4, v/v) (Torreblanca et al., 1986). Glycolipids were detected as purple spots by spraying with 0.5 % α-naphthol in methanol/water (1:1) and then with sulfuric acid/ethanol (1:1), followed by slight heating at 160 °C. Other lipids were detected as brown spots after prolonged heating.

For extraction of genomic DNA and determination of DNA G+C content, cells were harvested, washed and suspended in 0.15 M NaCl/0.1 M EDTA buffer (pH 8.0). Lysis was accomplished at 60 °C for 10 min by adding SDS at a final concentration of 2 % (w/v). DNA was extracted and purified by the method of Marmur (1961). Purity was assessed from A260/A280 and A230/A260 absorbance ratios (Johnson, 1994). DNA G+C content was determined from the mid-point value (Tm) of the thermal denaturation profile (Marmur & Doty, 1962), which was obtained by using a Perkin-Elmer UV-Vis 551S spectrophotometer at 260 nm. This instrument was programmed for temperature increases of 1.0 °C min⁻¹. The Tm was determined by a graphic method described by Ferragut & LeClerc (1976), and the G+C content was calculated from this temperature by using the equation of Owen & Hill (1979), in 0.1 × SSC buffer (0.15 M NaCl buffered with 0.015 M trisodium citrate, pH 7.0). The Tm value of reference DNA from Escherichia coli NCTC 9001T was taken as 74.6 °C in 0.1 × SSC (Owen & Pitcher, 1985).

DNA was labelled by the multiprime system with a commercial kit with deoxy(1',2',5'-3H)cytidine 5'-triphosphate (Amersham Biosciences). The mean specific activity obtained with this procedure was 8.4 × 10⁶ c.p.m. (mg DNA)⁻¹. Labelled DNA was denatured before hybridization by heating at 100 °C for 5 min and then placed on ice. DNA–DNA hybridization studies were performed by the competition procedure of the membrane method, as described by Johnson (1994). Competitor DNA was sonicated (B. Braun Melsungen) at 50 W for two intervals of 15 s. Membrane filters (HAHY; Millipore) that contained reference DNA (25 μg cm⁻²) were placed in 5 ml screw cap vials that contained labelled, sheared, denatured DNA and denatured, sheared competitor DNA. The ratio of the concentrations of competitor to labelled DNA was at least 150:1. The final volume and concentration were adjusted to 140 ml, 2 × SSC and 30 % formamide. The hybridization temperature was 57 °C, which is within the limits of validity for the filter method (De Ley...
VKM B-1733T was *Halorubrum distributum* (McGenity & Grant, 2001). The DNA G+C range of G+C content of 63 mol%, a very similar value to that reported previously (63.6 mol%) (McGenity & Grant, 2001). Results of DNA–DNA hybridization data, as well as 16S rRNA gene sequence comparison, we propose to place these strains in a novel species, with the name *Halorubrum terrestre* sp. nov.

Our results show that strains VKM B-1739, VKM B-1916, VKM B-1954 and VKM B-2151 are significantly different from other previously described species of the genus *Halorubrum*, on the basis of DNA–DNA hybridization data, as well as 16S rRNA gene sequence comparison, we propose to place these strains in a novel species, with the name *Halorubrum terrestre* sp. nov.

**Description of Halorubrum terrestre sp. nov.**

*Halorubrum terrestre* (ter.res' tre. L. neut. adj. *terreste* of the soil, from which the strains were isolated).

Cells are pleomorphic, flat and disc-shaped, 1–0–1.5 × 1.5–2.5 μm in size. Motile. Gas vacuoles are not produced. Colonies are orange-red. Growth occurs in media that contain 15–30% NaCl, with optimum growth at 25% NaCl. Growth occurs between 28 and 50°C (optimum, 37–45°C) and pH 5–9 (optimum, 7.5). Chemo-organotrophic. Aerobic. Oxidase- and catalase-positive. Acid is produced from glycerol, but not from arabinose, fructose, galactose, glucose, lactose, maltose, sucrose or trehalose. Nitrate is not reduced to nitrite. Indole is not produced from tryptophan. Voges–Proskauer test is negative. Starch, gelatin and casein are not hydrolysed. H₂S is not produced. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are not produced. The following compounds are not:

![Phylogenetic tree of strain VKM B-1739 and other related halobacterial species based on their 16S rRNA gene sequences (GenBank accession numbers are shown in parentheses). Numbers on branches represent confidence levels from 1000 replicate bootstrap resamplings; values > 70% are shown. Bar, 0.02 expected changes per site.](http://ijs.sgmjournals.org)
used as sole carbon and energy sources: arabinose, cellobiose, aesculin, fructose, fucose, gluconolactone, glucose, glucosamine, inulin, mannose, melibiose, raffinose, rhamnose, ribose, sucrose, trehalose, xylose, adonitol, dulcitol, erythritol, ethanol, glycerol, mannitol, meso-inositol, propanol, sorbitol, α-aminovalerate, butyrate, caprylate, citrate, fumarate, glutamate, glyceraldehyde, 2-oxoglutarate, malate, malonate, oxalate, propionate, saccharate and tartrate. The following compounds are not used as sole carbon, nitrogen or energy sources: L-alanine, L-arginine, L-asparagine, betaine, creatine, L-glutamine, glycine, L-histidine, L-lysine, L-methionine, L-ornithine, L-proline, putrescine, sarcosine, L-serine, L-threonine and L-valine. Susceptible to anisomycin, bacitracin and novobiocin; resistant to ampicillin, chloramphenicol, kanamycin, naldixic acid, penicillin G, polymyxin, streptomycin and tetracycline. Polar lipids are C20-G20 derivatives of phosphatidylglycerol, phosphatidylglycerophosphate methyl ester, phosphatidylglycerol sulfate and a sulfated diglycosyl diether. DNA G+C content is 64·2–64·9 mol% (Tm method).

Type strain is 4pT (=VKM B-1739T = JCM 10247T). DNA G+C content of this strain is 64·4 mol% (Tm method). Isolated from saline soils.

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References


