Brumimicrobium aurantiacum sp. nov., isolated from coastal sediment

Hui Zhang, Ji-Ru Han, Ming-Jing Shi, Zong-Jun Du and Guan-Jun Chen

Abstract

A Gram-negative, facultatively anaerobic, non-motile, rod-shaped and orange-coloured bacterium, designated N62T, was isolated from marine sediment of the coast of Weihai, PR China. Strain N62T was found to grow optimally at 28-30 ºC, pH 7.0-7.5 and with 2.0-3.0 % (w/v) NaCl. The dominant cellular fatty acids of strain N62T were iso-C15:0, iso-C16:0 G, iso-C17:0 3-OH and iso-C17:1 3-OH. The major respiratory quinone was MK-6, and the DNA G+C content was 35.3 mol%. The predominant polar lipids were phosphatidylethanolamine, two unidentified aminolipids, an unidentified glycolipid and three unidentified lipids. Phylogenetic analysis based on 16S rRNA gene sequences revealed that N62T was a member of the family Crocinitomicaceae and had a 16S rRNA gene sequence similarity of 95.8-97.2 % with recognized Brumimicrobium species. On the basis of the phylogenetic and phenotypic evidences, strain N62T represents a novel species of the genus Brumimicrobium, for which the name Brumimicrobium aurantiacum sp. nov. is proposed. The type strain is N62T (=KCTC 42589T=MCCC 1H00117T).

In early 2016, the novel family Crocinitomicaceae was proposed by Munoz et al. [1], including the genera Brumimicrobium, Crocinitomix [2], Flavicola [3], Lishizhenia [4], Wandonia [5] and Salintireps [6]. The genus Brumimicrobium was proposed for the classification of the species Brumimicrobium glaciale [2]. The genus Brumimicrobium comprised two recognized species: B. glaciale, isolated from the algal-rich Antarctic (or Southern) Ocean [2], and Brumimicrobium mesophilum, isolated from tidal flat sediment at Yeongheung-do on the coast of the Yellow Sea, Korea [7]. Bacteria in the genus Brumimicrobium are Gram-negative, heterotrophic, facultatively anaerobic, orange-pigmented, oxidase-negative, catalase-positive and motile by gliding or non-motile. In this study, we report a novel species, designated N62T, which was isolated from a marine sediment sample, as a member of the genus Brumimicrobium, for which the name Brumimicrobium aurantiacum sp. nov. is proposed.

Strain N62T was isolated from a sediment sample collected from the coast of Weihai, PR China (36° 54’ 32.6” N 122° 15’ 16.2” E) by plating 1:10 serial dilutions of the sample on marine agar 2216 (MA; BD) and cultivation at 28 ºC for 4 days. After primary isolation and purification, the strain was cultivated at 28 ºC on MA and stored at −80 ºC in sterile 1 % (w/v) saline supplemented with 15 % (v/v) glycerol.

B. mesophilum JCM 14063T and B. glaciale LMG 21434T, purchased from the JCM (Japan Collection of Microorganisms) and the LMG (BCCM/LMG Bacteria Collection, Laboratory voor Microbiologie, Ghent University, Belgium), respectively, were used as reference strains for physiological tests and chemotaxonomic comparisons (except for the polar lipids analysis).

Cell morphology was observed by light microscopy (Ci-L; Nikon), and Gram staining was performed as described by Smibert and Krieg [8]. Gliding motility was tested on marine broth 2216 (MB; BD) solidified with 0.3 % agar according to the methods of Bernardet et al. [9]. The temperature range for growth was investigated on MA at 4, 8, 15, 25, 28, 30, 33, 37, 42 and 45 ºC. The pH range for growth was tested in MB at pH 5.5–9.5 (at intervals of 0.5 pH units), supplemented with 20 mM MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5) and CAPSO (pH 9.0 and 9.5) buffers (Sangon). The effect of NaCl on the growth of the species was tested using a medium comprising 1 g l−1 yeast extract, 5 g l−1 peptone and 20 g l−1 agar, made with artificial seawater (3.2 g l−1 MgSO4, 2.2 g l−1 MgCl2, 1.2 g l−1 CaCl2, 0.7 g l−1 KCl, 0.2 g l−1 NaHCO3) at NaCl concentrations of 0.0–15.0 % (w/v, at intervals of 0.5 %). Anaerobic growth was determined after cultivation in an anaerobic chamber on
MA with or without 0.1 % (w/v) NaNO₃ for 2 weeks at 28 °C. Hydrolysis of agar, starch, alginate, CM-cellulose, Tween (20, 40, 60 and 80) and nitrate reduction were examined using MA according to methods described by Dong and Cai [10]. Catalase activity was determined by adding 3% (v/v) H₂O₂ solution to bacterial colonies. Oxidase activity was detected using the bioMérieux oxidase reagent kit according to the manufacturer’s instructions. Because strain N62ᵀ showed poor growth on Iso-Sensitest and MuellerHinton agars, susceptibility to antibiotics was investigated using antibiotic-impregnated discs (Binhe) on MA according to the disc diffusion method described by Du et al. [11] and procedures outlined by the Clinical and Laboratory Standards Institute (CLSI) [12]. Physiological and biochemical characteristics were tested using API 20E, API ZYM and API 50CHB fermentation kits (bioMérieux) and GEN III microPlates (Biolog) according to the manufacturers' instructions, with all tests performed in a suspension medium supplemented with 3% (w/v) NaCl (final concentration).

Cells cultured in MB at 28 °C for 2 days (end of the late-exponential phase) were used to analyse the cellular fatty acids, respiratory quinones and polar lipid composition, with the exception that fatty acids of B. glacieae LMG 21434ᵀ were determined by cells cultured at 20 °C for 2 days. The cellular fatty acids of strain N62ᵀ were analysed according to the methods of the Microbial Identification System (Microbial ID). Respiratory quinones were extracted and purified according to the methods of Minnikin et al. [13] and analysed by HPLC [14]. Polar lipids were analysed by the Identification Service of the Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. Genomic DNA was extracted and purified using a genomic DNA extraction kit (TaKaRa) following the manufacturer’s protocol. The G+C content of genomic DNA was determined by HPLC [15], with ADNA (Takara) used as a standard.

The 16S rRNA of this strain was amplified by PCR using the bacterial primers 27F and 1492R. The purified PCR product was ligated into the pGM-T vector (Tiangen) and cloned as described by Xia et al. [16]. Sequencing reactions were performed by Shanghai Sunny Biotechnology. The nearly complete 16S rRNA gene sequence (1448 bp) was submitted to the GenBank database to search for similar sequences using the BLAST algorithm. The EzTaxon server (http://www.ezbiocloud.net/) [17] was also used to assess the similarity of sequences. Multiple alignments of the sequences were determined using CLUSTAL_X (version 1.81) [18], and alignments were edited manually in BioEdit version 7.0 [19]. The phylogenetic tree of strain N62ᵀ and several closely related species based on 16S rRNA gene sequence similarities was reconstructed using the neighbour-joining [20] method implemented in MEGA version 7 [21]. The maximum-likelihood [22] and maximum-parsimony [23] methods were also used to reconstruct phylogenetic trees to verify the taxonomic position of the novel isolate. The distances within the trees were evaluated by Kimura’s two-parameter model [24]. Bootstrap values were evaluated based on 1000 replicates in each of three methods.

In the neighbour-joining phylogenetic tree (Fig. 1), strain N62ᵀ fell within a clade comprising the type strains of species of the genus Brumimicrobium, clustering with B. glaciæae LMG 21434ᵀ with a bootstrap resampling value of 86%. This placement of strain N62ᵀ and the type strains of

![Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain N62ᵀ. The tree was reconstructed using the neighbour-joining algorithm. Bootstrap values (based on 1000 replications) are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Chryseobacterium joostei lx 5aᵀ was used as an outgroup. Bar, 0.020 substitutions per nucleotide position.](attachment:image.png)
species of the genus Brumimicrobium was also observed in trees reconstructed using the maximum-likelihood and maximum-parsimony algorithms (Figs S1 and S2, available in the online Supplementary Material). The 16S rRNA gene sequence similarity values between the strain N62T and its closest relatives, B. mesophilum JCM 14063T and B. glaciale LMG 21434T, were 97.2 and 95.8 %, respectively. The phylogenetic tree clearly suggested that strain N62T was a novel species of the genus Brumimicrobium.

Colonies of strain N62T were circular with smooth, entire edges and orange in colour. Cells were rod-shaped and non-motile. Growth occurred at 4–40°C (optimally at 28–30°C), pH 5.5–9.0 (optimally at pH 7.0–7.5) and with 0.5–13.0 % (w/v) NaCl (optimum 2.0–3.0 %). The strain was susceptible to (µg per disc) erythromycin (15), penicillin (10), chloramphenicol (30), rifampicin (5), lincomycin (2), ceftriaxone (30), acetylsalicylamycin (30), ampicillin (10), cefotaxime sodium (30) and clindamycin (30). It was resistant to tobramycin (10), tetracycline (30), norfloxacin (10), neomycin (30), gentamycin (10), sulfamethoxazole (10), ofloxacin (5), kanamycin (30) and streptomycin (10). Strain N62T was found to contain phosphatidyethanolamine, the unidentified glycolipid and one of the unidentified aminolipids (AL1) as the major polar lipids [7]. The major respiratory quinone of strain N62T was MK-6, which is consistent with that of the members of the genus Brumimicrobium. The predominant cellular fatty acids (>5 % of the total) were iso-C15:0 (51.5 %), iso-C15:0 3-OH (24.0 %) and iso-C17:0 3-OH (6.3 %), which were similar to those of B. mesophilum JCM 14063T and B. glaciale LMG 21434T, although there were some differences in the proportions of these predominant fatty acids and minor fatty acids (Table S1). The genomic DNA G+C content of strain N62T was 35.3 mol%, which fell into the 34.3–40.0 mol% range of the genus Brumimicrobium. The main physiological and biochemical characteristics of strain N62T and related bacteria are presented in Table 1.

Strain N62T shared many phenotypic characteristics with members of the genus Brumimicrobium. All strains in this genus are Gram-negative, catalase-positive and oxidase-negative, form orange colonies on MA, contain the same major respiratory quinone and produce alkaline phosphatase and gelatinase. However, the novel isolate could be clearly distinguished from all recognized species of the genus Brumimicrobium by the growth temperature range (4–40°C for strain N62T, 11–36°C for B. mesophilum JCM 14063T and –2–25°C for B. glaciale LMG 21434T) and by the tolerance to NaCl (0.5–13.0 % for strain N62T, 0.4–7.0 % for B. mesophilum JCM 14063T and 0.5–8.0 % for B. glaciale LMG

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**Table 1. Differential phenotypic characteristics of Brumimicrobium species**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (µm)</td>
<td>0.3–0.4×0.8–1.5</td>
<td>0.3–0.5×0.8–1.7*</td>
<td>0.3–0.4×1.0–3.0†</td>
</tr>
<tr>
<td>Temperature range (optimum, °C)</td>
<td>4–40 (28–30)</td>
<td>11–36 (26)*</td>
<td>–2–25 (16–19)*</td>
</tr>
<tr>
<td>Salinity range (% w/v)</td>
<td>0.5–13.0</td>
<td>0.4–7.0*</td>
<td>0.5–8.0†</td>
</tr>
<tr>
<td>Gliding motility</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Tween 40</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trypsin</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>l-Serine, methyl pyruvate, dextrin</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Gentibiose, methyl β-mannoside</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C (mol%)</td>
<td>35.3</td>
<td>34.3*</td>
<td>38–40†</td>
</tr>
</tbody>
</table>

*Data taken from Yang et al. [7].
†Data taken from Bowman et al. [2].
2143\textsuperscript{4}). In contrast to its closest neighbour, \textit{B. mesophilum} JCM 14063\textsuperscript{3}, strain N62\textsuperscript{T} could produce nitrate reductase, trypsin, \textalpha{}-chymotrypsin and naphthol-AS-BI-phosphohydrolase, and hydrolysed Tween 40 and 80, but not starch. Strain N62\textsuperscript{T} could not oxidize gentiobiose and methyl \textbeta{}-D-glucoside and could not produce acid from \textbeta{}-D-glucose. In contrast to \textit{B. glacieae} LMG 2143\textsuperscript{4}, strain N62\textsuperscript{T} did not demonstrate gliding motility and could not oxidise \textalpha{}-serine, methyl pyruvate or dextrin.

Phylogenetic, phenotypic and genotypic data from this study clearly indicate that strain N62\textsuperscript{T} represents a novel species within the genus \textit{Brumimicrobium}, for which the name \textit{Brumimicrobium aurantiacum} sp. nov. is proposed.

**DESCRIPTION OF \textit{BRUMIMICROBIUM AURANTIACUM} SP. NOV.**

\textit{Brumimicrobium aurantiacum} (au.ran.ti.a.cum. N.L. neut. adj. aurantiacum, orange-coloured).

Cells are facultatively anaerobic, Gram-negative, and non-gliding, with straight or slightly curved rods, and approximately 0.3–0.4 \mu{}m wide and 0.8–1.5 \mu{}m long. Colonies on marine agar 2216 (BD) are circular, smooth, with entire edges, opaque, orange, and about 0.6–1.0 mm in diameter after incubation at 28 °C for 2 days. Growth occurs at 4–40 °C (optimally at 28–30 °C), pH 5.5–9.0 (optimally at pH 7.0–7.5) and with 0.5–13.0 % (w/v) NaCl (optimum 2.0–3.0 %). No growth is observed without NaCl. Nitrate is reduced to nitrite. Oxidase-negative and catalase-positive. Gelatin and Tween 20, 40, 60 and 80 are hydrolysed, but starch, CM-cellulose, alginate, and agar are not. The strain is positive for gelatinase, but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H\textsubscript{2}S production, urease, tryptophan deaminase, indole production, and Voges–Proskauer reaction. Altose, trehalose, raffinose, lactose, \textalpha{}-D-glucose, D-fructose, D-galactose, D-mannitol, L-alanine, D-galacturonic acid, D-gluconic acid, glucuronamide, L-lactic acid, sucrose, turanose, L-fucose, L-rhamnose, quinic acid and D-saccharic acid are oxidized as the sole carbon and energy sources. Acid is produced from D-ribose and potassium 5-ketogluconate. Alkaline phosphatase, esterase (C14), leucine arylamidase, cystine arylamidase, trypsin, \textalpha{}-chymotrypsin, acid phosphatase, and naphthol-AS-BI-phosphohydrolase activities are present, but \textbeta{}-galactosidase, \textbeta{}-glucosidase, \textbeta{}-galactosidase, \textbeta{}-glucuronidase, \textalpha{}-glucosidase, N-acetyl-\textbeta{}-glucosaminidase, \textalpha{}-mannosidase, and \textbeta{}-fucosidase activities are absent. The predominant cellular fatty acids are iso-C\textsubscript{15:0} \textit{a}, iso-C\textsubscript{15:0} \textit{c12} and C\textsubscript{17:0} \textit{3-OH}. The predominant polar lipids are phosphatidylethanolamine, two unidentified aminolipids, an unidentified glycolipid, and three unidentified lipids. The major respiratory quinone is MK-6.

The type strain, N62\textsuperscript{T} (=KCTC 42589\textsuperscript{T}=MCCC 1H00117\textsuperscript{T}), was isolated from marine sediment on the coast of Weihai, PR China. The genomic DNA G+C content of the type strain is 35.3 mol%.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Ethical statement**

This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

**References**


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