Zunongwangia atlantica sp. nov., isolated from deep-sea water

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A taxonomic study was carried out on strain 22II14-10F7T, which was isolated from the deep-sea water of the Atlantic Ocean with oil-degrading enrichment. The bacterium was Gram-stain-negative, oxidase- and catalase-positive and rod-shaped. Growth was observed at salinities from 0.5 to 15 % and at temperatures from 4 to 37 °C; it was unable to hydrolyse Tween 40, 80 or gelatin. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain 22II14-10F7T represented a member of the genus Zunongwangia, with highest sequence similarity of 97.3 % to Zunongwangia profunda SM-A87T, while the similarities to other species were all below 94.0 %. The DNA–DNA hybridization estimate of the similarity between strain 22II14-10F7T and Z. profunda SM-A87T was 27.20 ± 2.43 % according to their genome sequences. The principal fatty acids were iso-C15 : 0, anteiso-C15 : 0, iso-C15 : 1G, iso-C17 : 03-OH, summed feature 3 (C16 : 1v7c/v6c) and summed feature 9 (iso-C17 : 1v9c or C16 : 010-methyl). The G+C content of the chromosomal DNA was 35.5 mol%. The major respiratory quinone was determined to be MK-6. Phosphatidylethanolamine (PE), two aminolipids (AL1 and AL2) and five unknown lipids (L1–L5) were present. The combined genotypic and phenotypic data show that strain 22II14-10F7T represents a novel species of the genus Zunongwangia, for which the name Zunongwangia atlantica sp. nov. is proposed, with the type strain 22II14-10F7T (=CGMCC1.12470T =LMG 27421T =MCCC 1A06481T).

During attempts to investigate oil-degrading bacteria in the deep water of the Atlantic Ocean, many bacterial strains were isolated and characterized taxonomically. This study focuses on one of these isolates, designated strain 22II14-10F7T. Comparative 16S rRNA gene sequence analysis indicated that strain 22II14-10F7T was closely related to the genus Zunongwangia, which belongs to the family Flavobacteriaceae. The genus Zunongwangia was proposed by Qin et al., (2007) and, at the time of writing, includes only the type species Zunongwangia profunda. Consequently, the aim of the present work is to determine the exact taxonomic position of strain 22II14-10F7T by using polyphasic characterization.

Deep-sea water was sampled with Niskin bottles attached to a conductivity, temperature and depth (CTD) circular rosette in 2011 during cruise DY-115A of the R/V Da-Yang Yi-Hao. The sampling site was on the South Atlantic Ocean ridge, and numbered as 22II-S025-CTD14, the water sample from 2927 m depth was used for enrichment of oil-degrading bacteria with 1 % (v/v) sterilized crude oil. The bacterial isolation on 216L marine agar medium was done according to the method described by Lai et al. (2009). For morphological and biochemical characterization, strain 22II14-10F7T was cultivated on marine agar 2216 (BD; Difco) medium.

Genomic DNA was prepared according to the method of Ausubel et al. (1995) and the 16S rRNA gene was amplified by PCR using primers that have been described previously (Liu & Shao, 2005). Sequences of related taxa were obtained from the GenBank database. Phylogenetic analysis was performed using MEGA version 5.0 (Tamura et al., 2011). Distances (distance options determined according to the Kimura two-parameter model) and clustering with the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and minimum evolution methods (Rzhetsky & Nei, 1992, 1993) were determined by using bootstrap values based on 1000 replications.
A nearly full-length 16S rRNA gene sequence (1490 nt) of strain 22II14-10F7\textsuperscript{T} was determined. As shown in Fig. 1, the phylogenetic tree based on 16S rRNA gene sequences showed that strain 22II14-10F7\textsuperscript{T} and \textit{Z. profunda} SM-A87\textsuperscript{T} formed an independent monophyletic cluster, with high bootstrap support (100 %). The two strains shared 16S rRNA sequence similarity of 97.3 %. The sequences of type strains of other species had 94.0 % similarity to strain 22II14-10F7\textsuperscript{T}. This high similarity strongly confirmed that strain 22II14-10F7\textsuperscript{T} belonged to the genus \textit{Zunongwangia}.

The average nucleotide identity (ANI) between two genomes was calculated using JSpecies (V1.2.1) as described by Richter & Rossello-Móra (2009). The draft genome sequences of the novel strain 22II14-10F7\textsuperscript{T} (GenBank accession number JQ844757.1) and \textit{Z. profunda} SM-A87\textsuperscript{T} (Qin et al., 2010) were obtained from the NCBI database. The ANI value using MUMer between two strains was 85.6 %, which is below standard criteria for classifying strains as representing the same species (95–96 %) (Richter & Rossello-Móra, 2009). This proved that strain 22II14-10F7\textsuperscript{T} represented a novel species of the genus \textit{Zunongwangia}. The DNA–DNA hybridization (DDH) estimate value was analysed using the genome to genome distance calculator (GGDC2.0) (Auch et al., 2010a, b; Meier-Kolthoff et al., 2013). The DDH estimate value between strain 22II14-10F7\textsuperscript{T} and \textit{Z. profunda} SM-A87\textsuperscript{T} was 27.20 % ± 2.43 %. This result confirmed that strain 22II14-10F7\textsuperscript{T} represented a novel species. Gram staining was performed using a Gram stain kit (Hangzhou Tianhe MiReagent) according to the manufacturer’s instructions. The cell size, morphology and flagellation pattern were observed by transmission electron microscopy (JEM-1230; JEOL) using cells negatively stained with phosphotungstic acid grown on marine agar at 28 °C for 1 day. Cell motility was observed by the hanging-drop method (Skerman, 1967). Catalase and oxidase activities and hydrolysis of Tweens 20,
40, 80 and starch were tested according to the standard methods (Dong & Cai, 2001). The optimal growth temperature was determined over a temperature range of 4–55 °C in marine broth 2216. The pH range for growth was determined in marine broth 2216 adjusted to pH 2–12, at 1 pH unit intervals, with citrate/phosphate (pH 2.0–7.0), Tris/HCl (pH 8.0–9.0), or sodium carbonate/sodium bicarbonate (pH 10.0–12.0) buffers. Tolerance to NaCl was tested using LB broth with NaCl concentrations of 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 18 or 20 % (w/v). Anaerobic growth was examined on marine agar 2216 supplemented with nitrate (1 g l⁻¹) incubated in a jar with the Anaoxomat Mark II Anaerobic System (Mart Microbiology). Microaerobic growth (O₂, 6 %) was examined by incubation on marine agar 2216 in a jar with the Anaoxomat Mark II. The degradation of oil was determined in artificial seawater medium (ASM) at a concentration of 0.5 % (w/v) according to the method of Liu & Shao (2005). The presence of flexirubin-type pigments was examined using 20 % KOH (w/v) as described by Bernardet et al., (2002). Other biochemical tests were carried out using API 20NE and API ZYM strips (bioMérieux) according to the manufacturer’s instructions, except for adjusting the NaCl concentration in all tests to 3.0 %. Z. profunda SM-A87T was tested at the same time for comparison. These results are given in the species description and Table 1.

Fatty acids in whole cells grown on marine agar 2216 medium at 28 °C for 48 h were saponified, methylated and extracted using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analysed by GC (6850; Agilent Technologies) and identified by using the TSBA6.0 database of the Microbial Identification System (Sasser, 1990). The fatty acids profile of 22II14-10F7T was produced in parallel with that for Z. profunda SM-A87T in this study. The results for both strains are shown in Table S1, available in IJSEM Online. The major fatty acids in both strains were iso-C₁₅:₀ anteiso-C₁₅:₀ and iso-C₁₇:₁ 3-OH. The major fatty acids profile of Z. profunda SM-A87T was similar to that reported by (Qin et al., 2007), but they differed in the presence/absence of C₁₅:₀.

Analyses of respiratory quinones and polar lipids were carried out by the Identification Service of the DSMZ (Braunschweig, Germany). The quinones were extracted according to a previously described method (Tindall, 1990a, b). Respiratory lipoquinones were separated into their different classes (e.g. menaquinones, ubiquinones) by TLC on silica gel, then further analysed by HPLC. Polar lipids were extracted from 100 mg freeze-dried cellular material using a chloroform/methanol/0.3 % aqueous NaCl mixture [1 : 2 : 0.8 (by vol.)] (Bligh & Dyer, 1959). Polar lipids were separated by two-dimensional silica gel TLC and then identified according to a previously described method (Tindall et al., 2007). The major respiratory quinone of the strain 22II14-10F7T was determined to be MK-6 (95 %); some minor unidentified components were present. This trait is in accordance with the properties of Z. profunda SM-A87T. The polar lipid profile of strain 22II14-10F7T consisted of phosphatidylethanolamine (PE), two aminolipids (AL1 and AL2) and five unknown lipids (L1–L5) (Fig. S1). It was similar to that of Z. profunda SM-A87T, which only has one identified phospholipid (phosphatidylethanolamine) (Qin et al., 2007).

Antibiotic susceptibility tests were performed by disc diffusion methods as described by Shieh et al. (2003). Strain 22II14-10F7T and Z. profunda SM-A87T were tested at the same time in this study. Both of them were sensitive to carbenicillin (100 μg per disc; OXOID), chloromycetin (30), ciprofloxacin (5), clindamycin (2), erythromycin (15), lincomycin (2), minomycin (30), ofloxacin (5), piperacillin (100) and rifampicin (5) and resistant to ampicillin (10), cefalexin (30), cefazolin (30), cefobid (30), co-trimoxazole (25), gentamicin (10), kanamycin (30), metronidazole (5), oxacillin (1), penicillin G (10), poly¬myxin B (30 IU), rocebin (30), streptomyacin (10), vancomycin (30) and vibramycin (30). The different susceptibility patterns to three antibiotics of the two strains are shown in Table 1.

The DNA G+C contents of the novel isolate 22II14-10F7T was 35.5 mol% according to the draft genome sequence. It

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Table 1. Physiological characteristics of strain 22II14-10F7T and Zunongwanga profunda SM-A87T

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tr>
<td>Growth with:</td>
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<tr>
<td>0 % NaCl</td>
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<td>+</td>
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<td>15 % NaCl</td>
<td>+</td>
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<td>Hydrolysis of:</td>
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<tr>
<td>Gelatin</td>
<td>-</td>
<td>+</td>
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<td>Tween 80</td>
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<td>Malic acid</td>
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<tr>
<td>API ZYM</td>
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<tr>
<td>2-Mannosidase</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Lipase (C14)</td>
<td>w</td>
<td>-</td>
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<tr>
<td>Esterase (C4)</td>
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<td>w</td>
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<tr>
<td>Susceptibility to:</td>
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<tr>
<td>Cephradin, tetracycline</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Norfloxacin</td>
<td>+</td>
<td>-</td>
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<tr>
<td>DNA G + C content (mol%)*</td>
<td>35.5</td>
<td>36.2</td>
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*Data from genome sequence information.
Description of Zunongwangia atlantica sp. nov.

Zunongwangia atlantica (at.lan.ti’ca. L. fem. adj. atlantica referring to the Atlantic Ocean, where the strain was isolated).

Cells are Gram-stain-negative rod-shaped, 0.7–0.8 μm wide and 1.7–1.8 μm long and non-motile. Positive for oxidase, catalase, β-galactosidase, β-glucosidase (aesculin hydrolysis), D-glucose fermentation and hydrolysis of Tween 20, but negative for nitrate reduction, denitrification, indole production, gelatinase, urease, arginine dihydrolase and hydrolysis of Tween 40, 80 and starch. Cannot grow under anaerobic conditions. Cannot degrade oil. Alkane monooxygenase gene was not detected in the draft genome sequence. The differences in physiological, biochemical and chemotaxonomic characteristics shown in Table 1. On the basis of the data described above, strain 22I14-10F7T should be classified as representing a novel species of the genus Zunongwangia, for which a name Zunongwangia atlantica sp. nov. is proposed.

Acknowledgements

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References


Qin, Q. L., Zhao, D. L., Wang, J., Chen, X. L., Dang, H. Y., Li, T. G.,
Zhang, Y. Z. & Gao, P. J. (2007). Wangia profunda gen. nov., sp. nov.,
a novel marine bacterium of the family Flavobacteriaceae isolated
from southern Okinawa Trough deep-sea sediment. FEMS Microbiol
Lett 271, 53–58.

Qin, Q. L., Zhang, X. Y., Wang, X. M., Liu, G. M., Chen, X. L., Xie, B. B.,
genome of Zunongwangia profunda SM-A87 reveals its adaptation to
the deep-sea environment and ecological role in sedimentary organic
nitrogen degradation. BMC Genomics 11, 247.

standard for the prokaryotic species definition. Proc Natl Acad Sci
U S A 106, 19126–19131.

least-squares, generalized least-squares, and minimum-evolution

minimum-evolution method of phylogenetic inference. Mol Biol
Evol 10, 1073–1095.

425.

cellular fatty acids, MIDI Technical Note 101. Newark, DE: MIDI.

rubra sp. nov., a red, facultatively anaerobic, marine bacterium

Bacteria, 2nd edn. Baltimore, MD: Williams & Wilkins.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar,
S. (2011). MEGA5: molecular evolutionary genetics analysis using
maximum likelihood, evolutionary distance, and maximum par-

of Halobacterium saccharovorum from various sources. Syst Appl
Microbiol 13, 128–130.

Tindall, B. (1990b). Lipid composition of Halobacterium lacusprof-

Phenotypic characterization and the principles of comparative
systematics. In Methods for General and Molecular Microbiology, 3rd
dyn, pp. 330–393. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak,
Microbiology.