**Thalassospira alkalitolerans** sp. nov. and **Thalassospira mesophila** sp. nov., isolated from a decaying bamboo sunken in the marine environment, and emended description of the genus *Thalassospira*

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Two marine bacteria, designated strains MBE#61T and MBE#74T, were isolated from a piece of sunken bamboo in the marine environment in Japan. Both of these strains were Gram-stain-negative, but had different cell shapes: MBE#61T was spiral, whereas MBE#74T was rod-shaped. The temperature, pH and salt concentration ranges for growth of strain MBE#61T were 4–38 °C (optimal at 32 °C), pH 4.5–11.0 (optimal at pH 7.0–8.0) and 1–11 % (optimal at 2 %) NaCl, whereas those of strain MBE#74T were 4–36 °C (optimal at 30 °C), pH 4.0–10.5 (optimal at pH 7.0–8.0) and 1–12 % (optimal at 4 %) NaCl. Phylogenetic analysis based on partial 16S rRNA gene sequences revealed that both strains belong to the genus *Thalassospira* within the class *Alphaproteobacteria*. Similarity between the 16S rRNA gene sequence of strain MBE#61T and those of the type strains of species of the genus *Thalassospira* was 97.5–99.0 %, and that of strain MBE#74T was 96.9–98.6 %; these two isolates were most closely related to *Thalassospira lucentensis* QMT2T. However, the DNA–DNA hybridization values between *T. lucentensis* QMT2T and strain MBE#61T or MBE#74T were only 16.0 % and 7.1 %, respectively. The DNA G+C content of strain MBE#61T was 54.4 mol%, and that of strain MBE#74T was 55.9 mol%. The predominant isoprenoid quinone of the two strains was Q-10 (MBE#61T, 97.3 %; MBE#74T, 93.5 %). The major cellular fatty acids of strain MBE#61T were C18 : 1v7c (31.1 %), summed feature 3 comprising C16 : 0v7c/iso-C15 : 02-OH (26.1 %) and C16 : 0 (20.9 %); those of strain MBE#74T were C16 : 0 (26.2 %), C17 : 0 cyclo (19.9 %) and C18 : 1v7c (12.1 %). On the basis of these results, strain MBE#61T and strain MBE#74T are considered to represent novel species of the genus *Thalassospira*, for which names *Thalassospira alkalitolerans* sp. nov. and *Thalassospira mesophila* sp. nov. are proposed. The type strains are MBE#61T (=JCM 18968T=CECT 8273T) and MBE#74T (=JCM 18969T=CECT 8274T), respectively. An emended description of the genus *Thalassospira* is also proposed.

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**Plant biomass**, which consists of cellulose, hemicellulose and lignin, has attracted attention over the years as a promising resource to replace fossil fuels like petroleum. Lignin is one of the most abundant organic polymers on Earth and a complex phenolic compound with a great potential as a raw material for production of useful chemicals (Sjöström, 1993). Due to its persistency in chemical and biological

Abbreviation: PAH, polycyclic aromatic hydrocarbon.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strains MBE#61T and MBE#74T are AB786710 and AB786711, respectively.

Two supplementary tables and one supplementary figure are available with the online version of this paper.
attacks, its utilization has been little explored to date. Therefore, the efficient and effective utilization of lignin by biochemical industries is anxiously awaited. The first step of lignin degradation is driven by microbes like wood-decaying fungi (Gold & Alic, 1993; Deacon, 2009) and some members of the genera Streptomyces (Crawford et al., 1983) and Nocardia (Trojanowska et al., 1977). Then, the lignin-related compounds are decomposed to smaller molecules and are mineralized by some bacteria (Masai et al., 1999). During our search for microbes that show high metabolizing activity against lignin-related compounds, we have isolated a wide range of previously unidentified bacteria (Ohta et al., 2012). And recently, we isolated two bacterial strains, MBE#61T and MBE#74T, which metabolized lignin-related aromatic compounds effectively and were affiliated with the genus Thalassospira. So, in this article, we report the polyphasic taxonomic characterizations of these two isolates. The genus Thalassospira, which was originally proposed with Thalassospira lucentensis, the type species of the genus, is known to accommodate polycyclic aromatic hydrocarbon (PAH)-degrading bacteria. At the time of writing, strains representing five species (T. lucentensis QMT2T, T. xiamenensis M-5T, T. profundimarism WP0211T, T. tepidiphila 1-18T, and T. xianhensis P-4T) have been isolated as members of the genus Thalassospira, and all of them are able to degrade several PAHs like naphthalene and phenanthrene, each with specific properties (López-López et al., 2002; Liu et al., 2007; Kodama et al., 2008; Zhao et al., 2009; Zhao et al., 2010). In addition to the members of species with validly published names, ‘T. permensis’ SMB34 has been isolated (Plotnikova et al., 2011).

The two novel strains were isolated from a piece of sunken bamboo in the coastal area of Japan. Bamboo is a fast-growing plant and a significant bioresource in the east and south area of Asia. A portion (approx. 1 g) of the sunken bamboo was soaked in 2 ml sterile artificial seawater (ASW; Nihon Pharmaceutical) and shaken briefly on a vortex machine at room temperature. The immersion fluid was incubated at 25 °C for 1 day and then spread on 1.5 % (w/v) agar containing milled Japanese timber bamboo [MJTB; 2 % (w/v)] milled Japanese timber bamboo and 0.5 × ASW]. Both strains were isolated after incubation at 25 °C for 10 days. After incubation, small colonies appearing on MJTB plates were picked and streaked onto solid plates containing Marine Broth 2216 (MB; BD Difco) for purification. For routine cultivation, MB was used.

Cell morphology was observed under a light microscope equipped with phase-contrast optics at ×400 magnification (Olympus). The external structures of both strains were observed with a JSM-6700F scanning electron microscope (JEOL). Motility was assayed in semi-solid MB agar (0.5 % agar) for 4 days. Cells were inoculated by stabbing with a straight needle and the tube was incubated at 25 °C; 2, 4, 6, 8, 12, 16, 20, 24, 28, 30, 32, 36, 38, 40 and 44 °C, at different pH (pH 3–12; at intervals of 0.5 pH units), and at various NaCl concentrations (0–20.0 %, w/v, NaCl; at intervals of 1.0 %, w/v) was tested in handmade MB liquid medium. Growth under anaerobic conditions was determined in an anaerobic chamber filled up with nitrogen gas, using MB and MB medium supplemented with nitrate.

Colonies of both strains, MBE#61T and MBE#74T, were cream-coloured, smooth, nearly circular and 3.0–5.0 mm in diameter on MB agar after incubation at 25 °C for 1 week. Cells of strain MBE#61T were spirilla and approximately 1.3–3.0 μm in length and 0.4–0.5 μm in width, whereas those of strain MBE#74 were slightly curved rods and approximately 1.5–2.9 μm in length and 0.4–0.5 μm in width (Fig. 1). Both strains possessed a single polar flagellum and were Gram-stain-negative. Growth of strain MBE#61T occurred at 4–38 °C (optimum 32 °C), at pH 4.5–11 (optimum pH 7.0–8.0) and at NaCl concentrations of 1–11 % (optimum 2 %); strain MBE#74T grew at 4–36 °C (optimum 30 °C), at pH 4.0–10.5 (optimum pH 7.0–8.0) and at NaCl concentrations of 1–12 % (optimum 4 %). In anaerobic conditions, growth of the two strains was not observed after 2 weeks of cultivation in MB at 25 °C. Assimilation and acid production from various substrates was examined by using the Biolog GN2 MicroPlate assay according to the manufacturer’s instructions in the elementary sense (incubation at 25 °C for 1 week). Other physiological and biochemical properties were tested using the API 20NE kit (bioMérieux) at 25 °C for 1 week. Catalase activity was evaluated by observing the oxygen bubble production in a 3 % (v/v) aqueous hydrogen peroxide solution. Oxidase activity was evaluated by the oxidation of 1 % (w/v) p-aminodimethylaniline oxalate. To

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**Fig. 1.** Scanning electron microscope images of Thalassospira alkalitolferans sp. nov. MBE#61T (a) and Thalassospira mesophila sp. nov. MBE#74T (b) grown in MB liquid medium at 25 °C for 5 days. Bars, 1.0 μm.
assay the enzyme activities, APIZYM (bioMérieux) tests were performed at 25 °C for 1 week. Hydrolysis of starch (Smibert & Krieg, 1994) and Tween 80 was tested on MB agar plates supplemented with 0.5 % (w/v) soluble starch or 0.5 % (w/v) Tween 80. For acid production from carbohydrates, bromocresol purple was added to a final concentration of 30 mg l⁻¹. Sensitivity to antibiotics was checked on MB plates using antibiotics discs (BBL Sensi-Disc; BD) containing (μg per disc unless otherwise stated): bacitracin (10 IU), tetracycline (30), vancomycin (30), kanamycin (30), neomycin (30), streptomycin (10), novobiocin (30), gentamicin (10), rifampicin (5), chloramphenicol (30), erythromycin (15), ampicillin (10), penicillin G (10 U), lincomycin (15), polymyxin B (100 U) and carbenicillin (100).

Abilities of strains MBE#61T and MBE#74T to degrade several PAHs (naphthalene, phenanthrene, anthracene, pyrene and benzo[α]pyrene) were examined in TYV medium based on ASW (1 × ASW, 0.2 % (w/v) Tween 80, 0.1 % (w/v) yeast extract and 0.01 % (w/v) vitamin mix (Balch et al., 1979)) supplemented with single PAHs (5 μg ml⁻¹) using an HPLC instrument equipped with a XBridge BEH C18 column (4.6 mm × 100 mm; Waters). After 7 days of incubation, strain MBE#61T could degrade anthracene and benzo[α]pyrene obviously, and pyrene slightly. Strain MBE#74T degraded all of these three PAHs but weakly. No degradation occurred on naphthalene and phenanthrene.

In the same fashion, the ability of the two strains to degrade monomeric aromatic compounds was measured in TYV culture supplemented with the respective single compounds from the following ten monomeric aromatics; p-coumaric acid, ferulic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, protocatechuic acid, vanillin, syringaldehyde, catechol and benzoic acid. The initial substrate concentration for all compounds was 1 mM, ranging from 0.1 to 0.2 mg ml⁻¹ depending on their molar mass. Strains MBE#61T and MBE#74T degraded p-hydroxybenzoic acid, protocatechuate, catechol and benzoic acid completely, and oxidized vanillin and syringaldehyde to the corresponding acid derivatives.

The physiological properties of strain MBE#61T and MBE#74T are summarized in Table 1 and the species descriptions.

Genomic DNA of strains MBE#61T and MBE#74T was extracted and purified according to the method described by Wilson (1987). The G + C content of the genomic DNA of strains MBE#61T and MBE#74T was determined by an HPLC instrument (Waters 600 series; Nihon Waters; Mesbah et al., 1989) equipped with a Cosmosil 5C18-PAQ column (4.6 mm × 150 mm; Nacalai Tesque) and was found to be 54.4 ± 0.5 mol% and 55.9 ± 0.3 mol%, respectively.

DNA–DNA hybridization assays between strains MBE#61T, MBE#74T, T. lucentensis QMT2T, T. xiamenensis M-5T and T. tepidiphila 1-1BT were carried out with photobiotin-labelled probes in microplate wells as described by Ezaki et al. (1989) using an 1402 Multilabel Counter (Perkin Elmer) for fluorescence measurements. T. lucentensis QMT2T was the closest phylogenetic neighbour of the novel strains, and T. xiamenensis M-5T and T. tepidiphila 1-1BT were distantly related neighbours. The hybridization temperature was 47.1 °C and reciprocal experiments were performed for each pair of strains. The mean hybridization levels for the pairs of strains (n=8) are shown in Table S2 (available in IJSEM Online). Strains MBE#61T and MBE#74T exhibited relatively high levels of hybridization with T. lucentensis QMT2T (12.5–16.0 % and 7.1–11.0 %, respectively), T. xiamenensis M-5T (24.1–25.0 % and 8.0–15.8 %, respectively) and T. tepidiphila 1-1BT (11.3–19.4 % and 9.0–10.4 %, respectively). The DNA–DNA relatedness between the two isolates was 7.3–15.1 %. This suggested that the two isolates should be classified as novel species of the genus Thalassospira, according to the criteria for the delineation of bacterial species (Wayne et al., 1987).

PCR amplification of the 16S rRNA gene was performed using universal bacterial primers described by Lane (1991) with LA-Taq DNA polymerase (TaKaRa Bio). The purified PCR product was sequenced after TA-cloning using an ABI BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and an automated DNA sequencer (ABI 3730XL; Applied Biosystems), and the almost complete 16S rRNA gene sequences (1422 nt of both strains) were determined. The resultant 16S rRNA gene sequences of strains MBE#61T and MBE#74T were compared with those available from GenBank using the ARB software (Ludwig et al., 2004; Kumar et al., 2005, 2006) and then the sequences were aligned using CLUSTAL X version 2.0.12 (Larkin et al., 2007). Phylogenetic analyses were carried out using maximum-likelihood (Felsenstein, 1981), neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms. A phylogenetic tree was reconstructed using the maximum-likelihood algorithm and evolutionary distances were calculated with the general time-reversible model (Tavaré, 1986; Zwickl & Holder, 2004) with Azorhizobium caulindinos ORS 571T (AP009384) as an outgroup taxon. A bootstrap analysis (Felsenstein, 1985) was performed with 1000 resampled datasets to estimate tree topology (Fig. 2).

A maximum-likelihood tree based on 16S rRNA gene sequences indicated that these two strains, MBE#61T and MBE#74T, belong to the genus Thalassospira and are closely related to the type strain of T. lucentensis. This clade was supported by a high bootstrap value (99 %) and the tree topology was the same as obtained by the neighbour-joining and maximum-parsimony algorithms. The 16S rRNA gene sequences of strains MBE#61T and MBE#74T were highly similar (98.9 % similarity) and shared the highest levels of similarity with T. lucentensis QMT2T (99.0 % and 98.5 %, respectively). The following strains also shared high 16S rRNA sequence similarity with strains MBE#61T and MBE#74T: T. xiamenensis P-4T (97.9 % and 97.7 %, respectively), T. profundimaris WP0211T (97.7 % and 97.2 %, respectively), T. xiamenensis M-5T (97.5 % and 97.2 %, respectively) and T. tepidiphila 1-1BT (97.5 % and 96.9 %, respectively).
Table 1. Differential phenotypic characteristics of strains MBE#61^T and MBE#74^T, and the five type strains of the recognized species of the genus *Thalassospira*

<table>
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<tr>
<td>Citric acid</td>
<td>W</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Galacturonic acid</td>
<td>–</td>
<td>W</td>
<td>W</td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>W</td>
</tr>
<tr>
<td>D-Gluconic acid</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
The chemotaxonomic characteristics of strains MBE\#61$^\text{T}$, MBE\#74$^\text{T}$ and \textit{T. lucentensis} QMT2$^\text{T}$ were determined using cells cultured in MB liquid medium at the exponential phase of growth at 25\degree C for 1 week and harvested. Analyses of isoprenoid quinones, polar lipids and cellular fatty acids were carried out according to the methods described by Minnikin \textit{et al.} (1977, 1984), Kroppenstedt (1985) and Nishijima \textit{et al.} (1997) and compared to values obtained for \textit{T. lucentensis} QMT2$^\text{T}$, which is the type strain closely related to both strain MBE\#61$^\text{T}$ and strain MBE\#74$^\text{T}$. Isoprenoid quinones were determined by an HPLC instrument (Waters 600 series; Nihon Waters) equipped with a Cosmosil 5C18-PAQ column (4.6 mm × 150 mm; Nacalai Tesque). The polar lipids were separated by two-dimensional TLC with an aluminium-backed silica gel plate (no. 5514; Merck) in solvent systems described by Ivanova \textit{et al.} (2005). The polar lipid composition was determined in comparison to that of \textit{T. lucentensis} QMT2$^\text{T}$ with an authentic sample. Spot detection and percentage calculation were performed as described previously (Tsubouchi \textit{et al.}, 2013). For analysis of cellular fatty acids, cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification system (version 4.5; MIDI), and then the fatty acid methyl ester profile was determined by GC with reference to the TSBA40 MIS standard library.

The chemotaxonomic characteristics of strain MBE\#61$^\text{T}$ were as follows. The predominant isoprenoid quinone was Q-10 (97.3\%). Diphosphatidylglycerol (30.0\% of the total lipids), amino-group containing glycolipids (18.7\%),

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|c|c|}
\hline
Characteristic & 1 & 2 & 3 & 4 & 5 & 6 & 7 \\
\hline d-Glucosaminic acid & $-$ & + & + & + & + & + & + \\
d-Glucuronic acid & $-$ & w & + & $-$ & + & + & $-$ \\
\textit{\beta}-Hydroxybutyric acid & $-$ & w & + & + & + & $+$ & + \\
\gamma-Hydroxybutyric acid & $-$ & $-$ & + & + & + & + & + \\
p-Hydroxyphenylacetic acid & $-$ & $-$ & + & $-$ & $-$ & $-$ & $-$ \\
3-Ketogluartic acid & $-$ & + & + & $+$ & $-$ & $-$ & + \\
DL-Lactic acid & $-$ & $-$ & $+$ & + & $-$ & + & + \\
Propionic acid & $-$ & w & $-$ & + & $-$ & W & $-$ \\
Succinic acid & $-$ & $-$ & $+$ & + & $-$ & + & $+$ \\
L-Asparagine & $-$ & $-$ & $-$ & $-$ & $+$ & $+$ & $-$ \\
L-Asparatic acid & $-$ & $-$ & + & $-$ & $+$ & $-$ & $-$ \\
L-Histidine & $-$ & $-$ & + & $+$ & + & $+$ & $-$ \\
Hydroxy-L-proline & $+$ & $-$ & $-$ & $+$ & $+$ & $+$ & $-$ \\
L-Leucine & w & + & w & $-$ & $-$ & $-$ & $-$ \\
L-Ornithine & w & w & $-$ & $+$ & $-$ & $-$ & $-$ \\
L-Serine & w & + & + & $-$ & $-$ & $-$ & $-$ \\
L-Threonine & w & w & + & w & $-$ & $-$ & $-$ \\
\gamma-Aminobutyric acid & $+$ & $-$ & $+$ & $+$ & $+$ & $+$ & $-$ \\
Thymidine & $-$ & w & $-$ & $+$ & $-$ & $-$ & $-$ \\
Putrescine & $-$ & $-$ & + & $-$ & $-$ & $-$ & $-$ \\
2,3-Butanediol & $+$ & $-$ & ND & $-$ & $-$ & $-$ & $-$ \\
\alpha-D-Glucose 1-phosphate & $-$ & w & ND & $-$ & $-$ & $-$ & $-$ \\
Acid production & & & & & & & \\
d-Fructose & $-$ & $-$ & $-$ & + & + & $-$ & + \\
Glucose & $-$ & $-$ & $-$ & + & $+$ & $-$ & $-$ \\
Maltose & $+$ & $-$ & $+$ & $+$ & $+$ & + & $-$ \\
Mannitol & $-$ & $+$ & $-$ & $+$ & $+$ & + & $+$ \\
Sucrose & $-$ & $-$ & $-$ & $-$ & $-$ & $-$ & $-$ \\
Xylose & $-$ & $-$ & + & $-$ & $+$ & $-$ & $-$ \\
\alpha-D-Galactose & $-$ & $-$ & $+$ & $-$ & $-$ & $-$ & $-$ \\
Mannose & $-$ & $-$ & $+$ & $+$ & $+$ & + & $+$ \\
Arabinose & $-$ & $-$ & $-$ & $-$ & $+$ & $+$ & $-$ \\
Lactose & $-$ & $-$ & $-$ & $-$ & $-$ & $-$ & $-$ \\
\hline
DNA G+C content (mol\%) & 54.4 ± 0.5 & 55.9 ± 0.3 & 54.4 ± 0.9 & 52.9 ± 0.8 & 61.2 ± 1.0 & 53.5 ± 1.6 & 46.8 ± 1.2 \\
\hline
\end{tabular}
\end{table}

*S, spirilla; CR, curved rods; V, vibroid; M, monopolar single; C, cream; Y, yellowish; CY, cream–yellow; SY, slightly yellow; O, opalescent.

†Data were obtained in this study.

‡SA, strictly aerobic; FA, facultatively anaerobic.
Fig. 2. Phylogenetic tree indicating the relationship between *Thalassospira alkalitolerans* sp. nov. MBE#61\(^T\), *T. mesophila* sp. nov. MBE#74\(^T\) and related genera of the class *Alphaproteobacteria* based on 16S rRNA gene sequence analysis. The tree was reconstructed using the maximum-likelihood (Felsenstein, 1981) algorithm and the general time-reversible model (Tavaré, 1986). *Azorhizobium cauliformans* LMG 6465\(^T\) (AP009384) was used as an outgroup taxon. Single, double and triple asterisks respectively indicate that the corresponding branches were recovered in both neighbour-joining and maximum-likelihood trees, both maximum-parsimony and maximum-likelihood trees, and neighbour-joining, maximum-parsimony and maximum-likelihood trees. Bootstrap values greater than 50% are given at branch points. Bar, 0.05 substitutions per nucleotide position.
unidentified lipids (17.5%) and unidentified glycolipids (17.4%) were the major polar lipid constituents. The dominant cellular fatty acids making up more than 5% of the total were C₁₈:₃ω₇c (31.3%), summed feature 3 comprising C₁₆:₀ω7ciso-C₁₅:₀ 2-OH (26.05%), C₁₆:₀ (20.87%) and C₁₇:₀ cyclo (5.88%). The chemotaxonomic characteristics of strain MBE#7₄ᵀ were as follows: the predominant isoprenoid quinone was Q-10 (93.5%). Amino-group containing glycolipids (23.0%), unidentified glycolipids (22.1%), phosphatidylglycerol (18.0%) and phosphatidylethanolamine (13.4%) were the major polar lipids. The dominant cellular fatty acids making up more than 5% of the total were C₁₆:₀ (26.19%), C₁₇:₀ cyclo (19.88%), C₁₈:₁ω7c (12.12%), C₁₉:₁ cyclo ω8c (10.79%) and C₁₄:₀ (8.25%). As reference, the chemotaxonomic characteristics of T. lucentensis QMT2ᵀ were as follows: the isoprenoid quinone was Q-10 (100.0%). Phosphatidylglycerol (31.3%), unidentified glycolipids (22.2%), diphosphatidylglycerol (20.3%) and phosphatidylethanolamine (10.3%) were the major polar lipid constituents. The dominant cellular fatty acids making up more than 5% of the total were C₁₈:₁ω7c (44.01%), summed feature 3 comprising C₁₆:₀ω7ciso-C₁₅:₀ 2-OH (23.86%) and C₁₆:₀ (15.29%). The predominant cellular fatty acids of the two novel isolates were similar to those of other recognized species of the genus Thalassospira, even though the proportions were different. The chemotaxonomic characterizations of the two isolates and two recognized species of the genus Thalassospira mesophila examined in this study are summarized in Table S1 and Supplementary figure S1.

On the basis of their phenotypic, physiological and chemotaxonomic characteristics, in addition to their phylogeny based on 16S rRNA gene sequence analysis and their genomic DNA–DNA relatedness, the two isolates should be classified as novel members of the genus Thalassospira, and moreover, should be considered as distinct species from each other. So, we propose that these two new strains are designated as representatives of two novel species, Thalassospira alkalitolerans sp. nov. for strain MBE#61ᵀ and Thalassospira mesophila sp. nov. for strain MBE#7₄ᵀ.

Emended description of the genus Thalassospira

The genus Thalassospira described by López-López et al. (2002) was previously emended by Liu et al. (2007). The description is as given previously with the following modifications. The cells are strictly aerobic or facultatively anaerobic, oxidase variable and 0.8–5 μm wide. The principal cellular fatty acids are C₁₈:₁ω7c and C₁₆:₀. Isoprenoid quinone type is Q-9 or Q-10. The range of DNA G+C content is 46.8 ± 1.2–61.2 ± 1.0 mol%.

Description of Thalassospira alkalitolerans
sp. nov.

Thalassospira alkalitolerans sp. nov. [Tha.las.so.spra.de. kal.al.i.to.ler.an.s] N.L. n. alkalitolerans (from Arabic article al the; Arabic n. qaliy ashes of saltwort) alkali; L. part. adj. tolerans tolerating; N.L. part. adj. alkalitolerans alkali-tolerating, referring to the ability of the organism to tolerate alkaline media.

Cells are Gram-stain-negative, strictly aerobic, heterotrophic, salt-tolerant, alkali-tolerant, motile spirochaetes with a monopolar flagellum, approximately 1.3–3.0 μm in length and 0.4–0.5 μm in width. Colonies are cream-coloured, smooth, nearly circular and 3.0–5.0 mm in diameter on MB agar after incubation at 25 °C for 4 days. Growth occurs at 4–38 °C (optimum 32 °C), NaCl concentrations between 1 and 11% (optimum 2%) and at pH 4.5–11 (optimum pH 7.0–8.0). Hydrolysis of starch and Tween 80 are not observed. With API ZYM, positive for alkaline phosphatase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, weakly positive for esterase (C4) and valine arylamidase, but negative for esterase lipase (C4), lipase (C14), cyanine arylamidase, trypsin, α-chymotrypsin, x-galactosidase, β-galactosidase, β-glucuronidase, x-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, x-mannosidase and x-fucosidase. Catalase and oxidase are negative. With API 20NE assay, weakly positive for hydrolysis of aesculin, but negative for nitrate reduction, indole production, glucose fermentation, arginine hydrolyase, urease, β-galactosidase and hydrolysis of gelatin. Sensitive to kanamycin, gentamicin, chloramphenicol, penicillin G, tetracycline, chloramphenicol, penicillin G, polymixin B and carbenicillin; slightly sensitive to tetracycline, streptomycin, novobiocin, rifampicin and erythromycin; resistant to bacitracin, vancomycin, neomycin, ampicillin and lincomycin. Among the 95 carbon sources in Biolog assay, the following are utilized: dextrin, Tween 40, lactulose, L-rhamnose, citric acid, quinic acid, succinamic acid, L-alanine, L-alanlyglycine, L-glutamic acid, hydroxy-L-proline, L-arginine, L-proline, L-prolylg glutamic acid, D- and L-serine, L-threonine, γ-aminobutyric acid, 2,3-butanediol and glycerol. Degrades anthracene and benzo[a]pyrene obviously, and pyrene slightly. Acid is produced from maltose and sucrose. The predominant isoprenoid quinone is Q-10. The major fatty acids are C₁₈:₁ω7c, summed feature 3 comprising C₁₆:₀ω7ciso-C₁₅:₀ 2-OH and C₁₆:₀. The major polar lipids are diphosphatidylglycerol, amino-group containing glycolipid, unidentified lipids and unidentified glycolipids.

The type strain, MBE#61ᵀ (=JCM 18968ᵀ=CECT 8273ᵀ), was isolated from sunken bamboo in the shallow water of Japan. The genomic DNA G+C content of the type strain is 54.4 ± 0.5 mol%.

Description of Thalassospira mesophila
sp. nov.

Thalassospira mesophila [me.so phi.la. Gr. adj. mesos medium; N.L. adj. philus-a -um (from Gr. adj. philos -e- on) friend, loving; N.L. fem. adj. mesophila medium-temperature-loving, mesophilic].

Cells are Gram-stain-negative, strictly aerobic, heterotrophic, salt-tolerant, alkali-tolerant, motile curved rods with a monopolar flagellum, approximately 1.5–2.9 μm in length.
length and 0.4–0.5 μm in width. Colonies are cream-coloured, smooth, nearly circular and 3.0–5.0 mm in diameter on MB agar after incubation at 25 °C for 4 days. Growth occurs at 4–36 °C (optimum 30 °C), NaCl concentrations between 1 and 12% (optimum 4%) and at pH 4.0–10.5 (optimum pH 7.0–8.0). Hydrolysis of starch and Tween 80 are not observed. With API ZYM, positive for alkaline phosphatase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, slightly positive for esterase (C4), esterase lipase (C4), and naphthol-AS-BI-phosphohydrolase, slightly positive for alkaline phosphatase, leucine arylamidase, acid phosphatase

**References**


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