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EXPERIMENTAL ARTICLES

Thalassospira permensis sp. nov., A New Terrestrial Halotolerant Bacterium Isolated from a Naphthalene-Utilizing Microbial Consortium[1]

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Abstract—A halotolerant bacterium, strain SMB34^T, was isolated from a naphthalene-utilizing bacterial consortium obtained from primitive technogeneous soil (Verkhnekamsk salt deposit, Perm region, Russia) by enrichment procedure. The strain itself was unable to degrade naphthalene and grew at NaCl concentrations up to 11% (w/v). The 16S rRNA-based phylogenetic analysis showed that the strain belongs to the genus *Thalassospira*. The DNA-DNA hybridization values between SMB34^T and the type strains of phylogenetically closest species (*T. xiamenensis, T. profundimaris* and *T. tepidiphila*) did not exceed 50%. The novel strain could be distinguished from the above species by the cell motility, MALDI/TOF mass spectra of whole cells and a range of physiological and biochemical characteristics. SMB34^T also considerably differs from the recently described species *T. xianhensis*, with the most striking differences in the DNA G + C content (53. ± 1.0 vs. 61.2 ± 1.0 mol %) and predominant ubiquinones (Q-10 vs. Q-9). The data obtained suggest strain SMB34^T (=VKM B-2527^T = NBRC 106175^T), designated as the type strain, represents a novel species, named *Thalassospira permensis* sp. nov.

Keywords: proteobacteria, *Thalassospira,* new species, halotolerant bacterium, MALDI/TOF mass spectra.

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A bacterial consortium SMB3, capable of utilizing naphthalene under NaCl concentration up to 11%, was isolated from primitive technogeneous soil formed on salt-mine spoils at the Verchnekamsk deposit of potassium-magnesium salts (Berezniki, Perm region, Russia) by the enrichment culture technique [1]. The consortium consisted of two halophilic organisms of the family *Halomonadaceae*, namely, *Salinicola socius* SMB35^T [2, 3] and *Halomonas* sp. SMB31, four halotolerant actinobacteria of the genera *Arthrobacter*, *Microbacterium* and *Rhodococcus* and a bacterium (strain SMB34^T) tentatively identified as a member of the family *Rhodospirillaceae* on the basis of phenotypic characteristics [1]. Subsequent analysis of the *16S rRNA* gene of strain SMB34^T showed that it belonged to the genus *Thalassospira* [4]. The most organisms of this genus, including four recognized species, i.e., *T. lucentensis* (type species) [4], *T. profundimaris, T. xiamenensis* [5] and *T. tepidiphila* [6], are inhabitants of marine environments or seawater-based pools.

In the present study, strain SMB34^T was subjected to a polyphasic taxonomic analysis and shown to represent a new species of the genus *Thalassospira*.

MATERIALS AND METHODS

Isolation and culture conditions. The enrichment culture was incubated aerobically at 28°C with shaking in Raymond's mineral medium (RMM) [7] containing (g l^{-1}) NH_4NO_3 (2.0), $MgSO_4 \cdot 7H_2O$ (0.2), KH_2PO_4 (2), Na_2HPO_4 (3), $CaCl_2 \cdot 6H_2O$ (0.01), Na_2CO_3 (0.1), 2 ml of 1% $MnSO_4 \cdot 5H_2O$ and 2 ml of 1% $FeSO_4 \cdot 7H_2O$ that was supplemented with naphthalene (0.1%, w/v) and NaCl (6%, w/v) as described previously [1]. Strain SMB34^T and other bacteria composing the naphthalene-utilizing consortium SMB3 were isolated by plating the enrichment onto RMM agar supplemented with 0.5% (w/v) tryptone, 0.25% (w/v) yeast extract and 3% (w/v) NaCl (desig-

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nated complete Raymond's medium, CRM). The strain was routinely cultured on CRM agar and Marine agar 2216 (MA; Difco) at 28 or 30°C.

Bacterial strains. Along with strain SMB34^T, the following three type strains of the phylogenetically close *Thalassospira* species, namely, *T. xiamenensis* M-5^T, *T. profundimaris* WP0211^T and *T. tepidiphila* 1-1B^T were used in experiments concerned with genotypic and phenotypic characterization (physiological and biochemical properties, the cellular fatty acid and ubiquinone compositions, MALDI/TOF MS analysis, the G + C DNA content and DNA–DNA hybridization).

Morphological, physiological and biochemical characteristics. The cell morphology, motility and flagellation were studied in cultures grown in liquid CRM medium at 28°C for 1–3 days by using phase-contrast and transmission electron microscopy. For electron microscopy, cells were negatively stained with uranyl acetate. The Gram-stain type was examined by using the standard method [8]. The relation to temperature was determined on CRM agar at 4, 10, 20, 28, 30, 37, 40 and 45°C (pH 7.0). The salinity range and optimum were tested in liquid RMM medium supplemented with 0.5% (wt/vol) triptone and 0.25% (wt/vol) yeast extract and Nutrient broth (NB; Difco) which contained 0.5 and 1–15% (w/v) NaCl (at 1% intervals) and also in the same media without addition of NaCl. The ability of strain SMB34^T to grow at different pH values (5–9) was studied at Na^+ concentrations of 0.8–0.85 M in the buffer systems (acetate buffer for pH 5, phosphate buffer for pH 6, 7 and 8, and *Tris*-HCl buffer for pH 8 and 9) [8] based on liquid RMM with glucose (1%, w/v). The growth intensity was assessed in triplicate by measuring the change in optical density (OD) at 540 nm with a photocolorimeter in all the above experiments. Catalase and oxidase activities were tested with a 3% (v/v) H_2O_2 solution and 1% (w/v) tetramethyl-*p*-phenylenediamine, respectively. Assimilation of carbon sources, activities for arginine dihydrolase, urease and β-galactosidase, hydrolysis of aesculin, gelatin, and production of indole were determined by inoculating the API 20NE strip (bioMérieux) with cells suspended in 3% NaCl. Utilization of acetate and D-sorbitol as sole carbon sources was tested in RMM medium with 3% NaCl, supplemented with the above carbon sources (0.5%, w/v) and assessed by measuring the change in optical density as aforementioned. Acid production from carbohydrates under aerobic conditions, nitrate reduction, denitrification, phenylalanine deaminase activities, the formation of hydrogen sulfide were investigated using API 20E test system (bioMerieux) according to the manufacturer's instructions, except that 3% NaCl solution was used for inoculation. Assimilation of carbon sources was also characterized using Biolog GN MicroPlates (Biolog) according to the manufacturer's instructions, except that 3% NaCl solution was used for inoculation. In addition, acid production from carbohydrates under aerobic conditions and glucose fermentation/oxidization were examined using the Hugh-Leifson medium with bromthymol-blue as described [8]. Nitrate reduction, denitrification, the ability to hydrolyze gelatin were also tested according to [8].

Cellular fatty acids and respiratory lipoquinones were analyzed as reported previously [2]. Cells for analyses were grown in trypticase soy broth (Difco) containing 2% (w/v) NaCl at 28°C for 48 h (the later exponential growth phase).

For matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI/TOF MS) analyses of whole cells [9–11], the strains were grown on Marine agar (Difco) and R2A medium (Difco) supplemented with 3% NaCl ($R2A_{NaCl}$) at 28°C for 24 h (5 independent experiments for a strain grown on each medium). The cells (approx. 3 μl) were removed from plates using a sterile pipette tip, dissolved in 50 μl of freshly prepared 50% acetonitrile (Sigma-Aldrich) containing 2.5% trifluoroacetic acid and vortexed. The suspension (0.8 μl) was applied directly as a thin film onto a polished-steel MALDI sample target and immediately mixed with 0.8 μl of matrix solution (a saturated solution of α-cyano-4-hydroxycinnamic acid [HCCA] dissolved in 50% acetonitrile containing 0.1% trifluoroacetic acid). The matrix sample spots were crystallized by air drying. The samples were analyzed with a time-of-flight mass spectrometer (Autoflex II, Bruker Daltonics, Germany). A nitrogen laser (wavelength 337 nm, pulse energy 100 mkJ) was used for ionization. Positive ion mass spectra were collected in the linear mode using a delay time of 350 ns, an acceleration voltage of 20 kV. The mass spectra were recorded in the range 2–20 kDa. The spectra were externally calibrated using a standard calibration mixture (protein calibration standard I was supplied by Bruker Daltonics, Germany). The obtained MALDI/TOF mass spectra were compared manually and by using the BioTyper (Version 1.0, BrukerDaltonics) software package.

The DNA isolation and G + C mol % content. Genomic DNA was extracted, purified [2] and the DNA G + C mol % content was determined by the thermal denaturation method [12, 13].

REP-PCR (repetitive extragenic palindromic-PCR) and BOX-PCR (derived from the *box*A element) DNA banding patterns were generated following the protocol [14].

16S rRNA gene sequencing and phylogenetic analysis. Amplification and sequencing of the 16S rRNA gene were performed as described previously [15]. The sequences of the PCR products were determined using a CEQ Dye Terminator Cycle Sequencing Kit and a model DNA sequencer MegaBACE 1000 (JSC GE Healthcare, USA). Alignment of 16S rDNA sequences from strain SMB34^T and closely related

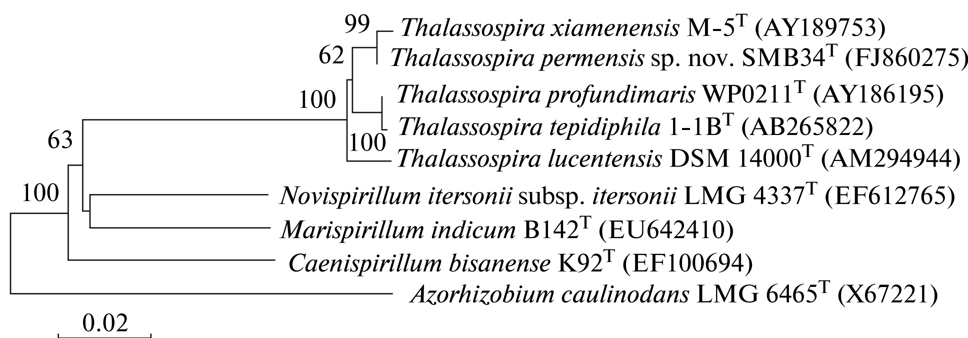

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain SMB34^T and the type strains of the *Thalassospira* species. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.02 nucleotide substitution rate (K_{nuc}) unit *Azorhizobium caulinodans* LMG 6465^T was used as the outgroup.

organisms retrieved from GenBank (http:/www.ncbi.nlm.nih.gov) was generated using CLUSTAL W software [16] and corrected manually. Evolutionary distances expressed in the estimated numbers of replacements per 100 nucleotides were calculated and phylogenetic trees were constructed using the neighbor-joining algorithm from TREECON [17]. Bootstrap analysis was performed using the functions of the TREECON program on the basis of 1000 alternative trees. Pairwise similarity values were calculated using the web-based tool provided by EzTaxon (www.eztaxon.org).

The DNA-DNA hybridization experiments were carried out with ^{3H}CTP-labeled probes by membrane filter method according to [18] as described previously [19].

RESULTS AND DISCUSSION

Strain SMB34^T and reference organisms formed similar colonies which were convex, circular, smooth, with entire edge, and yellowish on CRM agar and MA. Cells of strain SMB34^T were Gram-stain negative, slender curved rods or vibrioid, 0.23–0.42 × 1.23–2.0 μm in size, and motile by means of a single polar flagellum.

The 16S rRNA gene sequence of strain SMB34^T was 1398 bp long. Phylogenetic analysis performed with the sequences from nucleotide positions 41 to 1485, according to the *Escherichia coli* numeration [20], clearly indicated that the strain belonged to the genus *Thalassospira*, the family *Rhodospirillaceae*, class *Alphaproteobacteria* (Fig. 1). The phylogenetically nearest relatives with validly published names were *T. xiamenensis*M-5^T (99.6% 16S rRNA gene similarity), *T. profundimaris* WP0211^T (98.9%) and *T. tepidiphila* 1-1B^T (98.7%). The type species of this genus, *T. lucentensis*, was more distant from the above organisms (97.8–98.4% 16S rRNA gene similarities). It is also worth mentioning that different nucleotides (C or T) at the position 86 (according to the *E. coli* numbering) were revealed in the 16S rRNA gene nucleotide sequence of strain SMB34^T, which is rather indicative of the presence of at least two rRNA operons with different 16S rRNA genes. More than one rRNA operon and multiple 16S rRNA gene variants were revealed in a substantial number of bacterial genomes [21], but were not reported so far for the recognized species of the genus *Thalassospira*. Strain SMB34^T was also clearly distinguishable from the type strains of the aforementioned *Thalassospira* species by the DNA banding patterns produced with the REP-PCR and BOX-PCR fingerprinting techniques (data not shown).

The DNA G + C content of SMB34^T was 53.7 ± 1.0 mol %. The values obtained in this work for *T. xiamenensis* M-5^T (53.3 ± 1.1 mol %) and *T. tepidiphila* 1-1B^T (55.9 ± 1.0 mol. %) were consistent with those reported previously [5, 6]. A higher value (53.2 ± 1.0 mol %) than that provided in the original species description [5] was found for *T. profundimaris* WP0211^T (Table 1).

The mean DNA–DNA similarity value for SMB34^T towards *T. xiamenensis* M-5^T, *T. tepidiphila* 1-1B^T and *T. profundimaris* WP0211^T were 46.8 ± 1.8%, 34.7 ± 1.5% and 34.5 ± 0.1%), respectively. The results for reference strains were as follows: 49.3 ± 1.2% (the pair *T. profundimaris*–*T. tepidiphila*), 35.0 ± 0.5% (*T. xiamenensis*–*T. tepidiphila*), and 37.4 ± 1.9% (*T. xiamenensis*–*T. profundimaris*).

The cellular fatty acid pattern of strain SMB34^T was characteristic of the genus *Thalassospira* and included $C_{18:1}\omega7$ (37.4%), $C_{16:0}$ (20.1%), $C_{18:0}$ (10.9%), $C_{16:1}\omega7c$ (6.4%), *anteiso*-$C_{15:0}$ (5.8%), *iso*-$C_{15:0}$ (2.8%), $C_{14:0}$ 3-OH (2.56%), $C_{17:0}$ cyclo (3.10%), $C_{19:0}$ cyclo (1.50%), $C_{14:0}$ (2.06%), $C_{14:1}\omega7$ (1.45%), along with minor proportions (<1%) of other components. The major respiratory lipoquinone of SMB34^T and the type strains of *T. profundimaris, T. tepidiphila* and *T. xiamenensis* was ubiquinone with ten isoprene units (Q-10). In addition, minor amounts of Q-9 was detected in strain SMB34^T, while Q-11 was a minor component in *T. xiamenensis* M-5^T.

Table 1. Characteristics that differentiate SMB34^T from the type strains of *Thalassospira* species

Characteristic	SMB34^T	*T. xiamenensis* M-5^T	*T. profundimaris* WP0211^T	*T. tepidiphila* 1-1B^T	*T. lucentensis* QMT2^T*	*T. xianhensis* P-4^T**
Cell morphology*	Curved rods to vibrioid	Curved rods	Curved rods	Vibrioid to spiral	Vibrioid to spiral	Vibroid to spiral
Cell size, µm*	0.23–0.42 × 1.2–2.0	0.3–0.8 × 0.8–2.3	0.3–0.8 × 0.8–2.3	0.17–0.33 × 0.9–2.0	0.6 × 3–5	0.3–0.6 × 1.0–1.6
Flagellum*	Monopolar single	Monopolar single	–	Monopolar single	Monopolar single	–
Growth at 4°C	– or w	+	–	–	+	–
at 40°C	+	+	–	+	+	+
at 45°C	–	+	–	– / +†	ND	+
NaCl range for growth (%)	0–11.0 /0–9.0‡	0.5–10.0	2.0–9.0 /2.0–8.0†	0.5–12.0 /2.0–14.0†	2–10	0.1–17
Optimum NaCl (%)	2–5	2–4	3–4 /2–4†	3–4 /3†	ND	3–6
$NO_3^- \longrightarrow NO_2^-$	+	+	– /+†	+	– /+†	+
$NO_3^- \longrightarrow N_2$	+	+	– /+†	–	–	+
Growth with carbon source						
API 20NE test system:						
D-Maltose	+	+	–	+	+	ND
Capric acid	–	+	–	–	ND	ND
Citric acid	w	+	–	–	+	ND
Malic acid	+	–	v	+	+	ND
N-acetyl-glucosamine	+	+	–	–	+	ND
In RMM medium:						
Acetate	+	+	–	+	–	ND
D-Sorbitol	+	–	–	–	–	ND
Aerobic acid production††						
D-Glucose	+	w	–	–	ND	+
D-Mannitol	–	w	–	–	ND	+
Inositol	–	w	–	–	ND	ND
D-Sorbitol	–	w	–	–	ND	ND
D-Sucrose	–	+	–	–	ND	+
L-Arabinose	+	+	–	–	ND	ND
Degradation of naphthalene	–	ND	ND	+*	ND	+
Ubiquinones#	Q-10 (Q-9)	Q-10 (Q-11)	Q-10	Q-10	ND	Q-9
DNA G + C content (mol. %)	53.7 ± 1.0	53.3 ± 1.1 (52.6†)	53.2 ± 1.0 (47.0†)	55.9 ± 1.0 (55.1 ± 0.4†)	54.7	61.2 ± 1.0
Isolation source	Technogenous soil, salt mine	Seawater-based waste-oil pool	Deep-sea sediment	Petroleum-contaminated seawater	Sea water	Saline soil contaminated by crude oil

Note: +, positive reaction or growth; –, no reaction or growth; w, weakly positive reaction; v, variable; ND, no available data. All strains are positive for assimilation D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate and negative for hydrolysis of gelatin, aesculin and urea, indole production, assimilation adipic and phenylacetic (API 20NE test system). All strains are negative for acid production from L-rhamnose, D-melibiose and amygdalin, H_2S production, lysine and ornithine decarboxylase activity (API 20E test system). Similar data on acid production were obtained with conventional methods. Data for reference strains are from this study unless indicated.

* Data for *T. lucentensis* QMT2^T are taken from [4] and some characteristics for other reference species are from [5, 6].

** Data for *T. xianhensis* P-4^T are taken from [25].

†, Conflicting results reported in different publications [5, 6].

‡, Data obtained by using basal media NB and CRM, respectively.

†† According to API 20E test system and Hugh-Leifson test (see the text for details). Data for *T. xianhensis* are from [25], obtained using the SSDM with bromocresol purple as basal medium.

Minor ubiquinone is indicated in parentheses.

The MALDI/TOF mass spectrometry of whole bacterial cells is a rather new rapid and efficient method permitting the differentiation and identification of bacteria, particularly at the species level [9–11]. The protein identification studies have revealed that many of the signals of the bacterial mass patterns are derived from ribosomal or other abundant bacterial proteins [11]. In the present study, the protein peaks with a relative intensity >1% (unambiguously detectable peaks) were registered in the range of 3000 to 11000 *m/z* (mass/charge) and were resolved with ±2.0 Da reproducibility (manufacturer's specification) on the *m/z* scale. The peak patterns were influenced by the growth medium and to a lesser degree were dependent on individual experiment. In general, the spectra of cultures from $R2A_{NaCl}$ contained a little more peaks than cultures from MA. Over 80% peaks were reproduced in all replicates of a strain from each of the above media and usually had closely comparable relative (but not absolute) intensities. Five ion peaks (*m/z* 4912, 5138, 6754, 6812, 7253) of different relative intensities were registered in all the cultures irrespective of the growth medium, with the peaks being more pronounced with cells from $R2A_{NaCl}$ (Fig. 2, Table 2). These five peaks may probably serve as biomarkers of organisms of the genus *Thalassospira* or at least the species group considered.

Regardless of the growth medium and individual experiment, the spectra of SMB34^T and *T. xiamenensis* M-5^T always displayed a higher similarity, while the spectra of *T. tepidiphila* 1-1B^T were more similar to those of *T. profundimaris* WP0211^T. Clustering of the MALDI/TOF mass spectra acquired in each individual experiment by using the BioTyper Software (data not shown) was in excellent agreement with the phylogenetic grouping based on 16S rRNA gene sequences (Fig. 1). As shown in five experiments with the strains grown on $R2A_{NaCl}$, the proportion of similar peaks with a relative intensity >1% for SMB34^T and *T. xiamenensis* M-5^T was 79.0–86.6%), while strains of *T. profundimaris* and *T. tepidiphila* shared 73.2–87.0%) similar components. Some peaks were unique to each strain, though relative intensities of such peaks usually did not exceed 10% and sometimes the peaks were not detectable (relative intensity <1%). The unique peaks reproducible in 3–5 experiments for strain SMB34^T were registered at *m/z* 4477 and 10350, whereas the unique peaks for *T. xiamenensis* M-5^T were at *m/z* 3635 and 4519. Thus, strain SMB34^T could be distinguished both from the phylogenetically closest *T. xiamenensis* M-5^T and the remaining strains by its MALDI/TOF mass spectrum. The data obtained also showed that MALDI/TOF MS fingerprinting performed under standardized and controlled experimental conditions, combined with the specific peptide peak patterns, can be useful for identification of bacteria of this genus at the species level. Furthermore, members of *Thalassospira* clearly differ from other bacterial genera by the

Table 2. Regularly detectable components in MALDI/TOF mass spectra (molecular weight in Da per ion charge, *m/z*) of *Thalassospira* strains grown at 28°C for 24 h on R2A (Difco) supplemented with 3% (w/v) NaCl

m/z values	SMB34^T	*T. xiamenensis* M-5^T	*T. profundimaris* WPO211^T	*T. tepidiphila* 1-1B^T
3625	–	–	+	(+)
3635	–	(+)	–	–
3653	–	–	+	(+)
3684	(+)	+	–	–
4402	–	–	(+)	–
4466	–	–	(+)	(+)
4477	(+)	–	–	–
4481	–	–	–	(+)
4519	–	(+)	–	–
4629	+	+	–	–
4636	–	–	+	+
<u>4912</u>	+	+	+	+
<u>5138</u>	+	+	+	+
5159	–	–	+	(+)
5172	+	+	–	–
5223	–	–	+	(+)
6147	–	–	–	(+)
6303	–	–	–	(+)
6307	+	+	–	–
6318	-	-	+	+
<u>6754</u>	(+)	(+)	(+)	(+)
<u>6812</u>	+	+	(+)	(+)
<u>7253</u>	+	(+)	+	+
7264	–	–	+	+
7273	+	+	–	–
7310	–	–	+	+
7368	+	+	–	–
7509	+	+	–	–
7516	–	–	(+)	(+)
9217	+	+	–	–
9261	+	+	–	–
9277	–	–	+	+
10350	+	–	–	–

Note: + and (+), protein peaks with a relative intensity >1% unambiguously detectable in 5 and 3–4 independent experiments, respectively; –, not appeared in any spectrum of a strain. Underlined are components characteristic of all *Thalassospira* strains used in the study.

The protein peaks were resolved with ±2.0 Da reproducibility (manufacturer's specification).

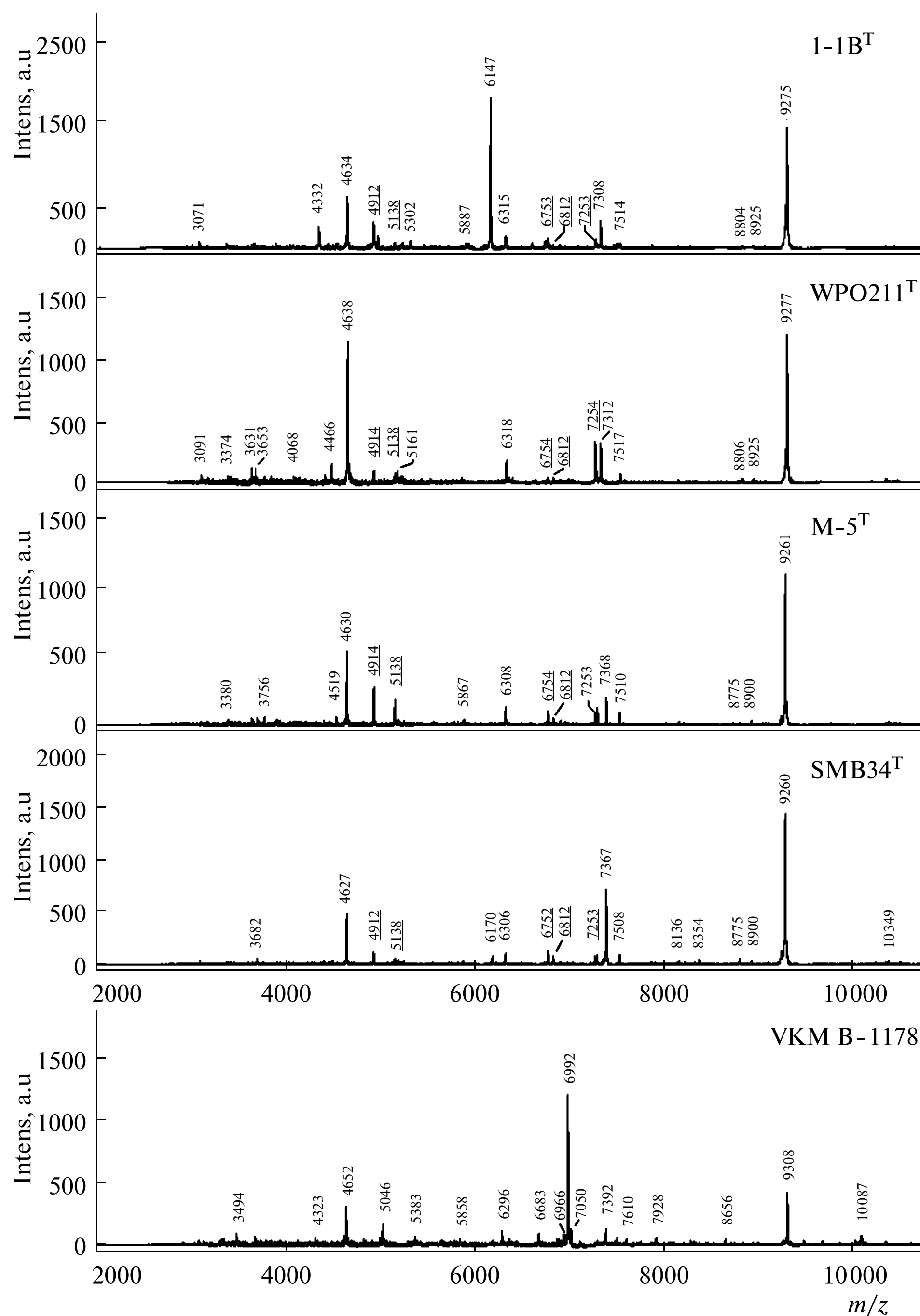

Fig. 2. MALDI/TOF mass spectra of strain SMB34^T and reference strains, *T. xiamenensis* M-5^T, *T. profundimaris* WP0211^T and *T. tepidiphila* 1-1B^T grown at 28°C for 24 h on R2A agar supplemented with 3% (w/v) NaCl and the spectrum of phylogenetically distant species, *Brevundimonas* sp. VKM B-1178, grown on R2A. Numerals indicate ions peaks' *m/z* values (molecular weight in Da per ion charge). Peaks with the underlined *m/z* values are characteristic of all *Thalassospira* strains analyzed (see Table 3 for details).

spectra, as show our preliminary results with representatives of environmental *Alphaproteobacteria* (exemplified by a strain of *Brevundimonas* Fig. 2).

Data on the physiological and biochemical characteristics are listed in Tables 1 and 3, and in the species description. Their comparison shows that the phenotypic features of strain SMB34^T are generally consistent with those of the genus *Thalassospira*. At the same time, strain SMB34^T is clearly distinguishable from the other species of this genus, including the phylogenetically closest *T. xiamenensis*. Notably, only strain SMB34^T was able to grow (moderately well) in media

Table 3. Characteristics that differentiate SMB34^T from the type strains of *Thalassospira* species according to Biolog GN MicroPlates (Biolog)

Substrate	SMB34^T	*T. xiamenensis* M-5^T	*T. profundimaris* WP0211^T	*T. tepidiphila* 1-1B^T	*T. lucentensis* QMT2^T	*T. xianhensis* P-4^T
Acetic acid	+	w	–	+	+	–
N-Acetyl-D-glucosamine	+	+	–	–	+	+
cis-Aconitic acid	+	+	–	+	w	+
γ-Amino butyric acid	+	–	–	+	+	+
L-Asparagine	+	+	–	+	–	+
Aspartic acid	+	w /–†	–	+	+	+
Citric acid	+	+ /–†	–	+	+	+
D–Galactose	+	+	–	+	+	+
D-Galacturonic acid	w	–	w	w	w	–
D-Gluconic acid	+	+	+	w /–†	+	+
D-Glucuronic acid	+	–	–	+	+	+
Glycerol	–	+	+	+	+	+
L-Histidine	+	+	w	+	+	+
Hydroxy-L-proline	+	+	–/ w†	+	–	+
p-Hydroxyphenylacetic acid	–	–	–	–	+	–
myo-Inositol	+	+	–	+	+	+
α-D-Lactose	–	–	–	– /+†	–	+
L-Leucine	w	w /–†	–	–	w	–
Maltose	+	+	–	–	+	+
D-Mannitol	+	+	+	+	–	+
L–Ornithine	+	+	–	–	–	+
Putrescine	–	–	–	–	+	+
D-Saccharic acid	+	–	–	–	+	+
L-Serine	+	+	–	+	+	–
D-Sorbitol	+	–	–	–	–	+
Sucrose	+	+	–	–	–	+
D-Trehalose	+	+	–	–	–	+
L-Threonine	w	w	–	–	–	–
Thymidine	+	+	–	–	–	–

Note: +, positive reaction; –, no reaction; w, weak reaction. All strains are positive for oxidization of L-arabinose, D-cellobiose, D-fructose, D-mannose, D-glucosaminic acid, γ-hydroxybutyric acid, DL-lactic acid, L-alanyl-glycine, L-alanine and L-glutamic acid. All strains are negative for D-melibiose.

Data for SMB34^T and *T. xiamenensis* M-5^T are from this study. Characteristics for *T. tepidiphila* 1-1B^T, *T. profundimaris* WP0211^T, *T. lucentensis* QMT2^T and *T. xianhensis* P-4^T are taken from [5, 6, 25].

†, Indicates conflicting results obtained in other studies.

not supplemented with NaCl. The other organisms tested required NaCl for their growth as follows from this study and previous reports [4–6]. It is also worth noting that the maximal and minimal NaCl concentrations supporting growth of some strains varied with basal media (Table 1).

Thus, although strain SMB34^T is fairly similar to *T. xiamenensis* M-5^T in terms of the *16Sr RNA* gene sequences (99.6% similarity), the results of DNA–DNA hybridization, together with the differences in phenotypic traits and the terrestrial origin of SMB34^T, suggest that this strain could be classified as a representative of novel species of the genus *Thalassospira*, according to the currently accepted criteria for the definition of bacterial species [22, 23].

It is interesting that, despite being isolated from the microbial consortium SMB3 capable of growth on naphthalene as a sole carbon and energy source, strain SMB34^T was unable to degrade this compound [1]. The strain is most likely involved in mutualistic inter-

actions with other members of the consortium and utilizes some carbon sources (e.g., acetate) derived from the decomposition of naphthalene by the other consortium members (e.g., by *Rhodococcus* strains that degrade naphthalene in pure cultures). It is also worth noting that SMB34^T, along with other strains composing the consortium, was recovered from primitive technogeneous soil formed on salt mine spoils at the Verkhnekamsk salt deposit (Perm region, Russia) which had been formed on the place of the ancient Permian sea about 280 millions years ago. In contrast, the absolute majority of organisms of the genus *Thalassospira* are marine inhabitants, as can be judged from publications [4–6, 24, 25] and public data bases. It might be speculated that *Thalassospira permensis* or its ancestor also originally inhabited the ocean and then survived, being trapped within salt crystals, subsequently evolving as a terrestrial bacterium, together with other members of the local microbial community.

When this work was completed and ready for publication, a new species of the genus *Thalassospira*, *T. xianhensis,* isolated from oil-contaminated saline soil in China, was proposed [26]. The type strain of this species exhibited 99.9% 16S rRNA gene sequence similarity to SMB34^T. However, the DNA G + C content of *T. xianhensis* is 61.2 ± 1.0 mol % (at least more than 5.5 mol % odds with that of SMB34^T). Such a considerable difference very probably indicates these strains to represent different species, as a bacterial species does not usually contain strains whose G + C mol % differs more than 5% [22, 27]. In addition, the type strain of *T. xianhensis* is significantly distinguished from SMB34^T and other named species of *Thalassospira* by phenotypic characteristics, including the predominant ubiquinone Q-9, non-motile cells, salt resistance, and a range of other physiological and biochemical features (Tables 1, 2). Among the differentiating phenotypic traits, at least the difference in predominant ubiquinone is considered to be of a high taxonomic value and believed to differentiate genera or species within a genus, as demonstrated in numerous publications. In other words, organisms with such differences in the respiratory lipoquinone system share less than 70% DNA–DNA hybridization value, the traditional standard of the current species definition [22, 27]. There are also examples of different species with identical or nearly identical sequences of the conservative 16S rRNA gene [28–30]. Further comparative studies of *Thalassospira permensis* sp. nov. and *T. xianhensis* at the phenotypic and genomic levels, involving *T. lucentensis* (the type species of this genus) and other recognized *Thalassospira* species, as well as relevant representatives of a large group of phylogenetically close organisms recently appearing in public data bases, will facilitate the development of taxonomy of this genus and provide persuasive arguments for the emendation of the genus description.

Description of Thalassospira permensis sp. nov.

Thalassospira permensis (per.men'sis. N.L. fem. adj. *permensis* pertaining to Perm, the geographical origin of the type strain).

Gram-stain-negative curved rods (1.23–1.98 × 0.23–0.42 μm). Motile by means of a single polar flagellum. Colonies are convex, circular, smooth, yellowish-colored and have entire edge on Marine agar and some other media. Mesophilic, grows in the temperature range of 10–40°C (optimum of 28–30°C). No growth occurs at 45°C; growth at 4°C varies with the test medium and, if any, is very weak. Cells grow at NaCl concentrations up to 11% (w/v) with optimum at 2–5%. Catalase and oxidase tests are positive. Negative reactions both in the classical Hugh-Leifson test and the API systems (API 20E and API 20NE) for glucose fermentation. Nitrate is reduced to nitrogen gas. According to the API 20NE test system, D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-maltose, gluconate, citrate (weak) and malic acid are used as carbon sources for growth. Growth also occurs with acetate and D-sorbitol as sole carbon and energy source in minimal salts medium. Acids are produced aerobically from D-glucose, L-arabinose (API 20E test system) and from D-maltose, D-mannose, D-trehalose, D-fructose, D-xylose and D-sorbitol (conventional tests). No acids are formed from lactose, D-raffinose, L-rhamnose and dulcitol. In Biolog GN2 MicroPlates the following carbon sources are utilized: N-acetyl-D-glucosamine, acetic acid, aconitic acid, adonitol, D-alanine, L-alanine, L-alanyl-glycine, γ-amino butyric acid, L-arabinose, D-arabitol, L-asparagine, aspartic acid, cellobiose, citric acid, D-fructose, D-galactose, D-gluconic acid, α-D-glucose, D-glucuronic acid, L-glutamic acid, glycyl-L-glutamic acid, L-histidine, γ-hydroxybutyric acid, β-hydroxybutyric acid, hydroxy-L-proline, myo-inositol, α-ketoglutaric acid, D,L-lactic acid, malonic acid, maltose, D-mannitol, D-mannose, mono-methyl-succinate, L-ornithine, L-proline, L-pyroglutamic acid, quinic acid, D-saccharic acid, L-serine, D-sorbitol, succinic acid, sucrose, thymidine, D-trehalose, turanose, urocanic acid and xylitol. The following are utilized weakly: bromosuccinic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, glycyl-L-aspartic acid, L-leucine, methyl pyruvate, propionic acid and L-threonine. Urea, gelatin, and starch are not hydrolyzed. Naphthalene is not degraded. The major respiratory lipoquinone is Q-10, with minor amount of Q-9. The predominant fatty acids (>10% of the total) are $C_{18:1}\omega7$, $C_{16:0}$, and $C_{18:0}$. The DNA G + C content is 53.7 ± 1.0 mol % (T_m). Other characteristics are as given in Tables 1, 2 and 3.

The type strain is SMB34^T (=VKM B-2527^T = NBRC 106175^T), isolated from the naphthalene-utilizing bacterial consortium obtained from the techno-

geneous soil of salt mines (Berezniki, Perm region, Russia).

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REFERENCES

1. Anan'ina, L.N., Altyntseva, O.V., and Plotnikova, E.G., Study of a Microbial Community Isolated from the Saltern Region, *Vestn. Perm. Univ.* [in Russian], 2005, vol. 6, pp. 109–114.
2. Anan'ina, L.N., Plotnikova, E.G., Gavrish, E.Yu., Demakov, V.A., and Evtushenko, L.I., *Salinicola socius* gen. nov., sp. nov., a Moderately Halophilic Bacterium from a Naphthalene-Utilizing Microbial Association, *Microbiology* (Engl. Transl.), 2007, vol. 76, pp. 324–330.
3. Anan'ina, L.N., Plotnikova, E.G., Gavrish, E.Yu., Demakov, V.A., and Evtushenko, L.I., *Salinicola socius* gen. nov., sp. nov., in *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSEM*, List No. 124, *Int. J. Syst. Evol. Microbiol.*, 2008, vol. 58, pp. 2471–2472.
4. López-López, A., Pujalte, M.J., Benlloch, S., Mata-Roig, M., Rosselló-Mora, R., Garay, E., and Rodriguez-Valera, F., *Thalassospira lucentensis* gen. nov., sp. nov., a New Marine Member of the α-*Proteobacteria, Int. J. Syst. Evol. Microbiol.*, 2002, vol. 52, pp. 1277–1283.
5. Liu, C., Wu, Y., Li, L., Ma, Y., and Shao, Z., *Thalassospira xiamenensis* sp. nov. and *Thalassospira profundimaris* sp. nov., *Int. J. Syst. Evol. Microbiol.,* 2007, vol. 57, pp. 316–320.
6. Kodama, Y., Stiknowati, L.I., Ueki, A., Ueki, K., and Watanabe, K., *Thalassospira tepidiphila* sp. nov., a Polycyclic Aromatic Hydrocarbon-Degrading Bacterium Isolated from Seawater, *Int. J. Syst. Evol. Microbiol.*, 2008, vol. 58, pp. 711–715.
7. Raymond, R.L., Hudson, J.O., and Jamison, V.W., Oil Degradation in Soil, *Appl. Environ. Microbiol.*, 1976, vol. 31, pp. 522–535.
8. *Manual of Methods for General Bacteriology*, Gerhardt, P., Murray, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R., and Phillips, G.B., Eds., Washington: Amer. Soc. Microbiol., 1981 [Russ. Transl. Moscow: Mir, 1984]
9. Holland, R.D., Wilkes, J.G., Rafii, F., Sutherland, J.B., Persons, C.C., Voorhees, K.J., and Lay, J.O., Rapid Identification of Intact Whole Bacteria Based on Spectral Patterns Using Matrix-Assisted Laser Desorption/Ionization with Time-of-Flight Mass Spectrometry, *Rapid Commun. Mass Spectrom.*, 1996, vol. 10, pp. 1227–1232.
10. Fenselau, C. and Demirev, P.A., Characterization of Intact Microorganisms by MALDI Mass Spectrometry, *Mass Spectrom. Rev.*, 2001, vol. 20, pp. 157–171.
11. Sauer, S. and Kliem, M., Mass Spectrometry Tools for the Classification and Identification of Bacteria, *Nature Rev.Microbiol.*, 2010, vol. 8, pp. 74–82.
12. Wolfrum, T., Gruner, G., and Stolp, H., Nucleic Acid Hybridization of Pink-Pigmented Facultative Methylotrophs and Pseudomonads, *Int. J. Syst. Bacteriol.*, 1986, vol. 36, pp. 24–28.
13. Owen, R.J. and Lapage, S.P., The Thermal Denaturation of Partly Purified Bacterial Deoxyribonucleic Acid and Its Taxonomic Applications, *J. Appl. Bacteriol.*, 1976, vol. 41, pp. 335–340.
14. Versalovic, J., Schneider, M., de Bruijn, F.J., and Lupski, J.R., Genomic Fingerprinting of Bacteria Using Repetitive Sequence-Based Polymerase Chain Reaction, *Meth. Cell Mol. Biol.,* 1994, vol. 5, pp. 25–40.
15. Gavrish, E.Yu., Krauzova, V.I., Potekhina, N.V., Karasev, S.G., Plotnikova, E.G., Altyntseva, O.V., Korosteleva, L.A., and Evtushenko, L.I., Three New Species of *Brevibacteria, Brevibacterium antiquum* sp. nov., *Brevibacterium aurantiacum* sp. nov., and *Brevibacterium permense* sp. nov., *Microbiology* (Engl. Transl.), 2004, vol. 73, pp. 176–183.
16. Thompson, J.D., Higgins, D.G., and Gibson, T.J., CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting, Position Specific Gap Penalties and Weight Matrix Choice, *Nucleic Acid Res.,* 1994, vol. 22, pp. 4673–4680.
17. Van de Peer, Y. and De Wachter, R., TREECON for Windows: a Software Package for the Construction and Drawing of Evolutionary Trees for the Microsoft Windows Environment, *Comput. Appl. Biosci.,* 1994, vol. 10, pp. 569–570.
18. Tijssen, P, Hybridization with Nucleic Acid Probes. Part II. Probe Labeling and Hybridization Techniques, in *Laboratory Techniques in Biochemistry and Molecular Biology*, Vliet, P.C., Ed., Amsterdam: Elsevier Science, 1993, pp. 391–406.
19. Evtushenko, L.I., Dorofeeva, L.V., Krausova, V.I., Gavrish, E.Y., Yashina, S.G., and Takeuchi, M., *Okibacterium fritillariae* gen. nov., sp. nov., a Novel Genus of the Family *Microbacteriaceae, Int. J. Syst. Evol. Microbiol.*, 2002, vol. 52, pp. 987–993.
20. Brosius, J., Palmer, M.L., Kennedy, P.J., and Noller, H.F., Complete Nucleotide Sequence of a 16S Ribosomal RNA Gene from *Escherichia coli, Proc. Natl. Acad. Sci. USA*, 1978, vol. 75, pp. 4801–4805.

21. Acinas, S.G., Marcelino, L.A., Klepac-Ceraj, V., and Polz, M.F., Divergence and Redundancy of 16S rRNA Sequences in Genomes with Multiple *rrn* Operons, *J. Bacteriol.*, 2004, vol. 186, pp. 2629–2635.
22. Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O., Krichevsky, M.I., Moore, L.H., Moore, W.E.C., Murray, R.G.E., Stackebrandt, E., Starr, M.P., and Trüper, H.G., International Committee on Systematic Bacteriology. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics, *Int. J. Syst. Bacteriol.,* 1987, vol. 37, pp. 463–464.
23. Stackebrandt, E., Frederiksen, W., Garrity, G.M., Grimont, P.A.D., Kämpfer, P., Maiden, M.C.J., Nesme, X., Rosseló-Mora, R., Swings, J., Trüper, H.G., Vauterin, L., Ward, A.C., and Whitman, W.B., Report of the Ad Hoc Committee for the Re-Evaluation of the Species Definition in Bacteriology, *Int. J. Syst. Evol. Microbiol*, 2002, vol. 52, pp. 1043–1047.
24. Wang, B., Lai, Q., Cui, Z., Tan, T., and Shao, Z., A Pyrene-Degrading Consortium from Deep-Sea Sediment of the West Pacific and Its Key Member *Cycloclasticus* sp. P1, *Environ. Microbiol.*, 2008, vol. 10, pp. 1948–1963.
25. Kaster, K.M., Bonaunet, K., Berland, H., Kjeilen-Eilertsen, G., and Brakstad, O.G., Characterisation of Culture-Independent and -Dependent Microbial Communities in a High-Temperature Offshore Chalk Petroleum Reservoir, *Antonie Van Leeuwenhoek*, 2009, vol. 96, pp. 423–439.
26. Zhao, B., Wang, H., Li, R., and Mao, X., *Thalassospira xianhensis* sp. nov., a Polycyclic Aromatic Hydrocarbon-Degrading Marine Bacterium, *Int. J. Syst. Evol. Microbiol.*, 2010, vol. 60, pp. 1125–1129.
27. Rosselló-Mora, R. and Amann, R., The Species Concept for Prokaryotes, *FEMS Microbiol. Rev.*, 2001, vol. 25, pp. 39–67.
28. Fox, G.E., Wisotzkey, J.D., and Jurtshuk, P., How Close Is Close: 16S rRNA Sequence Identity May not Be Sufficient to Guarantee Species Identity, *Int. J. Syst. Bacteriol.*, 1992, vol. 42, pp. 166–170.
29. Martinez-Murcia, A.J., Benlloch, S., and Collins, M.D., Phylogenetic Interrelationships of Members of the Genera *Aeromonas* and *Plesiomonas* as Determined by 16S Ribosomal DNA Sequencing: Lack of Congruence with Results of DNA-DNA Hybridizations, *Int. J. Syst. Bacteriol.*, 1992, vol. 42, pp. 412–421.
30. Wang, L.T., Lee, F.L., Tai, C.J., and Kasai, H., Comparison of *gyrB* Gene Sequences, 16S rRNA Gene Sequences and DNA-DNA Hybridization in the *Bacillus subtilis* Group, *Int. J. Syst. Evol. Microbiol.*, 2007, vol. 57, pp. 1846–1850.