Modestobacter marinus sp. nov., a psychrotolerant actinobacterium from deep-sea sediment, and emended description of the genus *Modestobacter*

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The taxonomic status of an actinobacterium that changed colour during growth, strain 42H12-1^T, isolated from deep-sea sediment collected from the Atlantic Ocean, was established using a combination of genotypic and phenotypic data. Strain 42H12-1^T formed a distinct branch in the 16S rRNA gene phylogenetic tree together with the type strains in the genus *Modestobacter*. The highest sequence similarity by BLAST analysis was to *Modestobacter versicolor* CP153-2^T (98.5 %) and the second-highest sequence similarity was to *Modestobacter multiseptatus* AA-826^T (97.5 %). DNA–DNA relatedness of only 12 % (sd 1.82 %) between strain 42H12-1^T and *M. versicolor* DSM 16678^T differentiated them as members of separate genomic species. Colonies of strain 42H12-1^T were black on oligotrophic medium, but orange to red, turning black, on copiotrophic medium. The peptidoglycan contained *meso*-diaminopimelic acid. The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and an unknown aminophospholipid. The predominant menaquinone was MK-9(H₄). The major fatty acids were iso-C_{16:0} and C_{17:1ω8c}. The DNA G + C content was 72.3 ± 1 mol%. Strain 42H12-1^T (=DSM 45201^T =CGMCC 4.5581^T) is assigned as the type strain of a novel species of the genus *Modestobacter*, for which the name *Modestobacter marinus* sp. nov. is proposed.

Modestobacter multiseptatus (Mevs *et al.*, 2000) and *Modestobacter versicolor* (Reddy *et al.*, 2007) are Gram-positive, non-spore-forming, short-rod- or coccoid-shaped psychrophilic actinobacteria that belong to the family *Geodermatophilaceae* (Normand, 2006). The former was isolated from Antarctic surface soil from the Linnaeus Terrace (1600 m) in the Asgard Range/Transantarctic Mountains. The latter was isolated from a biological soil crust sample collected from the Colorado Plateau, USA. Here, we report the polyphasic characterization of strain 42H12-1^T, which is considered to represent a novel species of the genus *Modestobacter*.

Strain 42H12-1^T was isolated from a deep-sea sediment sample (2983 m) collected from the Atlantic Ocean (14.7518° N 44.9782° W). The 16S rRNA gene was amplified from a single colony of the isolate using universal primers and PCR conditions as described by Chun & Goodfellow (1995). The almost-complete 16S rRNA gene sequence (1483 nt) of strain 42H12-1^T was obtained and analysed using BLAST searches against the GenBank and EzTaxon databases (Altschul *et al.*, 1997; Chun *et al.*, 2007). The 16S rRNA gene sequences of closely related taxa obtained from GenBank were aligned using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed by using the maximum-parsimony (Kumar *et al.*, 2004) and neighbour-joining (Saitou & Nei, 1987) methods with MEGA 3.1 software. The topologies of the trees were evaluated by performing a bootstrap analysis (Felsenstein, 1985) using 1000 replications.

The 16S rRNA gene sequence comparison clearly showed that strain 42H12-1^T belonged to the genus *Modestobacter*

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 42H12-1^T is EU181225.

Four supplementary figures and a supplementary table are available with the online version of this paper.

and formed a distinct subclade with *M. versicolor* CP153-2^T (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online). The 16S rRNA gene sequence similarities between strain 42H12-1^T and *M. versicolor* CP153-2^T and *M. multiseptatus* AA-826^T were 98.5 and 97.5 %, respectively. Lower 16S rRNA gene sequence similarities were recorded with strains of species of *Blastococcus* and *Geodermatophilus* (also in the family *Geodermatophilaceae*), such as *Blastococcus jejuensis* KST3-10^T (96.4 %), *Blastococcus saxobidens* BC444^T (96.0 %), *Blastococcus aggregatus* ATCC 25902^T (95.6 %), *Geodermatophilus obscurus* DSM 43160^T (94.2 %) and *Geodermatophilus ruber* CPCC 201356^T (95.0 %).

The reference strains *M. versicolor* DSM 16678^T and *M. multiseptatus* DSM 44406^T were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). *M. versicolor* DSM 16678^T was maintained on modified ISP2 medium (International *Streptomyces* Project medium 2; 0.4 % glucose, 0.4 % yeast extract, 1 % malt extract, pH 7.0–7.2) at 28 °C and *M. multiseptatus* DSM 44406^T was grown on R2A (DSMZ medium 830) at 20 °C.

There were obvious morphological differences between strain 42H12-1^T and *M. multiseptatus* DSM 44406^T. *M. multiseptatus* DSM 44406^T did not grow on ISP2 or trypticase soy agar (TSA). Growth of *M. multiseptatus* DSM 44406^T was visible after 20 days on R2A at 20 °C, but only as a thin lawn. In contrast, strain 42H12-1^T grew well on both ISP2 and TSA plates within 7 days at 28 °C. The maximum temperature for growth of *M. multiseptatus* DSM 44406^T was 28 °C, while that of strain 42H12-1^T was 35 °C. The obvious differences stated above support the conclusion that they represent different species.

Morphological characteristics of cells from exponentially growing cultures were observed by light microscopy (Olympus microscope CX21) and transmission electron microscopy (JEOL JEM-1230). Strain 42H12-1^T and *M. versicolor* DSM 16678^T were grown on rich medium (ISP2) and oligotrophic medium (1/10-strength ISP2; 0.04 % glucose, 0.04 % yeast extract, 0.1 % malt extract, pH 7.0–7.2), and the colours of the colonies were observed after incubating for 2, 4, 6, 10, 15 and 30 days at 28 °C. The temperature for growth was tested at 4, 10, 20, 28, 30, 35

and 37 °C on ISP2 agar over 15 days, while the pH for growth was investigated between pH 3.0 and 11.0 at intervals of 1 pH unit at 28 °C over 15 days using ISP2 broth with different buffers as described by Xu *et al.* (2005). NaCl tolerance was tested at 0–15 % NaCl (w/v) (at intervals of 1 %) using ISP2 broth at 28 °C over 15 days.

Gram staining was carried out by using the standard Gram reaction and was confirmed by using the KOH lysis test (Cerny, 1978). Catalase activity was determined using 3 % H₂O₂. Oxidase activity was observed by oxidation of tetramethyl-*p*-phenylenediamine. Biochemical tests such as nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β -glucosidase, gelatin hydrolysis and β -galactosidase were performed using an API 20NE kit (bioMérieux) and enzyme activities were determined using an API ZYM kit (bioMérieux) according to the manufacturer's instructions. Carbon assimilation and oxidation were determined using API 20NE and API 50CH kits (bioMérieux). Sensitivity to different antibiotics was determined by using antimicrobial susceptibility discs. Physiological properties are given in the species description and are compared with those of *M. versicolor* DSM 16678^T in Table 1.

Colonies of *M. versicolor* DSM 16678^T were initially colourless to pink and turned dark with incubation over 2–4 days on oligotrophic complex medium, whereas the colonies remained colourless or pink until a melanin-like pigment was produced in the stationary phase (after 2 weeks) on a rich complex medium (Reddy *et al.*, 2007). Colour-change tests on copiotrophic medium (ISP2) and oligotrophic medium (1/10-strength ISP2) for strain 42H12-1^T were observed for 2–30 days with *M. versicolor* DSM 16678^T as a comparison. Strain 42H12-1^T showed a similar pattern of colony colour change to *M. versicolor* DSM 16678^T on oligotrophic medium, but colonies of strain 42H12-1^T were deep-orange, whereas those of *M. versicolor* DSM 16678^T were pink. After 3 days, both strains started to turn darker, but the speed of melanin-like pigment production in strain 42H12-1^T was noticeably greater. After 15 days, colonies of the two strains had changed colour completely to black, but the amount of melanin-like pigment produced in strain 42H12-1^T was markedly greater than in *M. versicolor* DSM 16678^T (Supplementary Fig. S2).

Fig. 1. Phylogenetic dendrogram obtained by distance-matrix analysis of 16S rRNA gene sequences (1402 nt), showing the position of strain 42H12-1^T and its phylogenetic neighbours. Bootstrap values are expressed at branch points as percentages of 1000 replications; only values above 50 % are shown. Bar, 0.01 substitutions per nucleotide position.

Table 1. Characteristics that distinguish strain 42H12-1^T from *M. versicolor* DSM 16678^T

Data were obtained in this study. +, Positive; –, negative; (+), weakly positive.

Characteristic	42H12-1 ^T	<i>M. versicolor</i> DSM 16678 ^T
Colony colour (on ISP2 at initiation)	Orange to red	Pink
API 20 NE results		
Urease activity	–	+
Nitrate reduction	(+)	–
Gelatin hydrolysis	–	+
Assimilation of (API 20NE):		
<i>N</i> -Acetyl-D-glucosamine	–	+
Maltose	–	+
Enzyme activity (API ZYM)		
β-Galactosidase	(+)	–
β-Glucosidase	+	(+)
Acid produced from (API 50CH):		
Glycerol	+	–
D-Galactose	+	–
D-Mannose	+	–
D-Mannitol	+	–
Sucrose	+	–
Turanose	+	–
Presence of unknown aminophospholipid	+	–
Major fatty acids (>9.5%)*	i-C _{16:0} , C _{17:1} ω8c	i-C _{15:0} , i-C _{16:0} , C _{17:1} ω8c, C _{18:1} ω9c

*i, Iso-branched.

Biomass of strain 42H12-1^T and *M. versicolor* DSM 16678^T for chemical and molecular analysis was prepared by culturing in liquid ISP2 broth for 3–5 days at 28 °C in a rotary shaker and then harvesting by centrifugation.

Analysis of the isomer of diaminopimelic acid was done using freeze-dried cells, which were hydrolysed with 6 M HCl at 120 °C for 30 min (Lechevalier & Lechevalier, 1980). The cell hydrolysate was analysed by TLC (Hasegawa *et al.*, 1983). Polar lipids were extracted using procedures described by Komagata & Suzuki (1987) and separated by TLC on Merck silica gel 60 plates by two-dimensional development with polar lipid standards. Amino-group polar lipids were detected by spraying with ninhydrin and Dittmer & Lester reagent. Menaquinones were extracted according to Collins *et al.* (1977) and analysed by HPLC (Kroppenstedt, 1985). Freeze-dried biomass for fatty acid analysis was prepared by culturing in tryptic soy broth (TSB; BBL) for 4 days at 28 °C. Cellular fatty acid analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI Sherlock 6.0; library TSBA6). Genomic DNA for determination of the G+C content was prepared according to the method of Marmur (1961). The DNA G+C content was determined by reversed-phase HPLC according to Mesbah *et al.* (1989).

The polar lipids detected were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol and an unknown aminophospholipid (Supplementary Fig. S3). The predominant menaquinone

was MK-9(H₄). The major fatty acids were iso-C_{16:0} and C_{17:1}ω8c. Details of the cellular fatty acid profile are given in Supplementary Table S1.

The DIG-High Prime DNA Labelling and Detection Starter kit II (Roche) was used for DNA–DNA hybridization experiments between strain 42H12-1^T and *M. versicolor* DSM 16678^T. All procedures were performed in accordance with the manufacturer's directions. Detailed steps were followed as described previously (Xu *et al.*, 2009). The DNA–DNA relatedness was 12 % (SD 1.82 %).

The 16S rRNA gene sequence similarity between 42H12-1^T and *M. versicolor* DSM 16678^T and *M. multiseptatus* DSM 44406^T was above 97 %, which is the threshold that is generally recognized as delineating a genospecies (Stackebrandt & Goebel, 1994). Characteristics such as the cell morphology, respiratory quinone system and diagnostic diamino acid in whole-cell hydrolysates indicated that the isolate should be assigned to the genus *Modestobacter*. A low DNA–DNA relatedness of only 12 % to its closest phylogenetic relative, *M. versicolor* DSM 16678^T, in whole-genome association experiments and obvious phenotypic differences indicated that strain 42H12-1^T is a novel member of the genus. Strain 42H12-1^T contains an unknown aminophospholipid, while *M. versicolor* DSM 16678^T does not. There are obvious differences in the fatty acid profiles of the two strains (Supplementary Table S1). Phenotypic differences between the two strains are summarized in Table 1. On the basis of

all the data, strain 42H12-1^T is proposed to represent a novel species of the genus *Modestobacter*, for which we propose the name *Modestobacter marinus* sp. nov. An emended description of the genus is also presented.

Emended description of the genus *Modestobacter* Mevs *et al.* 2000

The genus description is as given by Mevs *et al.* (2000) and emended by Reddy *et al.* (2007), with the following further modifications. The major fatty acid (>10%) is iso-C_{16:0}. The DNA G + C content is in the range 68–73 mol%.

Description of *Modestobacter marinus* sp. nov.

Modestobacter marinus (ma.ri'nus. L. masc. adj. *marinus* pertaining to the sea, where the type strain was found).

Cells are non-spore-forming, Gram-stain-positive, short rods (straight, lightly curved, about 0.5–0.8 × 1.5–2.5 µm; Supplementary Fig. S4), motile by means of flagella. Colonies are orange to red on copiotrophic medium at the initial stage of growth and turn black after 14 days. Colonies are dark throughout growth on oligotrophic medium. Psychrotolerant, growing at 4–35 °C (but not at 37 °C), with optimal growth at 28 °C. Tolerant of only narrow variations in pH, with fast growth occurring at pH 6–8 and very slow growth at pH 9. Tolerates NaCl at concentrations less than 5% (w/v). Hydrolyses casein, cellulose and starch. Does not produce H₂S and cannot grow on DNase test plates. In API 20NE tests, positive for aesculin hydrolysis, *p*-nitrophenyl β-D-galactopyranosidase and assimilation of L-arabinose, D-mannitol, potassium gluconate, malic acid and phenylacetate. Negative for indole production, acid from glucose, arginine dihydrolase, urease and gelatinase. Nitrate reduction is weakly positive. Tested by the API ZYM enzyme assay, positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and β-glucosidase; weakly positive for cystine arylamidase, trypsin, α-chymotrypsin and β-galactosidase; negative for esterase (C4), lipase (C14), α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. In the API 50 CH test system, acids are produced from glycerol, L-arabinose, D-ribose, D-galactose, D-fructose, D-mannose, D-mannitol, aesculin, maltose, sucrose, trehalose, turanose and L-fucose. Weakly positive for acid production from L-rhamnose, salicin, cellobiose, lactose and D-lyxose. All the other API 50 CH test results are negative. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid as the diamino acid. Major fatty acids are iso-C_{16:0} and C_{17:1ω8}; the major respiratory quinone is MK-9(H₄). The diagnostic phospholipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol and an unknown aminophospholipid. The G + C content of the DNA of the type strain is 72.3 ± 1 mol%. Cells are sensitive to a wide variety of antibiotics, but are resistant to

discs containing ceftazidime (30 µg), trimethoprim (5 µg) and monobactam (30 µg).

The type strain, 42H12-1^T (=DSM 45201^T =CGMCC 4.5581^T), was isolated from deep-sea sediment collected from the Atlantic Ocean.

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