# *Polycladomyces abyssicola* gen. nov., sp. nov., a thermophilic filamentous bacterium isolated from hemipelagic sediment

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A novel filamentous bacterium, designated strain JIR-001$^T$, was isolated from hemipelagic sediment in deep seawater. This strain was non-motile, Gram-positive, aerobic, heterotrophic and thermophilic; colonies were of infinite form and ivory coloured with wrinkles between the centre and the edge of the colony on ISP2 medium. The isolate grew aerobically at 55–73 °C with the formation of aerial mycelia; spores were produced singly along the aerial mycelium. These morphological features show some similarities to those of the type strains of some species belonging to the family *Thermoactinomycetaceae*. Phylogenetic analysis based on 16S rRNA gene sequences confirmed that strain JIR-001$^T$ belongs to the family *Thermoactinomycetaceae* within the class *Bacilli*. Similarity levels between the 16S rRNA gene sequence of strain JIR-001$^T$ and those of the type strains of *Thermoactinomycetaceae* species were 85.5–93.5 %; highest sequence similarity was with *Melghirimyces algeriensis* NariEX$^T$. In the DNA–DNA hybridization assays between strain JIR-001$^T$ and its phylogenetic neighbours the mean hybridization levels with *Melghirimyces algeriensis* NariEX$^T$, *Planifilum fimeticola* H0165$^T$, *Planifilum fulgidum* 500275$^T$ and *Planifilum yunnanense* LA5$^T$ were 5.3–7.5, 2.3–4.7, 2.1–4.8 and 2.5–4.9 %, respectively. The DNA G+C content of strain JIR-001$^T$ was 55.1 mol%. The major fatty acids were iso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{16:0}$ and $C_{16:0}$. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, glucolipid, phosphatidylserine, an amino-group containing phospholipid, an unknown phospholipid and two unknown lipids. The predominant menaquinone was MK-7 and the cell-wall peptidoglycan contained *meso*-diaminopimelic acid, glutamic acid and alanine. On the basis of phenotypic characteristics and 16S rRNA gene sequence comparisons, strain JIR-001$^T$ is considered to represent a novel species in a new genus of the family *Thermoactinomycetaceae*, for which the name *Polycladomyces abyssicola* gen. nov., sp. nov. is proposed. The type strain of *Polycladomyces abyssicola* is JIR-001$^T$ (=JCM 18147$^T$=CECT 8074$^T$).

The genus *Thermoactinomyces*, which was proposed with the description of *Thermoactinomyces vulgaris*, the type species of the genus, was one of the earliest known actinomycete taxa (Tsilinsky, 1899).

Initially, *Thermoactinomyces* species were recognized as actinomycetes because of their morphological features of forming aerial and substrate mycelia as well as their biochemical properties such as aerobicity, Gram-stainability and filamentous growth. Traditionally, classification of the actinomycetes and their relatives has been based almost solely on morphological aspects, for instance life cycles, spore characteristics and branching pattern of mycelia (Gottlieb, 1974). However, some studies revealed that *Thermoactinomyces* species form endospores like bacilli and show lower DNA G+C content than those of

Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JIR-001$^T$ is AB688114.

Two supplementary figures and one supplementary table are available with the online version of this paper.

actinomycetes (Cross *et al.*, 1968, 1971; Lacey & Vince, 1971; Lacey & Cross, 1989). Moreover, 16S rRNA gene sequence analysis indicated that the genus *Thermoactinomyces* is more closely related to *Bacillus* species than to actinomycetes. Additionary, Stackebrandt & Woese (1981) suggested that possession of mycelia is not a certain phylogenetic indicator. It has been proposed that the genus *Thermoactinomyces* should no longer be classified within the order *Actinomycetales* and should be placed within the family *Bacillaceae* (Stackebrandt & Woese, 1981; Park *et al.*, 1993; Yoon & Park, 2000; Yoon *et al.*, 2000), and therefore *Thermoactinomyces* has been reclassified into the order *Bacillales* based on similarities in their manner of proliferation, thermal tolerance and the biochemical properties of their endospores, as well as deviation of their lineage from actinomycetes.

Subsequently, on the basis of phylogenetic and chemotaxonomic analyses, the genus *Thermoactinomyces* has been divided into six genera: *Thermoactinomyces sensu stricto*, *Laceyella*, *Seinonella*, *Thermoflavimicrobium*, *Planifilum* and *Mechercharimyces* in a strict sense (Yoon & Park, 2000; Yoon *et al.*, 2005; Hatayama *et al.*, 2005; Matsuo *et al.*, 2006; Zhang *et al.*, 2007). At the time of writing, with four new genera, *Shimazuella* (Park *et al.*, 2007), *Desmospora* (Yassin *et al.*, 2009), *Kroppenstedtia* (von Jan *et al.*, 2011) and *Melghirimyces* (Addou *et al.*, 2012) being added, rearranged and/or reclassified, the family *Thermoactinomycetaceae* now consists of 10 recognized genera. These bacteria are characterized by the formation of a single, non-stalked spore on the aerial or substrate hyphae, or consecutive spores on straight or branched sporephores. Members of the family *Thermoactinomycetaceae* are chemoheterotrophic and thermophilic, growing at 30–75 °C, except for four species, *Seinonella peptonophila* (Nonomura & Ohara, 1971; Yoon *et al.*, 2005), *Mechercharimyces mesophilus* (Matsuo *et al.*, 2006), *Mechercharimyces asporophorigenens* (Matsuo *et al.*, 2006) and *Shimazuella kribbensis* (Park *et al.*, 2007), which are mesophilic, growing at below 45 °C.

During our investigation of microbial community structure analysis in deep-seafloor sediment, we encountered a hitherto-unknown filamentous, branched, thermophilic bacterium, strain JIR-001$^T$, that produces endospores and does not correspond to any recognized taxon. We propose herein a novel genus and species belonging to the family *Thermoactinomycetaceae* on the basis of phylogenetic and polyphasic biochemical studies.

In this study, the taxonomic position of strain JIR-001$^T$ was determined. The bacterium was isolated from a deep-seafloor sediment subsample at a depth of 48 m below the seafloor (mbsf) off the Shimokita Peninsula of Japan in the north-western Pacific Ocean (Site C9001: water depth 1180 m). The sediment subsamples (0.1 g) were transferred into 5 ml artificial seawater (Nihon Pharmaceutical Co., Ltd) supplemented with 0.1 % (w/v) marine broth 2216 (MB; Difco) and incubated at 60 °C with shaking at 150 r.p.m. in a rotary shaker incubator for 5 days, and then spread onto a 1.5 % (w/v) agar plate containing basal seawater [BSW-1; 0.1 × artificial seawater, 0.5 % (w/v) MB and 0.5 % (w/v) NaCl]. The plate was incubated at 60 °C for 7 days, when strain JIR-001$^T$ was isolated. The morphological, physiological and biochemical characteristics of strain JIR-001$^T$ were investigated using routine cultivation on BSW-1 agar plates at 60 °C.

Cell morphology was observed under a light microscope equipped with phase-contrast optics at 400 × magnification (Olympus). For microscopic examination, cells were grown on malt extract-yeast extract (ISP2; Difco) agar plates for 7 days at 60 °C. For transmission electron microscopy, cell preparations were negatively stained with sodium phosphotungstate (Fardeau *et al.*, 1997). The micromorphology of cells of strain JIR-001$^T$ was examined with a JSM-6700F scanning electron microscope (JEOL) and is shown in Fig. S1 (available in IJSEM Online). Gram staining was carried out using a Gram stain kit (Wako) according to the manufacturer's instructions. Formation of aerial mycelium and substrate mycelium was determined by observation with the naked eye of cultures of the strain after 7 days incubation at 60 °C on the following different solid media: tryptone-yeast extract agar (ISP1; Difco), ISP2, oatmeal agar (ISP3), inorganic salt-starch agar (ISP4; Difco), glycerol-asparagine agar (ISP5) (Shirling & Gottlieb, 1966), nutrient agar (NA; Difco) and BSW-1 agar. Cultures on each medium were examined with respect to the development of aerial and substrate mycelium and its colour, and the production of diffusible pigments. Melanoid pigment production was examined on peptone-yeast extract iron agar (ISP6) and tyrosine agar (ISP7) (Shirling & Gottlieb, 1966).

Motility was assayed using a semi-solid ISP2 agar tube supplemented with 0.5 % agar. Cells were inoculated by stabbing with a straight needle and the tube was incubated at 60 °C for 7 days.

To determine the range of temperatures tolerated, strain JIR-001$^T$ was grown on ISP2 medium and incubated at 35, 40, 45, 50, 55, 60, 65, 70, 73 and 75 °C. The pH range was tested on ISP2 medium and adjusted to the designed pH (pH 5.0–11.0 at intervals of 1 pH unit) at 60 °C. Different buffers were used: citrate buffer for pH 5.0–6.0, Tris/HCl for pH 7.0–9.0, tetraborate buffer for pH 10.0 and $Na_2HPO_4$ buffer for pH 11.0. NaCl tolerance was tested on ISP2 medium by varying the salt concentration in the culture medium [physiological saline and 1–10 % (w/v) at intervals of 1.0 % salinity units] at 60 °C. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on ISP2 and on ISP2 supplemented with nitrate, both prepared anaerobically using nitrogen.

Strain JIR-001$^T$ was tested for the utilization of 17 carbohydrates, 18 amino acids and nine organic acids as sole carbon and energy sources by using amended basal medium (Tsukamura, 1966; Addou, *et al.*, 2012) and basal seawater medium [BSWA-1; 0.1 × artificial seawater, 1.5 %

(w/v) agar] (Table 1). Each of the carbohydrates, amino acids and organic acid sodium salts was added to the medium to a final concentration of 0.01 M. For the acid production assay, the basal medium supplemented with 0.02 % (w/v) yeast extract and 0. $8 \times 10^{-3}$ % (w/v) bromocresol blue was used as pH indicator (Tsukamura, 1966). As a control, Tsukamura basal medium and BSWA-1 without carbon source were used. In addition, liquid carbon utilization medium (ISP9) (Shirling & Gottlieb, 1966) was used for carbohydrate and organic acid utilization tests. Utilization of amino acid was tested in ISP9 medium without a nitrogen source.

Hydrolysis of starch was determined on nutrient agar medium supplemented with 1 % (w/v) starch. After

**Table 1.** Menaquinone, cellular fatty acid and polar lipid profiles of strain JIR-001$^T$ and the type strains of its closest related species among related genera.

Strains: 1, JIR-001$^T$ (data from this study); 2, *Melghirimyces algeriensis* NariEX$^T$ (data from this study); 3, *Planifilum fimeticola* H0165$^T$ (Hatayama *et al.*, 2005). Strain JIR-001$^T$ and *Melghirimyces algeriensis* NariEX$^T$, its closest related species among related genera, were grown in ISP2 liquid medium at the exponential phase of growth at 60 °C and harvested. Fatty acid results are presented as a percentage of the total fatty acids. −, Not detected; ND, no data available.

| Chemotaxonomic characteristic | 1 | 2 | 3 |
|---|---|---|---|
| Menaquinone composition (%) | | | |
| MK-6 | − | 2.0 | − |
| MK-7 | 97.0 | 94.0 | 98.6 |
| MK-8 | 3.0 | 2.0 | 1.2 |
| Fatty acid (%) | | | |
| $C_{10:0}$ | − | 0.39 | − |
| $C_{12:0}$ | − | 0.20 | 0.30 |
| $C_{14:0}$ | 0.52 | 3.05 | 0.20 |
| $C_{15:0}$ | − | 0.75 | 1.30 |
| $C_{16:0}$ | 4.74 | 4.68 | 5.20 |
| $C_{17:0}$ | − | − | 2.50 |
| $C_{18:0}$ | − | − | 0.80 |
| $C_{16:1}\omega7c$ alcohol | − | − | 0.40 |
| $C_{18:1}\omega9c$ | − | 0.31 | 0.30 |
| iso-$C_{17:1}\omega9c$ | 1.56 | − | − |
| iso-$C_{13:0}$ | − | 1.25 | − |
| iso-$C_{14:0}$ | 1.03 | 1.61 | 1.10 |
| iso-$C_{15:0}$ | 35.50 | 59.12 | 4.90 |
| iso-$C_{16:0}$ | 22.54 | 1.81 | 45.10 |
| iso-$C_{17:0}$ | 28.11 | 6.68 | 13.90 |
| iso-$C_{18:0}$ | 0.63 | − | 6.10 |
| iso-$C_{16:0}$ H | 1.52 | − | − |
| iso-$C_{18:0}$ H | 0.46 | − | − |
| anteiso-$C_{13:0}$ | − | 0.10 | − |
| anteiso-$C_{15:0}$ | 0.79 | 18.20 | 4.10 |
| anteiso-$C_{17:0}$ | 1.11 | 1.53 | 13.20 |
| Summed feature 3* | 0.34 | − | − |
| Summed feature 4* | 1.16 | − | − |
| Summed feature 5* | − | − | 0.40 |
| Polar lipid (%) | | | |
| Phosphatidylglycerol (PG) | 9.3 | 11.1 | ND |
| Diphosphatidylglycerol (DPG) | 38.7 | 17.1 | ND |
| Phosphatidylethanolamine (PE) | 20.8 | 39.7 | ND |
| Phosphatidylmonomethylethanolamine (methyl-PE) | 10.4 | 4.6 | ND |
| Phosphatidylserine (PS) | 3.7 | − | ND |
| Glucolipid (GL) | 5.4 | − | ND |
| Unknown lipid (L) | 8.1 | 6.0 | ND |
| Unknown phospholipids (PL) | 3.7 | 21.5 | ND |

*Summed features consist of one or more fatty acids that could not be separated by the Microbial Identification System. Summed feature 3: $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; summed feature 4: iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B; summed feature 5: $C_{18:2}\omega6,9c$ and/or anteiso-$C_{18:0}$.

incubation at 60 °C for 7 days, iodine solution was added to the culture, and the disappearance of the blue colour around the colony was then evaluated as a positive reaction (Barrow & Feltham, 1993). Degradation of casein and L-tyrosine was tested according to the method of Staneck & Roberts (1974). Degradation of aesculin (Gordon, 1966) was tested using aesculin agar (Addou *et al.*, 2012). Degradation of xanthine, hypoxanthine and gelatin was tested according to the methods of Gordon (1966) and Gordon & Mihm (1957).

Urease activity was determined according to the method of Lányí (1987). Catalase activity was investigated by immersing one 7-day-old culture of strain JIR-001$^T$ on ISP2 medium into an aqueous solution of $H_2O_2$ and observing the generation of bubbling. Indole production and nitrate/nitrite reduction were checked by using the methods of Gordon & Mihm (1957) and Joffin & Leyral (2006).

Sensitivity to antibiotics was checked on ISP2 agar plates using antibiotic discs (Becton Dickinson) containing: bacitracin, 10 IU; polymyxin B, 100 U; cephalothin, 30 μg; tetracycline, 30 μg; minocycline, 30 μg; vancomycin, 30 μg; kanamycin, 30 μg; neomycin, 30 μg; lincomycin, 15 μg; streptomycin, 10 μg; novobiocin, 30 μg; gentamicin, 10 μg; arbekacin, 30 μg; rifampicin, 5 μg; chloramphenicol, 30 μg; erythromycin, 15 μg; ampicillin, 10 μg; carbenicillin, 100 μg; penicillin G, 10 U; amoxicillin, 25 μg; imipenem, 10 μg; spectinomycin, 100 μg; cefmetazole, 30 μg; cefazolin, 30 μg; and cefotiam, 30 μg.

The chemotaxonomic characteristics of strains JIR-001$^T$ and *Melghirimyces algeriensis* NariEX$^T$, which was the most closely related species among related genera, were determined using cells cultured in ISP2 liquid medium at the exponential phase of growth at 60 °C and harvested. Analyses of isoprenoid quinones, polar lipids, cellular fatty acids and peptidoglycan composition of strain JIR-001$^T$ were carried out as described below. Isoprenoid quinones were extracted from freeze-dried cell pellets with methanol as described by Collins *et al.* (1977) and analysed using an HPLC system (Waters 600 series; Nihon Waters) with a Cosmosil 5$C_{18}$-PAQ (4.6 mm × 150 mm) column (Nacalai Tesque). Polar lipids were extracted according to Minnikin *et al.* (1984) as modified by Kroppenstedt & Goodfellow (2006), and were then separated by two-dimensional TLC with an aluminium-backed silica gel plate (Merck no.5514) in solvent systems described by Ivanova *et al.* (2005). The polar lipid composition was determined and compared with those of phylogenetically related type strains with an authentic sample. For detection, iodine (Merck) and molybdatophosphoric acid (Merck) for total lipids, Dittmer–Lester reagent (Merck) for phosphorus, ninhydrin reagent (Merck) for the amino group, Dragendorff reagent (Merck) for quaternary nitrogen, *p*-anisaldehyde reagent (Wako) for glycolipids and periodate-Schiff reagent for glycerolipids were used. These spots of the image captured with a scanner were digitalized based on their areas and intensities using ImageJ software (http://rsb.info.nih.gov/ij/). Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification system (version 6.0; MIDI), and the fatty acid methyl ester profile was then determined by GC with reference to the TSBA40 Microbial Identification System (MIS) standard library. Peptidoglycan preparations purified according to the method of Schleifer (1985) were held after cell breakage by shaking with glass beads and subsequent trypsin digestion. The amino acids in cell-wall hydrolysates were examined by two-dimensional ascending TLC on cellulose plates using a solvent system as described by Schleifer (1985). The molar ratios of amino acids were determined by GC-MS after modification as *N*-heptafluorobutyryl amino acid isobutyl esters (MacKenzie, 1987; Groth *et al.*, 1996).

The genomic DNA of strain JIR-001$^T$ was extracted and purified according to the method described by Wilson (2001) with slight modification. Sequencing and assembly of the 16S rRNA gene was performed as described by Lane (1991) with LA-*Taq* DNA polymerase (TaKaRa Bio). The resultant 16S rRNA gene sequence (1485 nt) of strain JIR-001$^T$ was compared with those available from GenBank using ARB software (Ludwig *et al.*, 2004; Kumar *et al.*, 2005, 2006) to determine an approximate phylogenetic affiliation, considering the secondary structure of the 16S rRNA gene. Phylogenetic analyses were carried out using neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms. A phylogenetic tree was constructed using the neighbour-joining algorithm and evolutionary distances were calculated using the model of Jukes & Cantor (1969) with *Bacillus subtilis* DSM 10$^T$ (AJ276351) and *Geobacillus stearothermophilus* DSM 22$^T$ (AJ294817) as outgroup taxa. A bootstrap analysis (Felsenstein, 1985) was performed with 1000 resampled datasets to estimate the reliability of the tree topology (Fig. 1).

The G+C content of the genomic DNA of strain JIR-001$^T$ was determined by HPLC (Mesbah *et al.*, 1989) with a Cosmosil 5$C_{18}$-PAQ (4.6 mm × 150 mm) column (Nacalai Tesque, Inc.). DNA–DNA hybridization assays between strain JIR-001$^T$ and its closest phylogenetic neighbours, namely *Melghirimyces algeriensis* NariEX$^T$ and *Planifilum* species (*Planifilum fimeticola* H0165$^T$, *Planifilum fulgidum* 500275$^T$, *Planifilum yunnanense* LA5$^T$), were carried out with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989) using a 1420 Multilabel Counter (Perkin Elmer) for fluorescence measurements. The hybridization temperature was 47 °C and reciprocal experiments were performed for each pair of strains.

On BSW-1 solid medium, strain JIR-001$^T$ formed colonies 3.0–5.0 mm in diameter that were cream-coloured, irregularly shaped, thin and flat, and formed substrate mycelium but no aerial mycelium. Colonies were inlaid

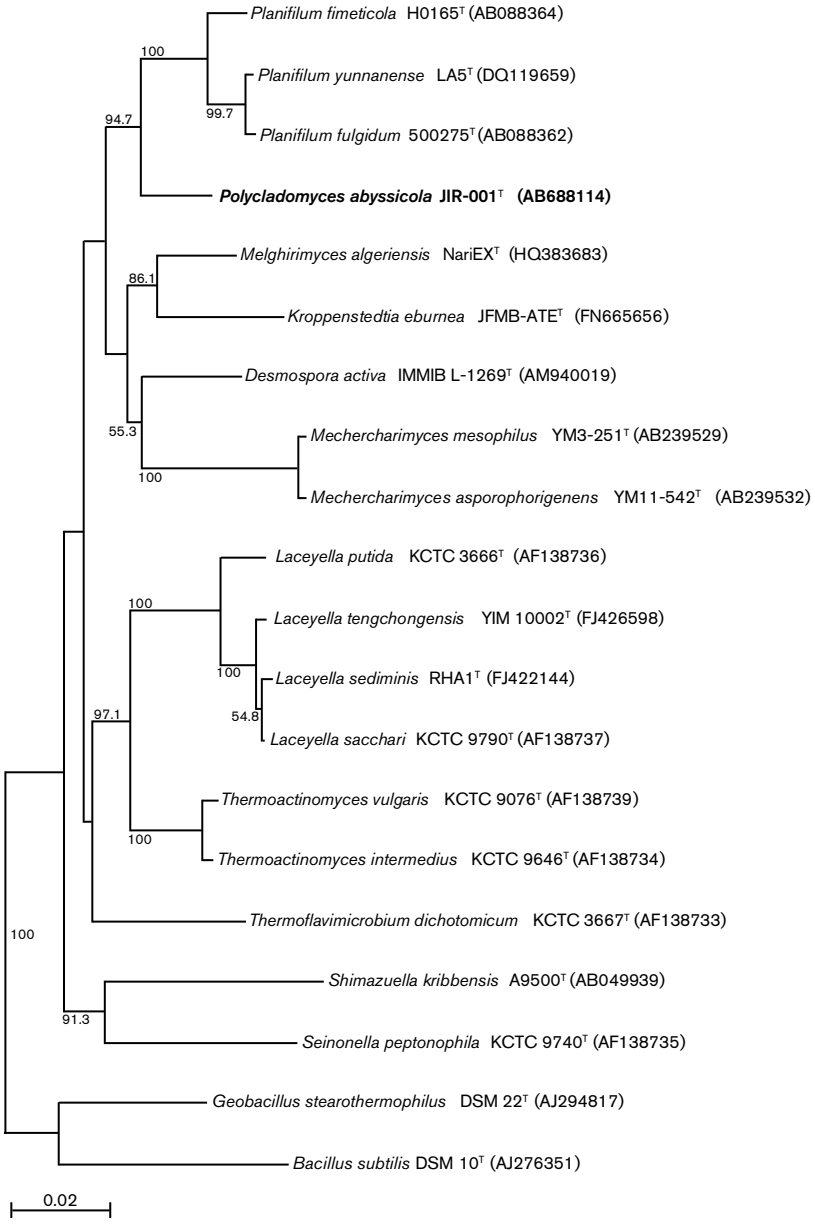


**Fig. 1.** Neighbour-joining phylogenetic tree showing the position of strain JIR-001$^T$ within the family *Thermoactinomycetaceae*. *Bacillus subtilis* DSM 10$^T$ and *Geobacillus stearothermophilus* DSM 22$^T$ were used as outgroups. Bootstrap values greater than 50 % are given at branch points. Bar, 0.02 substitutions per nucleotide position.

in the agar and difficult to detach. On ISP3 and NA medium, strain JIR-001$^T$ formed colonies that were irregular in shape and size, thin and flat and ivory-coloured but no aerial mycelium was observed. Colonies were easily detachable. On ISP2 medium, strain JIR-001$^T$ formed colonies 1.0–1.5 cm in diameter that were of infinite form and ivory-coloured with wrinkles between the centre and the edge of the colony, and formed white aerial mycelium. Colonies were slightly cohesive but easily detachable from the agar. No diffusible pigments were detected on the growth medium. Growth of this strain on ISP1, ISP4, ISP5 and ISP7 media was not observed. No melanoid pigments were produced on ISP6 medium.

Microscopic studies revealed that cells of strain JIR-001$^T$ were long, almost straight, but with moderately flexuous and branched hyphae, forming single endospores (Fig. S1a) (Cross *et al.*, 1968). Transmission electron microscopy showed a dense outer cell wall (Fig. S1b). Scanning electron microscopy showed detailed external structures, e.g. attachment sites of endospores (Fig. S1c and d). Cells were approximately 0.3–0.4 μm wide and 1.5–2.0 μm long at a minimum, and were separated by septal walls. One or at most two endospores, which were approximately 0.9–1.0 μm across and oval, were formed on a hypha (Fig. S1d) and arthrospores were not observed.

Growth of strain JIR-001$^T$ was observed at temperatures between 55 and 73 °C, the optimum growth temperature being 55–60 °C, at pH 6.5–8.5, the optimum being at pH 7.0–8.0, and with 1–2 % (w/v) NaCl. Growth under anaerobic conditions was not observed after 2 weeks of

cultivation in ISP2 at 60 °C. Cells were Gram-positive and catalase-negative.

On BSWA-1, strain JIR-001$^T$ utilized ribose, glycerol, xylose, mannose, glucose, alanine, glycine, asparagine, arginine and fumarate as sole carbon sources for growth, whereas on Tsukamura medium, growth of JIR-001$^T$ was not observed. No acid production was detected from the utilization of carbohydrates. Judgments based on the results of the tests on ISP9 liquid medium were difficult to make because strain JIR-001$^T$ showed poor growth and weak degradation of substrates in liquid medium.

Casein and gelatin were decomposed by this strain; however, xanthine, hypoxanthine, tyrosine and starch were not hydrolysed. Urease, indole production and nitrate/nitrite reduction were negative. The results of physiological and biochemical tests are summarized in Tables 1 and 2.

Strain JIR-001$^T$ was sensitive to bacitracin, gentamicin, rifampicin, erythromycin, ampicillin, penicillin, imipenem, amoxicillin, cefmetazole and cefazolin, slightly sensitive to vancomycin, kanamycin, neomycin, streptomycin, arbekacin, spectinomycin, cefotiam, minocycline, tetracycline and chloramphenicol, but resistant to novobiocin.

The predominant menaquinone of strain JIR-001$^T$ was MK-7 (97 %) and the minor was MK-8 (3 %). The polar lipid pattern of strain JIR-001$^T$ consisted of phosphatidylglycerol (PG, 9.3 %), diphosphatidylglycerol (DPG, 38.7 %), phosphatidylethanolamine (PE, 20.8 %), phosphatidylmonomethylethanolamine (methyl-PE, 10.4 %), phosphatidylserine (PS, 3.7 %), an amino-group containing phospholipid (NPL, 0.001 %), glucolipid (GL, 5.4 %), an unknown phospholipid (PL1, 3.7 %) and two unknown lipids (L1, 6.8 %; L2, 1.3 %) (Fig. S2 and Table 1). The cellular fatty acid profile of JIR-001$^T$ consisted of iso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{16:0}$ and $C_{16:0}$ as major components (35.5, 28.11, 22.54 and 4.74 %, respectively). Additionally, cellular fatty acid analysis revealed the presence of a number of minor components (Table 1). The hydrolysate (4 M HCl, 100 °C for 16 h) of the peptidoglycan preparation of strain JIR-001$^T$ contained *meso*-diaminopimelic acid (*meso*-DAP), alanine and glutamic acid in a molar ratio of 0.4 : 1.5 : 1.0, and no characteristic sugars were detected. Additionally, the partial hydrolysate (4 M HCl, 100 °C for 0.75 h) contained the peptide L-Ala–D-Glu. These results indicated that strain JIR-001$^T$ showed a peptidoglycan of type A1$\gamma$ (Schleifer & Kandler, 1972) and cell-wall chemotype III (Lechevalier & Lechevalier, 1970).

The neighbour-joining tree based on 16S rRNA gene sequences indicated that strain JIR-001$^T$ constitutes an independent clade within the family *Thermoactinomycetaceae*, and its topology was also supported by using the maximum-parsimony and maximum-likelihood algorithms. 16S rRNA gene sequence similarities between strain JIR-001$^T$ and the type strains of other members of the family *Thermoactinomycetaceae* were as follows: *Melghirimyces algeriensis*, 93.5 %; *P. fimeticola*, 92.9 %; *P. fulgidum*, 92.5 %; *Desmospora activa*, 92.4 %; *P. yunnanense*, 92.3 %; *Thermoactinomyces intermedius*, 92.3 %; *Thermoactinomyces vulgaris*, 92.0 %; *Thermoflavimicrobium dichotomicum*, 90.8 %; *Kroppenstedtia eburnea*, 90.6 %; *Mechercharimyces asporophorigenens*, 90.5 %; *Laceyella putida*, 89.8 %; *Laceyella sacchari*, 89.8 %; *Laceyella tengchongensis*, 89.5 %; *Laceyella sediminis*, 89.7 %; *Mechercharimyces mesophilus*, 88.0 %; *Seinonella peptonophila*, 87.5 %; and *Shimazuella kribbensis*, 85.5 % (Table S1).

The G+C content of the genomic DNA of strain JIR-001$^T$ was 55.1 mol%. Mean hybridization levels between strain JIR-001$^T$ and its phylogenetic neighbours were as follows: *Melghirimyces algeriensis* NariEX$^T$, 5.3–7.5 %; *P. fimeticola* H0165$^T$, 2.3–4.7 %; *P. fulgidum* 500275$^T$, 2.1–4.8 %; *P. yunnanense* LA5$^T$, 2.5–4.9 %.

Strain JIR-001$^T$ could be distinguished from members of the *Thermoactinomycetaceae* by several characteristics in addition to phylogenetic data (Table 2). In particular, strain JIR-001$^T$ is the only member of the family *Thermoactinomycetaceae* that contains glucolipid and phosphatidylserine as polar lipid components. Based on 16S rRNA gene sequences, strain JIR-001$^T$ was assigned to the family *Thermoactinomycetaceae* with *Melghirimyces algeriensis* NariEX$^T$ and *P. fimeticola* H0165$^T$ as its closest neighbours. DNA–DNA hybridization assays between strain JIR-001$^T$ and *Melghirimyces algeriensis* NariEX$^T$ showed a mean relatedness value of only 5.3–7.5 %, therefore indicating the wide difference between the two strains. The most crucial differential feature between strain JIR-001$^T$ and *Melghirimyces algeriensis* NariEX$^T$ was the presence of *meso*-DAP as the peptidoglycan component; only *Melghirimyces algeriensis* NariEX$^T$ and *Kroppenstedtia eburnea* IMMIB L-1269$^T$ in the family *Thermoactinomycetaceae* have the LL-DAP in the cell wall. In addition, *Melghirimyces algeriensis* NariEX$^T$, the closest relative of strain JIR-001$^T$ based on 16S rRNA gene sequence similarity, differed from strain JIR-001$^T$ in its menaquinone profile, salt torelance and colour of aerial mycelium. On the other hand, *P. fimeticola* H0165$^T$, the next closest relative of strain JIR-001$^T$ based on 16S rRNA gene sequence similarity, differed from strain JIR-001$^T$ in the formation of aerial mycelium, carbohydrate assimilation, range of growth temperature, salt tolerance and polar lipid component. In addition, DNA–DNA relatedness critically differentiated between strain JIR-001$^T$ and members of the genus *Planifilum* (*P. fimeticola* H0165$^T$, *P. fulgidum* 500275$^T$ and *P. yunnanense* LA5$^T$); each pair of strains shared only 2.3–4.7, 2.1–4.8, 2.5–4.9 %, respectively. *Desmospora activa* IMMIB L-1269$^T$ differed from strain JIR-001$^T$ by colour of aerial mycelium, carbohydrate assimilation, range of growth temperature and DNA G+C content. *Kroppenstedtia eburnea* JFMB-ATE$^T$ could be distinguished from strain JIR-001$^T$ by range of growth temperature, cellular fatty acid profile and DAP isomer. *Mechercharimyces* strains differ from strain JIR-001$^T$ by range of growth temperature, menaquinone, polar lipid composition, cellular fatty acid profile and DNA G+C

**Table 2.** Differential phenotypic and molecular characteristics of the new isolate and related taxa of the family *Thermoactinomycetaceae*

Taxa: 1, strain JIR-001$^T$ (data from this study); 2, *Melghirimyces algeriensis* NariEX$^T$ (Addou *et al.*, 2012); 3, *Planifilum* (data from Hatayama *et al.*, 2005; Zhang *et al.*, 2007; von Jan *et al.*, 2011); 4, *Desmospora activa* IMMIB L-1269$^T$ (Yassin *et al.*, 2009; von Jan *et al.*, 2011); 5, *Kroppenstedtia eburnea* IMMIB L-1269$^T$ (von Jan *et al.*, 2011); 6, *Mechercharimyces* (Matsuo *et al.*, 2006); 7, *Thermoactinomyces* (Yoon *et al.*, 2005); 8, *Thermoflavimicrobium dichotomicum* KCTC 3667$^T$ (Yoon *et al.*, 2005; von Jan *et al.*, 2011); 9, *Laceyella* (Yoon *et al.*, 2005); 10, *Seinonella peptonophila* KCTC 9740$^T$ (Yoon *et al.*, 2005); 11, *Shimazuella kribbensis* A9500$^T$ (Park *et al.*, 2007; von Jan *et al.*, 2011). +, Positive; −, negative; +/−, variable; NO, not observed; ND, no data available.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Colour of aerial mycelium | White | Yellow | NO | Yellow | White | White | White | Yellow | White | White | White |
| Degradation of: | | | | | | | | | | | |
| Gelatin | + | + | + | + | + | + | + | + | + | − | − |
| Starch | − | − | + | + | − | − | + | − | + | − | + |
| Casein | + | + | + | + | + | + | + | + | + | − | + |
| Xanthine | − | − | − | − | − | − | − | + | − | − | − |
| Hypoxanthine | − | − | − | − | − | − | − | + | − | − | − |
| Aesculin | +/− | +/− | + | + | + | − | + | − | +/− | ND | − |
| L-Tyrosine | − | − | +/− | − | − | − | +/− | − | +/− | − | − |
| Ranges for growth | | | | | | | | | | | |
| Temperature (°C) | 55–73 | 37–60 | 55–63 | 37–50 | 25–50 | 15–37 | 50–55 | 55 | 35–65 | 35 | 32 |
| NaCl (%) | 1–2 | 0–21 | 0–20* | ND | ND | ND | ND | <1 | 1 | ND | ND |
| Major menaquinone | MK-7 | MK-7 | MK-7 | MK-7 | MK-7 | MK-9 | MK-7 | MK-7 | MK-9 | MK-7 | MK-9 |
| Other detected menaquinones† | MK-8 | MK-6, MK-8 | MK-8 | NO | NO | MK-8 | MK-8 or MK-9 | NO | MK-7 or MK-8 or MK-10 | MK-8, MK-9, MK-10 | MK-10 |
| Major cellular fatty acids‡ | i-$C_{15:0}$, i-$C_{17:0}$, i-$C_{16:0}$, $C_{16:0}$ | i-$C_{15:0}$, ai-$C_{15:0}$* | i-$C_{17:0}$, ai-$C_{17:0}$, i-$C_{15:0}$ or i-$C_{16:0}$* | i-$C_{15:0}$, i-$C_{17:0}$, $C_{16:0}$ | i-$C_{15:0}$, ai-$C_{15:0}$ | i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:0}\omega 11c$, i-$C_{17:0}$ | i-$C_{15:0}$, i-$C_{17:0}$, ai-$C_{15:0}$ | i-$C_{15:0}$, ai-$C_{15:0}$, i-$C_{16:0}$ | i-$C_{15:0}$, ai-$C_{15:0}$ | i-$C_{14:0}$, ai-$C_{15:0}$, i-$C_{16:0}$ | i-$C_{15:0}$, ai-$C_{15:0}$, $C_{16:0}$, i-$C_{16:0}$, ai-$C_{17:0}$ |
| DAP isomer | *meso*-DAP | LL-DAP | *meso*-DAP | *meso*-DAP | LL-DAP | *meso*-DAP | *meso*-DAP | *meso*-DAP | *meso*-DAP | *meso*-DAP | *meso*-DAP |
| DNA G+C content (mol%) | 55.1 | 47.3 | 58.7–60.3 | 49.3 | 54.6 | 44.9–45.1 | 48.0 | 43.0 | 48.0–49.0 | 40.0 | 39.4 |

*Data from this study.
†Only components making up >1 % of the peak area ratio are shown.
‡i, iso; ai, anteiso.

content. Moreover, strain JIR-001$^T$ could be distinguished from members of the *Thermoactinomycetaceae*, which are positioned at the opposite clade in the neighbour-joining phylogenetic tree (*Laceyella* strains, *Thermoactinomyces* strains, *Thermoflavimicrobium dichotomicum* KCTC 3667$^T$, *Seinonella peptonophila* KCTC 9740$^T$ and *Shimazuella kribbensis* A9500$^T$) (Fig. 1), based on 16S rRNA gene sequence similarity and DNA G+C content in addition to several other characteristics.

Strain JIR-001$^T$ could be clearly distinguished from members of the family *Thermoactinomycetaceae* based on its range of growth temperature, salt tolerance, colour of aerial mycelium, assimilation of various carbon sources, DNA G+C content, DNA–DNA relatedness, menaquinones, peptidoglycan component, cellular fatty acids and polar lipid profile (Tables 1 and 2). Furthermore, phylogenetic analysis based on 16S rRNA gene sequences showed that strain JIR-001$^T$ constitutes an independent clade within the family *Thermoactinomycetaceae* (Fig. 1). The genotypic and phenotypic data acquired in this study show that strain JIR-001$^T$ should be assigned to a novel species in a new genus in the family *Thermoactinomycetaceae*, for which the name *Polycladomyces abyssicola* gen. nov., sp. nov. is proposed.

### Description of *Polycladomyces* gen. nov.

*Polycladomyces* (Pol.y.clado.my.ces. Gr. adj. *poly* many; Gr. masc. n. *klados* a branch; Gr. masc. n. *mukês* fungus; N. L. masc. n. *Polycladomyces* a fungus that possesses many branches).

Aerobic, Gram-positive, thermophilic and forms ubiquitous branched white aerial mycelium after 5 days at 60 °C on ISP2 medium. The predominant menaquinone is MK-7, but MK-8 is also present. Major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{16:0}$ and $C_{16:0}$. Cell-wall peptidoglycan contains *meso*-DAP, alanine and glutamic acid in addition to glucosamine and muramic acid but no characteristic sugars. The peptidoglycan type is A1$\gamma$ and cell-wall chemotype is III. The polar lipid profile consists of DPG, PE, methyl-PE, PG, GL, PS, PL and L1–2. The type species is *Polycladomyces abyssicola*.

### Description of *Polycladomyces abyssicola* sp. nov.

*Polycladomyces abyssicola* (a.bys.si′co.la. L. n. *abyssus* deep sea; L. masc. suffix *-cola* inhabitant; N.L. masc. n. *abyssicola* inhabitant of the deep sea, referring to the source of isolation of the type strain).

Exhibits the following properties in addition to those given in the genus description. On ISP2 medium, colonies are ivory in colour, star-shaped with wrinkles between the centre and the edge of the colony, and form white aerial mycelium. No diffusible pigment is detected. Growth occurs at temperatures ranging from 55 to 73 °C, at pH ranging from 6.5 to 8.5, and at NaCl concentrations between 1 and 2 % (w/v). Ribose, glycerol, xylose mannose, alanine, glycine, asparagine, arginine and fumarate are used as sole carbon sources for growth. Use of rhamnose, *myo*-inositol, ornithine, lysine, histidine, aspartic acid, acetate, citrate, formate and succinate as sole carbon source is doubtful. The following compounds are not utilized: maltose, cellobiose, erythritol, sucrose, fructose, arabinose, raffinose, lactose, cysteine, isoleucine, threonine, methionine, proline, serine, glutamic acid, valine, glutamine, benzoate, oxalate, malate and propionate. Acid production from organic compounds is not observed. Casein, gelatin and aesculin are degraded, whereas starch, xanthine, hypoxanthine and tyrosine are not hydrolysed. Catalase activity, indole production and nitrate/nitrite reduction are negative. Sensitive to bacitracin, gentamicin, imipenem, rifampicin, erythromycin, ampicillin, penicillin G, amoxicillin, cefazolin, vancomycin, kanamycin, neomycin, streptomycin, arbekacin, spectinomycin, cefmetazole, cefotiam and minocycline, slightly sensitive to tetracycline and chloramphenicol, but resistant to novobiocin.

The type strain, JIR-001$^T$ (=JCM 18147$^T$=CECT 8074$^T$), was isolated from a deep-seafloor sediment subsample collected from the Shimokita Peninsula of Japan at a depth of 48 mbsf. The DNA G+C content of the type strain is 55.1 mol%.

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