Melghirimyces algeriensis gen. nov., sp. nov., a member of the family Thermoactinomycetaceae, isolated from a salt lake.

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Ammara Nariman Addou,[1,2] Peter Schumann,[3] Cathrin Spröer,[3] Hocine Hacene,[2] Jean-Luc Cayol[1] and Marie-Laure Fardeau[1]

[1]Laboratoire de Microbiologie MIO-IRD, UMR 235, Aix-Marseille Université, case 925, 163 Avenue de Luminy, 13288 Marseille cedex 9, France

[2]Laboratoire de Biologie Cellulaire et Moléculaire (équipe de Microbiologie), Université des Sciences et de la Technologie, Houari Boumédiènne, Bab Ezzouar, Algiers, Algeria

[3]Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7B, 38124 Braunschweig, Germany

A novel filamentous bacterium, designated NariEX^T, was isolated from soil collected from Chott Melghir salt lake, which is located in the south-east of Algeria. The strain was an aerobic, halotolerant, thermotolerant, Gram-positive bacterium that was able to grow in NaCl concentrations up to 21 % (w/v), at 37–60 °C and at pH 5.0–9.5. The major fatty acids were iso- and anteiso-$C_{15:0}$. The DNA G+C content was 47.3 mol%. The major menaquinone was MK-7, but MK-6 and MK-8 were also present. The polar lipid profile consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylmonomethylethanolamine (methyl-PE). Results of molecular and phenotypic analysis led to the description of the strain as a new member of the family *Thermoactinomycetaceae*. The isolate was distinct from members of recognized genera of this family by morphological, biochemical and chemotaxonomic characteristics. Strain NariEX^T showed 16S rRNA gene sequence similarities of 95.38 and 94.28 % with the type strains of *Desmospora activa* and *Kroppenstedtia eburnea*, respectively, but differed from both type strains in its sugars, polar lipids and in the presence of methyl-PE. On the basis of physiological and phylogenetic data, strain NariEX^T represents a novel species of a new genus of the family *Thermoactinomycetaceae* for which the name *Melghirimyces algeriensis* gen. nov., sp. nov. is proposed. The type strain of *Melghirimyces algeriensis*, the type species of the genus, is NariEX^T (=DSM 45474^T=CCUG 59620^T).

The genus *Thermoactinomyces* was described by Tsilinsky (1899) and accommodates Gram-positive, aerobic species with filamentous growth similar to that of actinomycetes (Hatayama *et al.*, 2005). Thus, members of this genus were initially considered to be actinomycetes. However, due to similarities in the development, heat resistance and other properties of the endospores to those of *Bacillus* strains (Cross *et al.*, 1968) and due to phylogenetic differences from actinomycetes, thermoactinomycetes have been affiliated to the '*Bacillales*' (Stackebrandt & Woese, 1981).

Abbreviations: DAP, diaminopimelic acid; DPG, diphosphatidylglycerol; methyl-PE, phosphatidylmonomethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NariEX^T is HQ383683.

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

Subsequently, the genus *Thermoactinomyces* has been divided into six genera on the basis of phylogenetic and chemotaxomomic studies: *Thermoactinomyces sensu stricto* (Yoon *et al.*, 2005), *Laceyella*, *Thermoflavimicrobium*, *Seinonella*, *Planifilum* (Hatayama *et al.*, 2005) and *Mechercharimyces* (Matsuo *et al.*, 2006). These genera have been grouped into the same family, *Thermoactinomycetaceae*, which was described by Matsuo *et al.* (2006). Other genera in this family have recently been described, including *Shimazuella* (Park *et al.*, 2007) and *Desmospora* (Yassin *et al.*, 2009), which has made emendation of the description of the family *Thermoactinomycetaceae* necessary (Yassin *et al.*, 2009). The description of this family was further emended by von Jan *et al.* (2011). The authors included the new genus *Kroppenstedtia*, members of which contain the isomer LL-diaminopimelic acid (DAP) in the cell wall; previous members of the family *Thermoactinomycetaceae* had only been found to contain *meso*-DAP.

All species in the family are thermophilic or thermotolerant, except for *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 all mesophilic.

Here, we characterize a novel member of the family *Thermoactinomycetaceae* isolated from soil of an Algerian salt lake during our investigations on extremophilic actinomycetes with the aim to explore their potential as producers of original bioactive metabolites.

Samples were collected from soil taken from an Algerian salt lake named Chott Melghir located in the south-east of Algeria.

A portion (1 g) of the collected soil sample was transferred into 100 ml ISP 2 liquid medium (Shirling & Gottlieb, 1966) supplemented with 10 % NaCl in 500 ml flasks and incubated in a rotary shaker incubator at 55 °C with shaking at 160 r.p.m. for 5 days. From this culture, an aliquot of 0.1 ml was taken daily and inoculated onto the surface of ISP 2 agar containing 10 % NaCl; plates were incubated at 55 °C and monitored after 48, 72 and 96 h. Strain NariEX^T was isolated by this procedure and maintained on ISP 2 agar with 10 % NaCl.

The biomass used for chemotaxonomic and phylogenetic studies was obtained from 72-h-old liquid cultures of strain NariEX^T in ISP 1 and 2 media supplemented with 10 % NaCl and incubated at 55 °C.

Morphological characteristics and purity of strain NariEX^T were checked under an Optiphot (Nikon) phase-contrast microscope. For transmission electron microscopy studies, cell preparations were negatively stained with sodium phosphotungstate, as described previously (Fardeau *et al.*, 1997). For microscopic examination, cells were grown on nutrient broth and agar supplemented with 10 % NaCl for 3 days at 55 °C. The study of cultural characteristics was conducted as described by Shirling & Gottlieb (1966). The colours of aerial and substrate mycelia were determined by observation with the naked eye of cultures after 72 h incubation at 55 °C on the following different semi-solid media: tryptone-yeast extract agar (ISP 1); malt extract-yeast extract agar (ISP 2); inorganic salt-starch agar (ISP 4); and glycerol-asparagine agar (ISP 5) (Shirling & Gottlieb, 1966). All media were supplemented with 10 % NaCl. Cultures on each medium were examined with respect to the development of growth, colour of aerial and substrate mycelia, and production of diffusible pigments.

Melanoid pigment production was examined on peptone-yeast extract-iron agar (ISP 6) and tyrosine agar (ISP 7) (Shirling & Gottlieb, 1966). Both were supplemented with 10 % NaCl.

The strain was tested for the utilization of 17 carbohydrates, 19 amino acids and 10 organic acids (Table 1) as sole carbon and energy sources by using the amended basal medium of Tsukamura (1966). The medium contained 10 % NaCl and the following (g l^{-1}): $(NH_4)_2SO_4$, 2.64; KH_2PO_4, 0.5; $MgSO_4 . 7H_2O$, 0.5; agar, 20. Each of the organic acid sodium salts, carbohydrates and amino acids was added to the medium to a final concentration of 0.02 M. For the detection of acid production from carbohydrates, the same medium was used and supplemented with 0.2 g yeast extract l^{-1} and 20 ml 0.04 % bromocresol blue solution l^{-1} as pH indicator (Tsukamura, 1966). Acid production was demonstrated by a change in the pH indicator from purple to yellow, indicating acidification of the medium. Basal medium without carbon source was used as a control. ISP 9 liquid medium was used to confirm carbohydrate and organic compound utilization tests. Utilization of amino acids was tested in ISP medium 9 without a nitrogen source. HPLC was used to detect degradation of substrates.

The degradation of casein and tyrosine was determined according to the method of Staneck & Roberts (1974). Hydrolysis of starch was determined on nutrient agar medium supplemented with 1 % starch. After incubation at 55 °C for 5 to 7 days, cultures have been flooded with iodine solution. The disappearance of blue colour around the colonies is scored as positive result (Cowan, 1974). The ability of the strain to degrade aesculin was examined according to the method of Gordon *et al.* (1974) modified by using aesculin agar instead of aesculin broth. The medium was composed of (g per litre distilled water): aesculin, 1; ferric citrate, 0.5; peptone, 10; agar, 20. Hydrolysis of xylan (0.4 %, w/v) was detected on the basal medium of Tsukamura (1966). Degradation of xanthine, hypoxanthine, adenine and gelatin was determined according to the method of Gordon (1966) and Gordon & Mihm (1957).

To determine the range of temperatures tolerated, strain NariEX^T was grown on ISP 2 medium supplemented with 10 % NaCl and incubated at 35, 37, 40, 50, 55 and 60 °C. The NaCl requirement for growth was determined on the ISP medium 2 by varying the salt concentration in the culture medium. NaCl concentrations tested were 0, 2, 5, 7, 10, 12, 15, 16, 17, 18, 20 and 21 % and cultures were incubated at 55 °C.

The pH range was tested on ISP 2 medium supplemented with 10 % NaCl buffered and adjusted to the desired pH (4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0). Different buffers were used: citrate buffer for pH 4.0–6.0, Tris/HCl for pH 7.0–9.0, tetraborate buffer at pH 9.5–10.0 and Na_2HPO_4 buffer for pH 11.0. Cultures were incubated at 55 °C.

Catalase activity was tested by immersing one loopful of 3-day-old culture of strain NariEX^T on ISP 2 medium supplemented with 10 % NaCl into an aqueous solution of H_2O_2 and observing the occurrence of bubbling. Urease activity was determined according to the method of Lányí (1987). Indole production, Voges–Proskauer reaction and nitrate reduction/denitrification were investigated by using the methods of Gordon & Mihm (1957), Guérin-Faublée *et al.* (1992) and Joffin & Leyral (2006).

DNA purification, and PCR amplification and sequencing of the 16S rRNA gene were performed as described

Table 1. Morphological and physiological characteristics of strain NariEX^T

Characteristic	NariEX^T
Colour of aerial mycelium	Yellow
Colour of substrate mycelium	Yellow
Utilization of carbohydrates as sole carbon source:	
Glycerol	+
Maltose	+
Cellobiose	+
Rhamnose	+
Erythritol	−
Sucrose	+
myo-Inositol	+
Xylose	−
Fructose	+
Arabinose	−
Ribose	+
Mannose	+
Glucose	+
Raffinose	+
Lactose	−
Acid production from carbohydrate	−
Utilization of:	
Ornithine	−
Cysteine	−
Isoleucine	−
Alanine	+
Threonine	+
Methionine	−
Proline	+
Serine	−
Glycine	+
Asparagine	+
Lysine	−
Glutamic acid	+
Histidine	−
Arginine	+/−
Aspartic acid	+
Valine	−
Glutamine	−
Acetate	+
Citrate	−
Formate	−
Benzoate	−
Oxalate	+
Fumarate	−
Succinate	+
Malate	+
Propionate	−
Hydrolysis of:	
Cellulose	−
Starch	−
Decomposition of:	
Urea	−
Casein	+
Tyrosine	−
Gelatin	+
Hypoxanthine	−
Xanthine	−
Adenine	−
Catalase	−
Growth conditions	
Temperature range (°C)	37–60
pH range	5.0–9.5
NaCl (%)	0–21
DNA G+C content (mol%)	47.3

previously (Ben Dhia Thabet *et al.*, 2004). The partial sequences generated were assembled using BioEdit version 5.0.9 (Hall, 1999) and the consensus sequence of 1344 nt was manually corrected for errors. The most closely related sequences in GenBank (version 178), and the Ribosomal Database Project (release 10) were identified using BLAST (Altschul *et al.*, 1997) and the Sequence Match program (Cole *et al.*, 2009). These sequences were retrieved and aligned and the alignment was adjusted manually according to the 16S rRNA secondary structure using BioEdit. Evolutionary distances were calculated by using the Jukes and Cantor correction (Jukes & Cantor, 1969). Dendrograms were constructed with the program TREECON using the neighbour-joining method (Saitou & Nei, 1987). Tree topology was evaluated by the bootstrap method (1000 replications) of resampling (Felsenstein, 1985). Its topology was also supported by using the maximum-parsimony and maximum-likelihood algorithms. Phylogenetic trees were inferred by maximum-parsimony (Fitch, 1971), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) algorithms, using the program TREECON. Tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings.

Genomic DNA for analysis of DNA G+C content and for DNA–DNA hybridization was isolated after disruption of bacterial cells by using a French press (Thermo Spectronic) and purified by chromatography on hydroxyapatite using the procedure of Cashion *et al.* (1977). The G+C content was determined by HPLC as described by Mesbah *et al.* (1989). DNA–DNA hybridizations were carried out as described by De Ley *et al.* (1970) considering the modifications described by Huß *et al.* (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with *in situ* temperature probe (Varian).

For analysis of cellular fatty acids, cells were cultivated on ISP 1 medium supplemented with 10 % NaCl at 55 °C for 72 h under aerobic conditions. Fatty acid methyl esters were obtained following the method of Stead *et al.* (1992) and were analysed by GC using the Microbial Identification System (MIDI, Sherlock Version 6.1; database, TSBA40; GC model 6890N, Agilent Technologies). Polar

lipids were determined according to the method described by Minnikin *et al.* (1979) and separated by two-dimensional TLC. To identify spots, ninhydrin reagent, Zinzadze reagent and molybdophosphoric acid were used (Embley & Wait, 1994). Isoprenoid quinones were extracted as described by Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1996)

On ISP 2 medium, strain NariEX^T formed colonies of 3–5 cm in diameter that were yellow-coloured with wrinkles formed between the centre and the edge of the colony, with a small projection in the centre, dull, flat and dry. After prolonged incubation, the diameter of the colony could reach that of the Petri dishes containing the medium. Colonies were inlaid in the agar and difficult to detach.

On ISP 1 medium, strain NariEX^T formed pale yellow aerial and substrate mycelium after 48 to 72 h at 55 °C. Colonies were pale yellow, shiny, flat, smooth with regular margins, easily detachable from the agar and could be removed as a whole. No diffusible pigments were detected on this growth medium. The strain grew well on ISP 1 and ISP 2 media, but weak growth was observed on ISP 4, 5, 6 and 7. No melanoid pigments were produced on ISP 6 or 7 media. Microscopic examination revealed long, straight to flexuous, moderately branched septate hyphae that developed single endospores (Fig. S1, available in IJSEM Online). Electron microscopy showed a dense outer cell wall (Fig. S2).

Strain NariEX^T was aerobic. It tolerated 0–21 % (w/v) NaCl, with optimal growth occurring at 7–12 % (w/v) NaCl. Growth occurred at 37–60 °C and pH 5.0–9.5. Good growth was observed at 40–55 °C and pH 6–8 with 10 % NaCl.

On Tsukamura medium, strain NariEX^T utilized glycerol, maltose, cellobiose, rhamnose, sucrose, *myo*-inositol, fructose ribose, mannose, glucose, raffinose, alanine, threonine, proline, glycine, asparagine, glutamic acid, aspartic acid, acetate, oxalate, succinate and malate as sole carbon sources for growth. No acid production was detected from the utilization of carbohydrate. Results of the tests carried out on ISP 9 liquid medium were difficult to interpret because of the weak growth and weak degradation of substrates in liquid medium.

Casein and gelatin were decomposed by the strain. Xanthine, hypoxanthine, tyrosine, adenine and starch were not hydrolysed. Urease and Voges–Proskauer reactions were negative. Indole was not produced. Nitrate was reduced to nitrite. Results of physiological and biochemical tests are summarized in Tables 1 and 2.

Strain NariEX^T contained LL-DAP and the quinones MK-7, MK-6 and MK-8 (94 : 2 : 2). The fatty acid profile of strain NariEX^T consisted of iso- and anteiso-$C_{15:0}$ as main components (59.13 % and 18.18 % respectively). In addition, cellular fatty acid analysis revealed the presence of a number of minor fatty acids (Table 2). The phospholipid pattern consisted of phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (methyl-PE), unknown phospholipids (PL1–5) and an unknown lipid (L), indicating phospholipid pattern type PII (*sensu* Lechevalier *et al.*, 1977) (Figs S3 and S4). The total hydrolysate of the peptidoglycan of strain NariEX^T (4 M HCl, 100 °C, 0.75 h) contained LL-DAP, alanine, glycine and glutamic acid in a molar ratio of 1.1 : 1.4 : 0.5 : 1.0. In addition to these amino acids, the partial hydrolysate contained the peptides L-Ala–D-Glu, D-Ala–Gly and L-Lys–D-Ala. The results indicated that strain NariEX^T showed the peptidoglycan type A3γ LL-DAP–Gly (Schleifer & Kandler, 1972; type A41.1 according to http://www.peptidoglycan-types.info). Cell wall sugars consisted of xylose, mannose, a small amount of galactose and traces of glucose.

Table 2. Fatty acid composition of strain NariEX^T

Fatty acid	Composition (%)
$C_{10:0}$	0.39
$C_{12:0}$	0.21
iso-$C_{13:0}$	1.25
anteiso-$C_{13:0}$	0.20
iso-$C_{14:0}$	1.59
$C_{14:0}$	3.12
iso-$C_{15:0}$	59.13
anteiso-$C_{15:0}$	18.18
$C_{15:0}$	0.73
iso-$C_{16:0}$	1.84
$C_{16:0}$	4.62
iso-$C_{17:0}$	6.66
anteiso-$C_{17:0}$	1.54
$C_{18:1}\omega 9c$	0.26

The DNA G+C content of strain NariEX^T was 47.3 mol%. Hybridization was done with *Kroppenstedtia eburnea* DSM 45196^T and the two strains shared only 9.6 % DNA–DNA relatedness. Sequence alignment and subsequent comparisons with sequences of representative members of the domain '*Bacteria*' consistently placed strain NariEX^T within the phylum '*Firmicutes*', class *Bacilli*, order *Bacillales*, family *Thermoactinomycetaceae* with *Desmospora activa* and *K. eburnea* as its closest phylogenetic neighbours (95.38 and 94.28 % similarities, respectively, with the type strains of these two species) (Fig. 1).

Based on 16S rRNA gene sequences, strain NariEX^T was assigned to the family *Thermoactinomycetaceae* with *K. eburnea* and *D. activa* as the closest relatives. DNA–DNA hybridization between the type strain of *K. eburnea* and strain NariEX^T revealed a relatedness value of only 9.6 %, thus indicating the great difference between the two strains. The novel isolate could be distinguished from members of the *Thermoactinomycetaceae* by several characteristics in addition to phylogenetic data (Table 3). The most important distinguishing feature of strain NariEX^T was the presence of the LL-DAP isomer in the cell wall; all described genera of the family *Thermoactinomycetaceae*, except *K. eburnea*, contain *meso*-DAP. *K. eburnea* and *D. activa*, the

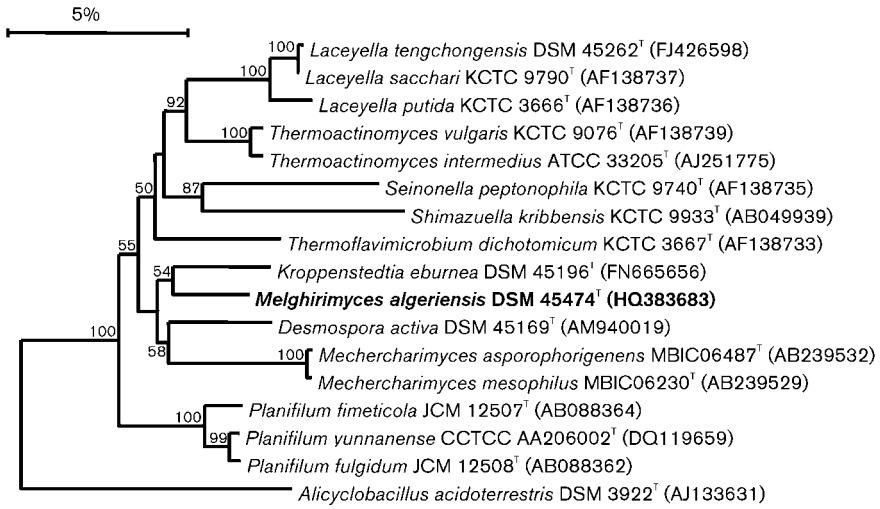

Fig. 1. Neighbour-joining phylogenetic dendrogram based on 1344 unambiguous nucleotides showing the relationship between strain NariEX^T and organisms belonging to the family *Thermoactinomycetaceae*. The dendrogram was reconstructed based 16S rRNA gene sequences of the type strains. GenBank accession numbers are shown in parentheses. Numbers at branch points specify the reliability of the branching order determined for 1000 resamplings; only bootstrap values $\geqslant$50 % are shown. Bar, 5 substitutions per 100 nucleotide positions.

closest relatives of strain NariEX^T based on 16S rRNA gene sequences, differed from strain NariEX^T in their menaquinone profiles: strain NariEX^T, in addition to MK-7 as major menaquinone, also contains MK-6 and MK-8, whereas *K. eburnea* and *D. activa* contain exclusively MK-7. In addition, strain NariEX^T is the only member of the family *Thermoactinomycetaceae* that contains menaquinone MK-6, thus distinguishing it from all taxa of the family *Thermoactinomycetaceae* with validly published names. Like *K. eburnea*, which contains only glucose and ribose, *D. activa* lacks any characteristic cell-wall sugars; the presence of xylose, mannose and galactose in the cell wall sugars of strain NariEX^T differentiated it from its two closest neighbours. The fatty acid profiles of strain NariEX^T, *K. eburnea* and members of the genus *Laceyella* all contain iso-$C_{15:0}$ and anteiso-$C_{15:0}$ as major fatty acids, which distinguishes the strain from all other genera of the family *Thermoactinomycetaceae*. *Laceyella* strains differ from NariEX^T by menaquinone, colour of aerial mycelium and presence of *meso*-DAP in the peptidoglycan. Another unique feature of strain NariEX^T is its halotolerance; the strain was able to grow at NaCl concentrations of up to 21 %. This characteristic has not previously been reported for members of the family *Thermoactinomycetaceae*. Strains of the genera *Laceyella* and *Thermoflavimicrobium* are not capable of growing in salinities greater than 1 %.

Phenotypic and molecular studies indicate that strain NariEX^T can be clearly distinguished from members of other genera of the family *Thermoactinomycetaceae*. Examination of the growth temperature, DNA G+C content, menaquinone composition and cellular fatty acids revealed that strain NariEX^T did not belong to the genera *Thermoactinomyces sensu stricto*, *Laceyella*, *Thermoflavimicrobium*, *Seinonella*, *Planifilum* or *Mechercharimyces*. On the basis of the results presented in this study, strain NariEX^T represents a novel species in a new genus in the family *Thermoactinomycetaceae*, for which the name *Melghirimyces algeriensis* gen. nov., sp. nov. is proposed.

Description of *Melghirimyces* gen. nov.

Melghirimyces (Mel.ghi.ri′my.ces. N.L. n. *Melghir* a salted lake named Chott Melghir in south-east of Algeria, from where the organism was isolated; Gr. masc. n. *mukês* fungus; N.L. masc. n. *Melghirimyces* a fungus of Chott Melghir).

Aerobic, Gram-positive, forms extensively branched yellow aerial and substrate mycelia after 72 h at 55 °C on ISP 1 and ISP 2 media supplemented with 10 % (w/v) NaCl. Major cellular fatty acids are iso- and anteiso-$C_{15:0}$. The major menaquinone is MK-7, but MK-6 and MK-8 are also present. Cell-wall peptidoglycan contains LL-DAP. The phospholipid pattern consisted of PG, DPG, PE, methyl-PE, PL1–5 and an unknown lipid. The type species is *Melghirimyces algeriensis*.

Description of *Melghirimyces algeriensis* sp. nov.

Melghirimyces algeriensis (al.ge.ri.en′sis. N.L. masc. adj. *algeriensis* of or belonging to Algeria).

Exhibits the following properties in addition to those given in the genus description. On ISP 2 medium, colonies are yellow, flat, dull with regular margins and radial wrinkles formed on the surface. Glycerol, maltose, cellobiose, rhamnose, sucrose, *myo*-inositol, fructose, ribose, mannose, glucose, raffinose, alanine, threonine, proline, glycine, asparagine,

Table 3. Differential phenotypic and molecular characteristics of the new isolate and related genera of the family *Thermoactinomycetaceae*

Taxa: 1, strain DSM 45474^T (data from this study); 2, *Kroppenstedtia eburnea* (von Jan *et al.*, 2011); 3, *Desmospora activa* (Yassin *et al.*, 2009); 4, *M. mesophilus* YM3-251 (Matsuo *et al.*, 2006); 5, *Thermoactinomyces* (Yoon *et al.*, 2005); 6, *Laceyella* (Yoon *et al.*, 2005); 7, *Thermoflavimicrobium* (Yoon *et al.*, 2005); 8, *Planifilum* (Hatayama *et al.*, 2005); 9, *Seinonella* (Yoon *et al.*, 2005); 10, *Shimazuella kribbensis* (Park *et al.*, 2007). +, Positive; −, negative; +/−, variable; ND, no data available; NO, not observed.

Characteristic	1	2	3	4	5	6	7	8	9	10
Colour of aerial mycelium	Yellow	White	Yellow	White	White	White	Yellow	ND	White	White
Degradation of:										
Casein	+	+	+	+	+	+	+	+	−	+
Hypoxanthine	−	−	−	−	−	−	+	−	−	−
Xanthine	−	−	−	−	−	−	+	−	−	−
L-Tyrosine	−	−	−	−	−	+/−	−	+/−	−	−
Gelatin	+	+	+	+	+	+	+	+	−	−
Ranges for growth:										
Temperature (°C)	37–60	25–50	37–50	15–37	50–55	48–55	55	55–63	35	32
NaCl (%)	0–21	ND	ND	ND	ND	1	<1	ND	ND	ND
Major menaquinone	MK-7	MK-7	MK-7	MK-9	MK-7	MK-9	MK-7	MK-7	MK-7	MK-9
Other detected menaquinones	MK-6, MK-8	NO	NO	MK-8	MK-8 or MK-9	MK-7, MK-8 or MK-10	NO	NO	MK-8, MK-9, MK-10	MK-10
Major fatty acids	i-$C_{15:0}$, ai-$C_{15:0}$	i-$C_{15:0}$, ai-$C_{15:0}$	i-$C_{15:0}$, i-$C_{17:0}$, $C_{16:0}$	i-$C_{15:0}$, i-$C_{17:1}\omega 11c$, ai-$C_{15:0}$	i-$C_{15:0}$, i-$C_{17:0}$, $C_{15:0}$	i-$C_{15:0}$, ai-$C_{15:0}$	i-$C_{15:0}$, ai-$C_{15:0}$ i-$C_{16:0}$	i-$C_{17:0}$ ai-$C_{17:0}$	i-$C_{14:0}$ ai-$C_{15:0}$ i-$C_{16:0}$	ai-$C_{15:0}$ i-$C_{16:0}$ $C_{16:0}$
DAP isomer	LL	LL	*meso*	*meso*	*meso*	*meso*	*meso*	*meso*	*meso*	*meso*
DNA G+C content (mol%)	47.3	54.6	49.3	45.0	48.0	48.0–49.0	43.0	58.7–60.3	40.0	39.4

glutamic acid, aspartic acid, acetate, oxalate, succinate and malate are used as sole carbon sources for growth. The following compounds are not utilized: erythritol, lactose, arabinose, xylose, ornithine, serine, cysteine, isoleucine, methionine, lysine, histidine, valine, glutamine, citrate, formate, benzoate, fumarate and propionate. Utilization of arginine as a sole carbon source is doubtful. Acids are not produced from organic compounds. Decomposes casein and gelatin. Cellulose, tyrosine, xanthine, hypoxanthine, adenine, urea and starch are not hydrolysed. Nitrate reduction is positive. Indole production and Voges–Proskauer reaction are negative. Growth occurs at 37–60 °C, in 0–21 % (w/v) NaCl and at pH 5.0–9.5.

The type strain, NariEX^T (=DSM 45474^T=CCUG 59620^T), was isolated from a soil sample collected from a salt lake in the south-east of Algeria. The DNA G+C content of the type strain is 47.3 mol%.

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References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST, a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Ben Dhia Thabet, O., Fardeau, M.-L., Joulian, C., Thomas, P., Hamdi, M., Garcia, J.-L. & Ollivier, B. (2004). *Clostridium tunisiense* sp. nov., a new proteolytic, sulphur-reducing bacterium isolated from an olive mill wastewater contaminated by phosphogypse. *Anaerobe* **10**, 185–190.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Marsh, T. & other authors (2009). The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* **37** (Database issue), D141–D145.

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.

Cowan, T. (1974). *Cowan and Steel's Manual for the Identification of Medica1 Bacteria*. Cambridge: Cambridge University Press.

Cross, T., Walker, P. D. & Gould, G. W. (1968). Thermophilic actinomycetes producing resistant endospores. *Nature* **220**, 352–354.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Embley, T. M. & Wait, R. (1994). Structural lipids of *Eubacteria*. In *Chemical Methods in Prokaryotic Systematics*, pp. 141–147. Edited by M. Goodfellow & A. G. O'Donnell. New York: Wiley.

Fardeau, M.-L., Ollivier, B., Patel, B. K. C., Magot, M., Thomas, P., Rimbault, A., Rocchiccioli, F. & Garcia, J.-L. (1997). *Thermotoga hypogea* sp. nov., a xylanolytic, thermophilic bacterium from an oil-producing well. *Int J Syst Bacteriol* **47**, 1013–1019.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Gordon, R. E. (1966). Some criteria for the recognition of *Nocardia madurae* (Vincent) Blanchard. *J Gen Microbiol* **45**, 355–364.

Gordon, R. E. & Mihm, J. M. (1957). A comparative study of some strains received as nocardiae. *J Bacteriol* **73**, 15–27.

Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H. N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.

Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.

Guérin-Faublée, V., Karray, S., Tilly, B. & Richard, Y. (1992). [*Actinomyces pyogenes*: conventional and API system bacteriologic study of 103 strains isolated from ruminants]. *Ann Rech Vet* **23**, 151–160 (in French).

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

Hatayama, K., Shoun, H., Ueda, Y. & Nakamura, A. (2005). *Planifilum fimeticola* gen. nov., sp. nov. and *Planifilum fulgidum* sp. nov., novel members of the family '*Thermoactinomycetaceae*' isolated from compost. *Int J Syst Evol Microbiol* **55**, 2101–2104.

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Joffin, J.-N. & Leyral, G. (2006) *Microbiologie Technique. Tome 1: Dictionnaire des Techniques*, 4th edn. Bordeaux: CRDP d'Aquitaine.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.

Matsuo, Y., Katsuta, A., Matsuda, S., Shizuri, Y., Yokota, A. & Kasai, H. (2006). *Mechercharimyces mesophilus* gen. nov., sp. nov. and *Mechercharimyces asporophorigenens* sp. nov., antitumour substance-producing marine bacteria, and description of *Thermoactinomycetaceae* fam. nov. *Int J Syst Evol Microbiol* **56**, 2837–2842.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Minnikin, D. E., Collins, M. D. & Goodfellow, M. (1979). Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* **47**, 87–95.

Nonomura, H. & Ohara, Y. (1971). Distribution of actinomycetes in soil. X. New genus and species of monosporic actinomycetes. *J Ferment Technol* **49**, 895–903.

Park, D. J., Dastager, S. G., Lee, J. C., Yeo, S. H., Yoon, J. H. & Kim, C. J. (2007). *Shimazuella kribbensis* gen. nov., sp. nov., a mesophilic representative of the family *Thermoactinomycetaceae*. *Int J Syst Evol Microbiol* **57**, 2660–2664.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.

Stackebrandt, E. & Woese, C. R. (1981). Towards a phylogeny of the actinomycetes and related organisms. *Curr Microbiol* **5**, 197–202.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to the identification of aerobic *Actinomycetes* by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Stead, D. E., Sellwood, J. E., Wilson, J. & Viney, I. (1992). Evaluation of a commercial microbial identification system based on fatty acid profiles for rapid, accurate identification of plant pathogenic bacteria. *J Appl Bacteriol* **72**, 315–321.

Tsilinsky, P. (1899). On the thermophilic moulds. *Ann Inst Pasteur* **13**, 500–505 (in French).

Tsukamura, M. (1966). Adansonian classification of mycobacteria. *J Gen Microbiol* **45**, 253–273.

von Jan, M., Riegger, N., Pötter, G., Schumann, P., Verbarg, S., Spröer, C., Rohde, M., Lauer, B., Labeda, D. P. & Klenk, H. P. (2011). *Kroppenstedtia eburnea* gen. nov., sp. nov., a thermoactinomycete isolated by environmental screening, and emended description of the family *Thermoactinomycetaceae* Matsuo *et al.* 2006 emend. Yassin *et al.* 2009. *Int J Syst Evol Microbiol* **61**, 2304–2310.

Yassin, A. F., Hupfer, H., Klenk, H.-P. & Siering, C. (2009). *Desmospora activa* gen. nov., sp., nov., a thermoactinomycete isolated from sputum of a patient with suspected pulmonary tuberculosis, and emended description of the family *Thermoactinomycetaceae* Matsuo *et al.* 2006. *Int J Syst Evol Microbiol* **59**, 454–459.

Yoon, J.-H., Kim, I.-G., Shin, Y.-K. & Park, Y.-H. (2005). Proposal of the genus Thermoactinomyces sensu stricto and three new genera, *Laceyella*, *Thermoflavimicrobium* and *Seinonella*, on the basis of phenotypic, phylogenetic and chemotaxonomic analyses. *Int J Syst Evol Microbiol* **55**, 395–400.