International Journal of Systematic and Evolutionary Microbiology (2015), **65**, 000–000 **DOI** 10.1099/ijs.0.000107-0

Myceligenerans cantabricum sp. nov., a barotolerant actinobacterium isolated from a deep cold-water coral

Aida Sarmiento-Vizcaíno,[1] Verónica González,[1] Alfredo F. Braña,[1] Axayacatl Molina,[2] José L. Acuña,[2] Luis A. García[3] and Gloria Blanco[1]

Correspondence
Gloria Blanco
gbb@uniovi.es

[1]Departamento de Biología Funcional, Área de Microbiología, e Instituto Universitario de Oncología del Principado de Asturias. Universidad de Oviedo, 33006 Oviedo, Spain

[2]Departamento de Biología de Organismos y Sistemas. Área de Ecología Universidad de Oviedo, Spain

[3]Departamento de Ingeniería Química y Tecnología del Medio Ambiente. Universidad de Oviedo, Spain

2

An actinobacterium strain (M-201^T) was isolated from a deep-sea scleractinian coral (*Fam. Caryophillidae*) collected at 1500 m depth in the Avilés Canyon in the Cantabrian Sea, Asturias, Spain. Strain M-201^T grew at pH 6.0–9.0 (optimum pH 7.0), between 4 and 37 °C (optimum 28 °C) and at salinities of 0.5–10.5 % (w/v) NaCl (optimum 0.5–3.0 %). The peptidoglycan contained the amino acids Lys, Ala, Thr, Glu and one unknown amino acid component, and belonged to type A4α, and the whole-cell hydrolysates contained glucose, mannose and galactose. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, an unknown phosphoglycolipid and seven unknown glycolipids. The predominant menaquinones were MK-9(H_4) and MK-9(H_6). Major cellular fatty acids were anteiso-$C_{15:0}$, iso-$C_{15:0}$ and anteiso-$C_{17:0}$. The genomic DNA G+C content was 72.4 mol%. The chemotaxonomic properties supported the affiliation of strain M-201^T to the genus *Myceligenerans*. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the organism was most closely related to *Myceligenerans crystallogenes* CD12E2-27^T (98.2 % 16S rRNA gene sequence similarity). However, it had a relatively low DNA–DNA relatedness value with the above strain (48 %). The isolate showed antibiotic activity against *Escherichia coli, Micrococcus luteus* and *Saccharomyces cerevisiae*. To the best of our knowledge, this is the first report of antibiotic production in the genus *Myceligenerans*. The differences in phenotypic, metabolic, ecological and phylogenetic characteristics justify the proposal of a novel species of the genus *Myceligenerans*, *Myceligenerans cantabricum* sp. nov., with M-201^T (=CECT 8512^T=DSM 28392^T) as the type strain.

The genus *Myceligenerans* was described by Cui *et al.* (2004). At the time of writing, this genus comprises three species with validly published names: *Myceligenerans xiligouense* (Cui *et al.*, 2004), *Myceligenerans crystallogenes* (Groth *et al.*, 2006) and *Myceligenerans halotolerans* (Wang *et al.*, 2011), and another not yet validly published: '*Myceligenerans salitolerans*' (Guan *et al.*, 2013). Chemotaxonomically, members of the genus *Myceligenerans* contain L-Lys–L-Thr–D-Glu in the cell-wall peptidoglycan (variation A4α), MK-9(H_4) and MK-9(H_6) as the predominant menaquinones, anteiso-$C_{15:0}$ and iso-$C_{15:0}$ as the major fatty acids, and have DNA G+C contents of 72–72.3 mol% (Wang *et al.*, 2011). Members of the genus *Myceligenerans* are Gram-positive and aerobic.

During a cruise expedition (BIOCANT3) in the Cantabrian Sea in April–May 2013 aboard the Sarmiento de Gamboa oceanographic ship, a disk solitary coral (*O. Scleractinia, Fam. Caryophillidae*) was collected in the sub-marine Avilés Canyon (Asturias, Spain). The sample was taken using a 5 m-long Agassiz trawl with a beam width of 5 m and towed during 1 h at 1500 m depth (P3 station, GPS coordinates 43° 38′ 58″ N 6° 30′ W). After collection, the coral was aseptically transferred to a sterile plastic bag, washed with

1 The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain M-201^T is HG965210.

Two supplementary figures are available with the online Supplementary Material.

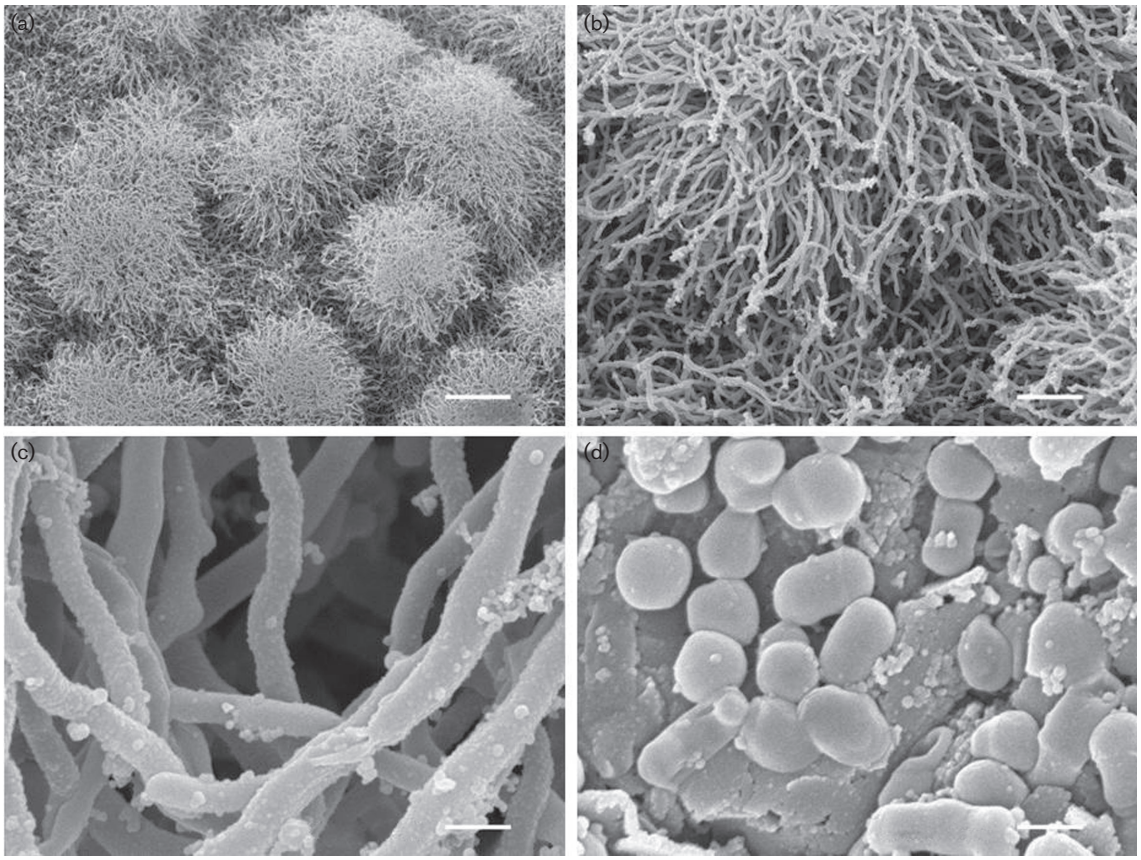

Fig. 1. Scanning electron micrographs of strain M-201^T showing the well-developed mycelium at different magnifications (a, ×750; b, ×3000; c, ×30000) and the spore morphology at ×30000 magnification (d). Bars, 20 μm (a), 5 μm (b), 0.5 μm (c, d).

sterile marine water, and immediately processed in the onboard laboratory as described previously (Braña *et al.*, 2015).

Strain M-201^T (=CECT 8512^T=DSM 28392^T) was isolated on selective media, comprising 1/3 tryptic soy broth (TSA, Merck) and 1/6 M-BLEB [9 g MOPS BLEB base (Oxoid) in 1 l Cantabrian Sea water] agar, containing the antifungal cycloheximide (80 μg ml^{-1}) and anti-Gram-negative bacteria nalidixic acid (20 μg ml^{-1}) under aerobic conditions (Braña *et al.*, 2015). The strain was maintained on TSA medium at 4 °C and as suspensions of mycelial fragments and spores in glycerol (20 %, v/v) at −20 °C and −80 °C. Biomass for chemical and molecular studies was obtained by cultivation using TSA at 28 °C for 1 week. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the organism was most closely related to *Myceligenerans crystallogenes* CD12E2-27^T (98.2 %). Therefore a taxonomic study using a polyphasic approach was carried out to establish the taxonomic position of the novel isolate.

Cultural characteristics were determined after incubation on TSA for 2–3 weeks at 28 °C. Cell morphology and cell dimensions were examined by light microscopy (model CX41; Olympus) after growth for 7 days on TSA medium. Scanning electron microscopy was performed with cells from old cultures grown in R5A (Fernández *et al.*, 1998) as previously described (Cui *et al.*, 2004; Rheims *et al.*, 1998) using a JSM-6610LV (JEOL) scanning electron microscope.

Growth was tested at 4, 10, 15, 20, 25, 28, 37, 40 and 50 °C on TSA. For NaCl tolerance experiments, TSA was used as the basal medium, and the NaCl concentration range used was 0.5–18.0 %, at intervals of 2.5 %. The pH range for growth was investigated between pH 4.0 and 11.0 at intervals of 1 pH unit, using the following buffer systems: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH_2PO_4/0.1 M NaOH; pH 9.0–11.0, 0.1 M $NaHCO_3$/0.1 M Na_2CO_3 (Wang *et al.*, 2011). *Myceligenerans xiligouense* DSM 15700^T, *Myceligenerans crystallogenes* DSM 17134^T, *Myceligenerans halotolerans* DSM 21949^T, and '*Myceligenerans salitolerans*' KCTC 29128, were used as reference strains for phenotypic tests. Metabolic properties and enzyme activities were determined by means of the API 50 CHB and API ZYM systems (bioMérieux) according to the manufacturer's instructions. Oxidation of 95 different substrates was tested using GP2 microplates (Biolog) within the density range specified by the manufacturer (20 % turbidity). Susceptibility to antibiotics was examined by placing antibiotic discs (Oxoid)

Table 1. Differential phenotypic characteristics between strain M-201^T and its closest relatives in the genus *Myceligenerans*

Strains: 1, M-201^T; 2, *Myceligenerans xiligouense* DSM 15700^T; 3, *Myceligenerans crystallogenes* DSM 17134^T; 4, *Myceligenerans halotolerans* DSM 21949^T; 5, '*M. salitolerans*' KCTC 29128. All data are from this study, except for strain 5, which were taken from Guan et al. (2013). All strains are positive in tests for the decomposition of aesculin. All strains are negative for activity of α-mannosidase and α-fucosidase. Strain M-201^T and all strains of species of the genus *Myceligenerans* with validly published names are susceptible to vancomycin (5 μg), cefamandol (30 μg), gatifloxacin (5 μg), chloramphenicol (50 μg), erythromycin (15 μg), doripenem (10 μg), streptomycin (10 μg) and clarithromycin (5 μg), but resistant to nystatin (100 U) and trimethoprim (1.25 μg). +, Positive; −, negative; (+), weakly positive; ND, no data available.

Characteristic	1	2	3	4	5
Pigmentation	Yellow	Yellow	White to cream	White to cream	ND
NaCl tolerance (w/v, %)	0.5–10.5	0.5–8.0*	0.5–3.0*	0.5–5.5*	0–16
Temperature range for growth(°C)	4–37	4–37*	4–37*	10–37*	15–40
pH range for growth	6–9	5–11	6–8	6–8	5–10
Enzyme assay (API ZYM)					
Alkaline phosphatase	−	+	+	−	(+)
Leucine arylamidase	+	+	+	−	+
Cystine arylamidase	−	+	+	−	+
Acid phosphatase	−	+	+	−	(+)
α-Galactosidase	−	+	+	−	+
β-Galactosidase	−	+	+	−	+
N-Acetyl-β-glucosaminidase	−	+	+	−	+
Trypsin	−	+	+	−	(+)
α-Glucosidase	+	+	+	+	−
β-Glucosidase	+	+	+	+	−
α-Chymotrypsin	−	−	−	−	+
β-Glucuronidase	−	−	−	−	+
Decomposition of:					
Tween 80	(+)	+	+	−	+
Starch	−	+	+	+	+
Antibiotic susceptibility					
Ampicillin (10 μg)	+	−	−*	+	−
Penicillin (10U)	+	−	+	+	−
Antibiotic production	+	−	+	−	ND

*Differences with data from Wang *et al.* (2011).

on soft agar plates that were seeded with suspensions of the test strains grown for 3 days at 28 °C. Antibiotic production was determined by bioassay against the following micro-organisms as indicators: the Gram-positive bacteria *Micrococcus luteus*, the Gram-negative *Escherichia coli* ESS, and the yeast *Saccharomyces cerevisiae*. The bioassays were carried out in 1/2-strength TSA or 1/2-strength Sabouraud (Difco), against bacteria and yeast, respectively. These analyses were performed with ethyl acetate extracts under acid conditions (1 % formic acid) from solid cultures. Oxygen requirements were studied with the Gaspack AnaeroGen incubation system (Oxoid) according to the manufacturer's instruction.

The peptidoglycan was isolated and its structure was determined as well as the cell-wall sugars according to published protocols (Schleifer & Kandler, 1972; Schumann, 2011). Analysis of respiratory quinones and polar lipids were carried out by the Identification Service of the Leibniz Institute Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany using previously described procedures for extraction and examination (Tindall, 1990a, b; Tindall *et al.*, 2007). Cellular fatty acids analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI).

For sequence analysis of the 16S rRNA gene, extraction of genomic DNA, PCR-mediated amplification of 16S rRNA gene, and sequencing of the amplified DNA fragment were carried out as described previously (Arahal *et al.*, 2008; Lucena *et al.*, 2010). The nucleotide sequence of strain M-201^T was compared in databases using the BLAST program (Basic Local Alignment Search Tool) (Zhang *et al.*, 2000) against the National Center for Biotechnology Information (NCBI) and submitted to the EMBL nucleotide database. Phylogenetic analysis was performed using MEGA software version 6.0 (Tamura *et al.*, 2013) after multiple alignment of data by CLUSTAL O (Sievers *et al.*, 2011). Distances (distance options according to Kimura's two-parameter model; Kimura, 1980) and clustering with the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods were determined using bootstrap values

Table 2. Results of the Biolog test for strain M-201^T and close relatives in the genus *Myceligenerans* (GP2 MicroPlates, 24 h incubation)

Strains: 1, M-201^T; 2, *Myceligenerans xiligouense* DSM 15700^T; 3, *Myceligenerans crystallogenes* DSM 17134^T; 4, *Myceligenerans halotolerans* DSM 21949^T. Data for reference strains from Wang *et al.* (2011) and Groth *et al.* (2006). All strains are positive for the utilization of dextrin, D-mannose, D-xylose and pyruvic acid. All strains are negative for utilization of β-cyclodextrin, mannan, *N*-acetyl-D-mannosamine, L-fucose, D-galacturonic acid, methyl β-D-galactoside, methyl α-D-mannoside, D-psicose, raffinose, xylitol, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, D-malic acid, L-malic acid, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl L-glutamic acid, putrescine, adenosine 5′-monophosphate, glucose 1-phosphate and glucose 6-phosphate. −, Negative; +, positive; (+), weakly positive; V, variable.

Characteristic	1	2	3	4
α-Cyclodextrin	−	−	−	V
Glycogen	−	+	−	−
Inulin	−	−	−	V
Tween 40	(+)	+	+	+
Tween 80	(+)	+	+	−
N-Acetyl-D-glucosamine	−	−	−	V
Amygdalin	+	+	V	V
L-Arabinose	−	+	V	V
D-Arabitol	−	V	−	V
Arbutin	+	+	−	+
Cellobiose	(+)	+	+	V
D-Fructose	(+)	+	V	+
D-Galactose	(+)	+	V	V
Gentiobiose	(+)	+	V	+
D-Gluconic acid	−	−	−	+
α-D-Glucose	+	+	V	+
myo-Inositol	−	−	−	+
α-Lactose	−	+	V	+
Lactulose	−	−	−	+
Maltose	(+)	+	V	V
Maltotriose	(+)	+	+	+
D-Mannitol	−	−	−	+
Melezitose	−	−	−	V
Melibiose	−	−	−	+
α-Methyl D-galactoside	−	+	−	+
3-Methyl glucose	−	V	−	V
Methyl α-D-glucoside	−	V	−	V
Methyl β-D-glucoside	−	−	−	+
Palatinose	−	+	V	+
L-Rhamnose	−	−	−	+
D-Ribose	−	V	V	+
Salicin	+	+	V	+
Sedoheptulosan	−	−	−	+
D-Sorbitol	−	+	−	+
Stachyose	−	−	−	V
Sucrose	−	+	V	−
D-Tagatose	−	V	−	+
Trehalose	+	+	V	+
Turanose	(+)	+	V	+

Table 2. cont.

Characteristic	1	2	3	4
Acetic acid	−	V	V	−
α-Hydroxybutyric acid	−	V	−	−
β-Hydroxybutyric acid	−	V	−	−
α-Ketovaleric acid	−	V	+	−
Lactamide	−	−	−	+
D-Lactic acid methyl ester	−	−	−	V
L-Lactic acid	−	+	−	−
Methyl pyruvate	−	+	+	−
Monomethyl succinate	−	V	V	V
Propionic acid	−	V	−	+
Succinamic acid	−	−	−	+
Succinic acid	−	−	−	V
N-Acetyl-L-glutamic acid	−	−	−	V
L-Alaninamide	−	+	−	V
L-Pyroglutamic acid	−	−	−	V
L-Serine	−	−	−	V
2,3-Butanediol	−	−	−	V
Glycerol	+	+	+	V
Adenosine	+	+	V	+
2′-Deoxyadenosine	−	+	V	−
Inosine	(+)	+	V	+
Thymidine	(+)	+	+	+
Uridine	(+)	+	−	V
Thymidine 5′-monophosphate	−	V	−	−
Uridine 5′-monophosphate	−	−	−	+
Fructose 6-phosphate	−	−	−	V
DL-α-Glycerol phosphate	−	−	−	V

based on 1000 replications (Felsenstein, 1985). For the determination of the DNA G+C content and DNA–DNA hybridization, DNA isolation was performed as described by Cashion *et al.* (1977). The DNA G+C content was analysed by reversed-phase HPLC as described previously (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984). DNA–DNA relatedness values were determined according to the optical renaturation method (De Ley *et al.*, 1970; Huss *et al.*, 1983).

Colonies of the isolate under study were yellow on TSA, R5A and M-BLEB, and irregular, with diameters of about 2–4 mm. Colony morphology changed significantly depending on the media and culture conditions (Fig. S1, available in the online Supplementary Material). Scanning electron micrographs are shown in Fig. 1. A well-developed mycelium (about 0.35 μm in diameter) (Fig. 1a, b, c) and abundant spores of similar diameter (Fig. 1d) were observed. The morphological features of strain M-201^T were consistent with those of recognized members of the genus *Myceligenerans*.

The organism could be distinguished from recognized members of the genus *Myceligenerans* using phenotypic properties (Table 1). In the API ZYM system, leucine arylamidase, α-glucosidase and β-glucosidase were positive, but esterase (C4), esterase lipase (C8), alkaline phosphatase, lipase (C14), valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase,

N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase were negative. Acid was produced from aesculin, but not from all other carbon sources (API 50 CHB). Oxidation results for 95 different substrates (Biolog GP2 MicroPlates) are listed in Table 2.

Strain M-201^T contained glucose, mannose and galactose as the major cell-wall sugars. The peptidoglycan of strain M-201^T contained the amino acids Lys, Ala, Thr, Glu and one unknown amino acid component (in a molar ratio of 1.0 : 1.6 : 0.6 : 1.0 : 0.9 for L-Lys : D-Glu : L-Thr : L-Ala : D-Ala) and belonged to type A4α: L-Lys–L-Thr–D-Glu [variation A11.57, according to Schumann (2011)]. The partial hydrolysate contains the peptides L-Ala–D-Glu, L-Lys–L-Thr, D-Ala–L-Lys–L-Thr and L-Lys–D-Ala. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, an unknown phosphoglycolipid and seven unknown glycolipids (Fig. S2). The predominant menaquinones were MK-9(H_4) (44 %) and MK-9(H_6) (39 %), and minor amounts of MK-9(H_8) and MK-9(H_2) were detected. Strain M-201^T had a cellular fatty acid profile that contained major amounts of branched fatty acids. The major fatty acids ~~were anteiso-$C_{15:0}$ (52.0 %), iso-$C_{15:0}$ (27.0 %), anteiso-$C_{17:0}$ (9.2 %) and iso-$C_{16:0}$ (4.6 %).~~ The chemotaxonomic data for strain M-201^T were consistent with its assignment to the genus *Myceligenerans*.

The results of 16S rRNA gene sequence comparisons clearly demonstrated that strain M-201^T belonged to the genus *Myceligenerans*. The relationship between the novel strain and their nearest phylogenetic relatives is shown in Fig. 2. The 16S rRNA gene sequence similarities between strain M-201^T and the type strains of *Myceligenerans crystallogenes* and *Myceligenerans xiligouense* were 98.2 and 97.8 % respectively. The G+C content of the DNA was 72.4 mol%. According to DNA–DNA hybridization experiments, strain M-201^T showed a DNA–DNA relatedness value of 48±2 % to *Myceligenerans crystallogenes* DSM 17134^T. This value was well-below the 70 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne *et al.*, 1987).

The isolate showed antibiotic activity against *E. coli*, *Micrococcus luteus* and *S. cerevisiae* when cultivated in M-BLEB. Within the reference strains, only *Myceligenerans crystallogenes* DSM 17134^T had similar results against *E. coli*, in the same media. To the best of our knowledge, this is the first report of antibiotic production in the genus *Myceligenerans*.

Therefore, on the basis of phenotypic, metabolic, phylogenetic and ecological differences, strain M-201^T (=CECT 8512^T=DSM 28392^T) should be classified as representing a novel species of the genus *Myceligenerans*, for which the name *Myceligenerans cantabricum* sp. nov. is proposed.

Description of *Myceligenerans cantabricum* sp. nov.

Myceligenerans cantabricum (can.ta′bri.cum. N.L. neut. adj. *cantabricum* pertaining to the Cantabrian Sea in the North Spain, where the type strain was isolated).

Aerobic, Gram-positive, mycelium- and spore-forming organism. Mycelia are well-developed. Colonies are yellow on R5A, M-BLEB and TSA media after 7 days incubation at 28 °C. In its natural ecosystem the organism lives in an

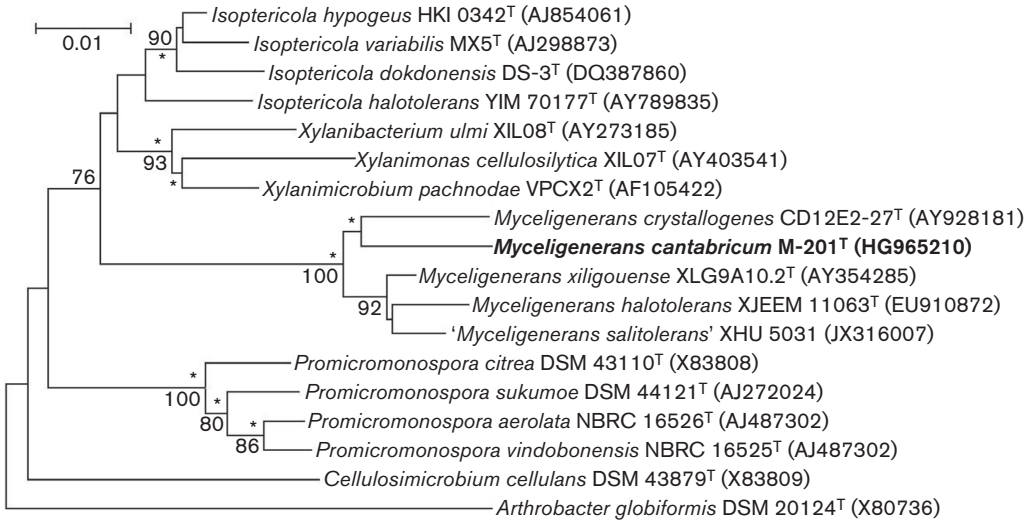

Fig. 2. Neighbour-joining phylogenetic tree obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain M-201^T and its most closely related phylogenetic neighbours. Numbers on branch nodes are bootstrap values (1000 resamplings; only values >70 % are given). The sequence of *Arthrobacter globiformis* DSM 20124^T (GenBank accession no. X80736) was used as an outgroup. Asterisks indicate that the corresponding nodes were also recovered in the maximum-likelihood tree. Bar, 1 % sequence divergence.

extreme habitat at 1500 m depth, so can grow at 150 atm of hydrostatic pressure and at 4–5 °C. Grows between 4 and 37 °C (optimum 28 °C) and at pH 6–9. NaCl in the culture medium is well-tolerated up to 10.5 %. Physiological characteristics (utilization of carbohydrates, enzymic activities, susceptibility to antibiotics and antibiotic production) are listed in Tables 1 and 2. In the API ZYM system, leucine arylamidase, α-glucosidase and β-glucosidase are positive, but esterase (C4), esterase lipase (C8), alkaline phosphatase, lipase (C14), valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-βglucosaminidase, α-mannosidase and α-fucosidase are negative. Acid is produced from aesculin, but not from all other carbon sources (API 50 CHB). Oxidation results for 95 different substrates (Biolog GP2) are listed in Table 2. The peptidoglycan contains the amino acids Lys, Ala, Thr, Glu and one unknown amino acid component, and belongs to type A4α, and the whole-cell hydrolysates contain glucose, mannose and galactose. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, an unknown phosphoglycolipid and seven unknown glycolipids. The predominant menaquinones are MK-9(H_4) and MK-9(H_6). Major cellular fatty acids are anteiso-$C_{15:0}$, iso-$C_{15:0}$ and anteiso-$C_{17:0}$.

The type strain is M-201^T (=CECT 8512^T=DSM 28392^T), which was isolated from a deep-sea coral (*O. Scleractinia, Fam. Caryophillidae*) at 1500 m depth in the sub-marine Avilés Canyon (Cantabrian Sea, Asturias, Spain). The genomic DNA G+C content of the type strain is 72.4 mol%.

Acknowledgements

This study was financially supported by Gobierno del Principado de Asturias (SV-PA-13-ECOEMP-62), Ministerio de Economía y Competitividad, Proyecto DOSMARES/BIOCANT (MICINN-10-CTM2010-21810-C03-02). The authors are grateful to Ricardo Anadón and all other participants in the BIOCANT3 campaign. We also thank José L. Caso and José A. Guijarro for their valuable help and continuous support; M.C. Macián, from the CECT (Colección Española de Cultivos Tipo), for her important contribution to this work and Alfredo J. Quintana for his excellent scanning electron microscopy technical assistance. This is a contribution of the Asturian Marine Observatory.

References

Arahal, D. R., Sánchez, E., Macián, M. C. & Garay, E. (2008). Value of *recN* sequences for species identification and as a phylogenetic marker within the family ''*Leuconostocaceae*''. *Int Microbiol* **11**, 33–39.

8

Braña, A. F., Fiedler, H.-P., Nava, H., González, V., Sarmiento-Vizcaíno, A., Molina, A., Acuña, J. L., García, L. A. & Blanco, G. (2015). Two *Streptomyces* species producing antibiotic, antitumor, and anti-inflammatory compounds are widespread among intertidal macroalgae and deep-sea coral reef invertebrates from the central Cantabrian Sea. *Microb Ecol*.

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.

Cui, X. L., Schumann, P., Stackebrandt, E., Kroppenstedt, R. M., Pukall, R., Xu, L. H., Rohde, M. & Jiang, C. L. (2004). *Myceligenerans xiligouense* gen. nov., sp. nov., a novel hyphae-forming member of the family *Promicromonosporaceae*. *Int J Syst Evol Microbiol* **54**, 1287–1293.

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

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.

Fernández, E., Weissbach, U., Sánchez Reillo, C., Braña, A. F., Méndez, C., Rohr, J. & Salas, J. A. (1998). Identification of two genes from *Streptomyces argillaceus* encoding glycosyltransferases involved in transfer of a disaccharide during biosynthesis of the antitumor drug mithramycin. *J Bacteriol* **180**, 4929–4937.

Groth, I., Schumann, P., Schütze, B., Gonzalez, J. M., Laiz, L., Suihko, M. L. & Stackebrandt, E. (2006). *Myceligenerans crystallogenes* sp. nov., isolated from Roman catacombs. *Int J Syst Evol Microbiol* **56**, 283–287.

Guan, T. W., Wu, N., Tang, S. K., Che, Z. M. & Chen, X. G. (2013). *Myceligenerans salitolerans* sp. nov., a halotolerant actinomycete isolated from a salt lake in Xinjiang, China. *Extremophiles* **17**, 147–152.

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.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Lucena, T., Pascual, J., Garay, E., Arahal, D. R., Macián, M. C. & Pujalte, M. J. (2010). *Haliea mediterranea* sp. nov., a marine gammaproteobacterium. *Int J Syst Evol Microbiol* **60**, 1844–1848.

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.

Rheims, H., Schumann, P., Rohde, M. & Stackebrandt, E. (1998). *Verrucosispora gifhornensis* gen. nov., sp. nov., a new member of the actinobacterial family *Micromonosporaceae*. *Int J Syst Bacteriol* **48**, 1119–1127.

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

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 16.

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

Schumann, P. (2011). Peptidoglycan structure. *Methods Microbiol* **38**, 101–129.

Sievers, F., Wilm, A., Dineen, D. G., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M. & other authors (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using CLUSTAL Omega. *Mol Syst Biol* **7**, 539.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* **30**, 2725–2729.

Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Tindall, B. J., Sikorski, J., Smibert, R. M. & Kreig, N. R. (2007). Phenotypic characterization and the principles of comparative systematics. In *Methods for General and Molecular Microbiology*, 3rd edn, pp. 330–393. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. Marzluf, T. M. Schmidt & L. R. Snyder. Washington, DC: American Society for Microbiology.

Wang, Y., Tang, S. K., Li, Z., Lou, K., Mao, P. H., Jin, X., Klenk, H. P., Zhang, L. X. & Li, W. J. (2011). *Myceligenerans halotolerans* sp. nov., an actinomycete isolated from a salt lake, and emended description of the genus *Myceligenerans*. *Int J Syst Evol Microbiol* **61**, 974–978.

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. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on the reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *J Comput Biol* **7**, 203–214.

Dear Authors,

Please find enclosed a proof of your article for checking.

When reading through your proof, please check carefully authors' names, scientific data, data in tables, any mathematics and the accuracy of references. Please do not make any unnecessary changes at this stage. All necessary corrections should be marked on the proof at the place where the correction is to be made; please mark up the correction in the PDF and return it to us (see instructions on marking proofs in Adobe Reader).

Any queries that have arisen during preparation of your paper for publication are listed below and indicated on the proof.

Please provide your answers when returning your proof.

Please return your proof by email (sgmprod@charlesworth-group.com) within 2 days of receipt of this message.

Query no.	Query
1	A sequence data footnote has been added with the details for the 16S rRNA gene sequence of strain M-201T because it is journal style that any new sequence data determined in this study are listed here. Please check and confirm ok.
2	Geographical information has been removed from the article title as this is preferred journal style. Please check and confirm ok.
3	In the paragraph detailing the selective media, the abbreviation for tryptic soy broth is usually TSB rather than TSA (used for tryptic soy agar). Please check and amend if necessary.
4	The genus name Myceligenerans has been written in full in species names because there is another genus name in the paper with the same initial letter and this is journal style in such cases. Please check and confirm ok.
5	Please add strain numbers for the micro-organisms used in antibiotic production assays.
6	Please check the edits to the sentence listing media for antibiotic production bioassays, and confirm ok or amend if necessary.
7	It is preferred journal style that 'Gram-stain-positive' or Gram-reaction-positive' is used to describe species rather than 'Gram-positive'. Please amend in species description, depending on the method used in this study.
8	Please check reference Brana et al., 2015. Is 2015 the correct year, the DOI links to a 2014 article? If it is for an in press paper, please add '(in press)' after journal title. If volume and page numbers are available, please update accordingly.
9	In Figure 2, superscript T to indicate type strain were missing from all strains. Copyeditor has asked typesetters to add this to all strains except 'M. salitolerans' XHU 5031. Please check and ensure the superscript T have been added correctly.
10	In the legend to Table 2, please indicate which data are from which reference.

Ordering reprints for SGM journals

As a result of declining reprint orders and feedback from many authors who tell us they have no use for reprints, **SGM no longer provides free reprints to corresponding authors**; instead, corresponding authors will receive two emails:

i) An email including a link to download the published PDF of their paper. You can forward this link to co-authors or others, and they can also use it to download the published PDF. The link can be used up to 25 times. This email will be sent out at around the time your article is published online.

ii) An email including a link to the SGM Reprint Service. You can forward this email to your co-authors if you wish, so that they can order their own reprints directly, or to your finance or purchasing department, if orders are placed centrally. This email will be sent out at around the time that your article is finalized for printing.

When you click on the link in this second email, you will be taken to an order page to place your reprint order. Like most online ordering sites, it is necessary to set up an account and provide a delivery address while placing your order, if you do not already have an account. Once an account and delivery address have been set up, these details will be stored by the system for use with future orders. Payments can be made by credit card, PayPal or purchase order.

As reprint orders are despatched by courier, there is a charge for postage and packing.

SUMMARY

- You can create or update your reprint account at any time at http://sgm-reprints.charlesworth.com/
- You will be sent an email when the reprints of this paper are ready for ordering
- **You cannot order reprints of this paper before this email has been sent**, as your paper will not be in the system
- Reprints can be ordered at any time after publication
- You will also receive an email with a link to download the PDF of your published paper

The reprint ordering details will be emailed to the author listed as the corresponding author on the journal's manuscript submission system. If your paper has been published (the final version, not the publish-ahead-of-print version) but you have not received this notification, email reprints@sgm.ac.uk quoting the journal, paper number and publication details.

If you have any questions or comments about the reprint-ordering system or about the link to your published paper, email reprints@sgm.ac.uk