Promicromonospora flava sp. nov., isolated from sediment of the Baltic Sea

Yi Jiang,[1,2] Jutta Wiese,[1] Yan-Ru Cao,[2] Li-Hua Xu,[2] Johannes F. Imhoff[1] and Cheng-Lin Jiang[2]

[1]Leibniz-Institut für Meereswissenschaften, IFM-GEOMAR, Düsternbrooker Weg 20, D-24105 Kiel, Germany

[2]Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China

Correspondence
Cheng-Lin Jiang
lihxu@ynu.edu.cn
Johannes F. Imhoff
jimhoff@ifm-geomar.de

A Gram-positive, non-spore-forming actinomycete, designated strain CC 0387^T, was isolated from a sediment sample from the Baltic Sea, Germany. Classification using a polyphasic approach and comparative 16S rRNA gene sequencing showed that strain CC 0387^T belonged to the genus *Promicromonospora* and displayed more than 3 % 16S rRNA gene sequence divergence from all *Promicromonospora* species with validly published names. Strain CC 0387^T did not produce aerial mycelium. Substrate mycelia were yellowish white to pale orange-yellow and fragmented into bacillary or coccoid elements. The cell wall contained lysine and alanine. Whole-cell hydrolysates contained galactose, glucose, rhamnose and ribose. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylglycerol, an unknown phospholipid and an unknown glycolipid. The predominant menaquinone was MK-9(H_4) (86 %). The G+C content of the DNA was 71.87 mol%. Based on morphological, chemotaxonomic, phenotypic and genetic characteristics, strain CC 0387^T (=CCTCC AA208024^T=DSM 21481^T) represents a novel species, for which the name *Promicromonospora flava* sp. nov. is proposed.

The genus *Promicromonospora* was proposed by Krasil'nikov *et al.* (1961). Members of the genus are characterized by the production of substrate mycelia that fragment into bacillary or coccoid elements, no branched fatty acids, MK-9(H_4) as the major menaquinone and a DNA G+C content of 70–75 mol%. The genus comprises the recognized species *Promicromonospora aerolata* (Busse *et al.* 2003), *P. citrea* (Krasil'nikov *et al.* 1961), *P. sukumoe* (Takahashi *et al.* 1987), *P. vindobonensis* (Busse *et al.* 2003) and *P. kroppenstedtii* (Alonso-Vega *et al.* 2008). As part of a research programme on actinomycete diversity in the Baltic Sea, Germany, taxonomic and comparative studies using a polyphasic approach were carried out with strain CC 0387^T and related species and genera. A novel species of the genus *Promicromonospora* is proposed.

Strain CC 0387^T was isolated from a sediment sample collected from the Baltic Sea, Germany, on fucose-proline medium made with Baltic Sea water [containing (l^{-1}): 5 g fucose, 1 g proline, 1 g $(NH_4)_2SO_4$, 1 g NaCl, 2 g $CaCl_2$, 1 g K_2HPO_4, 1 g $MgSO_4.7H_2O$, 20 g agar; pH 7.2] with 20 mg nalidixic acid and 100 mg nystatin as inhibitors of bacteria and fungi, after incubation at 28 °C for 21 days. The strain was maintained on medium YIM 83 (Difco marine broth 37 g, peptone 2 g) at 28 °C for 5 days.

Mycelial morphology was observed by using two models of light microscopy (Olympus microscope BH-2 and LY-SUPER HP CCD IMAGING) after incubation at 28 °C for 3 and 7 days, respectively. Cultural characteristics were determined after growth on six media, ISP (International *Streptomyces* project) 2, ISP 4, ISP 5 (Shirling & Gottlieb, 1966) Czapek's agar and nutrient agar for 14 days at 28 °C. The results were recorded following incubation at 28 °C for 14 days. Colours and hues were determined according to Kelly (1964). Strain CC 0387^T was examined for a range of phenotypic properties using standard procedures (Williams *et al.*, 1983). Enzyme activities were determined by using API ZYM system test kits (bioMérieux).

Biomass for the molecular systematic and most of the chemotaxonomic studies was obtained after incubation of cultures in yeast extract-malt extract broth (ISP 2; Shirling & Gottlieb, 1966) supplemented with the vitamin mixture of HV medium (Hayakawa & Nonomura, 1987) and 20 g NaCl l^{-1} at 28 °C for 5 days with shaking. Cell walls were purified and the amino acids of peptidoglycan were analysed by TLC (Lechevalier & Lechevalier, 1980; Jiang *et al.*, 2001). Analysis of whole-cell sugar composition followed procedures described by Becker *et al.* (1965) and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC 0387^T is AM992980.

A table showing the chemotaxonomic characteristics of strain CC 0387^T and other species of the genus *Promicromonospora* is available as supplementary material with the online version of this paper.

Lechevalier & Lechevalier (1980). Phospholipid analysis was carried out as described by Lechevalier *et al.* (1981). Menaquinones were determined by using the procedure of Collins *et al.* (1977). Biomass for quantitative fatty acid analysis was prepared by scraping colonies from 3 % (w/v) trypticase soy (BBL) plates (1.5 %, w/v, Bacto agar; Difco) after incubation at 28 °C for 5 days. The fatty acids were extracted, methylated and analysed using the standard Microbial Identification system (Sasser, 1990). The chromosomal DNA of strain CC 0387^T was extracted as described by Marmur (1961). The DNA G+C content was determined by using HPLC (Tamaoka & Komagata, 1984) with an Agilent 1000 LC system (IRIS Technologies). The sequence of the 16S rRNA gene (1612 nucleotides) of strain CC 0387^T was determined. Phylogenetic analysis based on 16S rRNA gene sequences was carried out using the procedures previously described (Jiang *et al.*, 2008).

Strain CC 0387^T was aerobic and Gram-positive. No aerial mycelium was produced on any of the six media tested (ISP 2, ISP 3, ISP 4, ISP 5, Czapek's agar and nutrient agar). Substrate mycelia fragmented into non-motile, coccoid, Y-shaped, V-shaped or curved bacillary elements. No sessile spores or other spore-like elements were observed. Cultures developed weakly on Czapek's agar (Dong & Cai, 2001) but developed well on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5) and nutrient agar (Dong & Cai, 2001). The substrate mycelium on these media was yellowish white to pale orange-yellow.

Physiological and biochemical characteristics, utilization of carbon and nitrogen sources and acid production by strain CC 0387^T are described in Table 1 and the species description. Enzyme activities are shown in Table 1. The cell wall of strain CC 0387^T contained lysine and alanine.

Table 1. Characteristics that differentiate strain CC 0387^T and other species of the genus *Promicromonospora*

Strains: 1, *Promicromonospora flava* sp. nov. CC 0387^T; 2, *P. vindobonensis* V45^T; 3, *P. aerolata* V54A^T; 4, *P. sukumoe* NBRC 14650^T; 5, *P. citrea* NBRC 12397^T; 6, *P. kroppenstedtii* RS16^T (Alonso-Vega *et al.*, 2008). Data are from this study except for column 6. +, Positive; −, negative; W, weak reaction; ND, no data available.

Characteristic	1	2	3	4	5	6
Menaquinones	MK-9(H_4), MK-8(H_4), MK-9(H_2), MK-9	MK-9(H_4), MK-9(H_2), MK-9(H_6)	MK-9(H_4), MK-9(H_2)	MK-9(H_4), MK-9, MK-9(H_2), MK-9(H_6)	MK-9	MK-9(H_4), MK-9(H_6), MK-8(H_4), MK-9(H_2)
Cell-wall composition	Lys : Ala (A3α)	Glu : Gly : Ala : Lys (A3α)	Glu : Gly : Ala : Lys (A3α)	Lys (A3α)	Lys : Ala (A3α)	Ala : Glu : Lys (A4α)
Cell-wall sugars*	Rib, Gal, Glu, Rha	Rha, Gal, Glu	Rha, Gal, Glu	ND	Gal	Gal, Rha
Oxidase	−	+	+	−	−	+
Nitrate reduction	+	+	+	−	+	+
Gelatin hydrolysis	−	−	W	+	+	+
API ZYM assays						
Acid phosphatase	−	+	−	+	+	+
α-Chymotrypsin	−	−	W	−	−	−
Trypsin	−	W	W	+	+	W
Assimilation of:						
D-Arabinose	−	+	−	W	−	−
D-Fructose	−	+	+	+	+	−
Galactose	−	−	W	+	+	−
D-Glucose	+	−	−	+	+	+
Lactose	+	+	+	W	+	+
Maltose	−	+	+	+	+	−
Mannitol	−	−	+	+	+	+
D-Mannose	−	+	+	+	+	−
Raffinose	−	+	−	−	+	−
Rhamnose	−	W	−	−	+	+
Ribose	+	+	+	+	W	+
Sorbitol	−	−	−	W	−	+
L-Sorbose	−	−	−	W	−	+
Starch	+	+	+	+	+	−
Sucrose	+	−	−	+	+	+
Trehalose	+	−	+	+	+	+

*Gal, Galactose; Glu, glucose; Rha, rhamnose; Rib, ribose.

Whole-cell hydrolysates contained ribose, galactose, glucose and rhamnose. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylglycerol, an unknown phospholipid and an unknown glycolipid. The predominant menaquinone was MK-9(H_4) (86 %) with minor amounts of MK-8(H_4) (6.65 %), MK-9(H_0) (2.64 %) and MK-9(H_2) (4.80 %). The genomic DNA G+C content of strain CC 0387^T was 71.87 mol% (HPLC).

Phylogenetic analysis was conducted using 16S rRNA gene sequences of *Promicromonospora* species and representatives of other genera in the family *Promicromonosporaceae*. The database search (BLAST) and neighbour-joining tree showed that strain CC 0387^T belonged to the genus *Promicromonospora* (Krasil'nikov *et al.* 1961) and displayed more than 3 % sequence divergence from all recognized species of the genus *Promicromonospora*: *P. sukumoe* NBRC 14650^T (96.86 % similarity), *P. aerolata* NBRC 16526^T (96.54 %), *P. citrea* DSM 43110^T (96.56 %), *P. vindobonensis* V45^T (96.55 %) and *P. kroppenstedtii* RS16^T (96.56 %) (Fig. 1). The similarities between strain CC 0387^T and each of the five recognized species were lower than the similarities among the five species. The maximum-likelihood tree supported this result (data not shown).

Strain CC 0387^T was isolated on a medium prepared with natural marine water and grew well in media containing 2–5 % (w/v) NaCl. Strain CC 0387^T did not produce acid from any of the 25 carbon sources tested, which distinguishes it from all other species of *Promicromonospora*. Fatty acid components of the five recognized species of the genus *Promicromonospora* and strain CC 0387^T were different, especially in the proportion of several major fatty acids: iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The proportion of anteiso-$C_{15:0}$ in strain CC 0387^T was 57.20 %, whereas it was below 51.1 % in other *Promicromonospora* species, and the proportion of iso-$C_{15:0}$ in strain CC 0387^T was 16.26 % whereas it was more than 30.8 % in the other *Promicromonospora* species (Supplementary Table S1, available in IJSEM Online). To date, 18 genera of actinobacteria, *Aeromicrobium*, *Aureobacterium*, *Corynebacterium*, *Dermacoccus*, *Dietzia*, *Gordonia*, *Kokuria*, '*Marinomyces*', *Micromonospora*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Salinispora*, *Salinibacterium*, *Streptomyces*, *Tsukamurella*, *Verrucosispora* and *Williamsia* have been isolated and characterized from marine samples (Jiang *et al.*, 2007). No member of the genus *Promicromonospora* from marine samples has yet been described. However, on the basis of morphological, chemotaxonomic, phenotypic and genetic characteristics, strain CC 0387^T represents a novel species of the genus *Promicromonospora*, for which the name *Promicromonospora flava* sp. nov. is proposed.

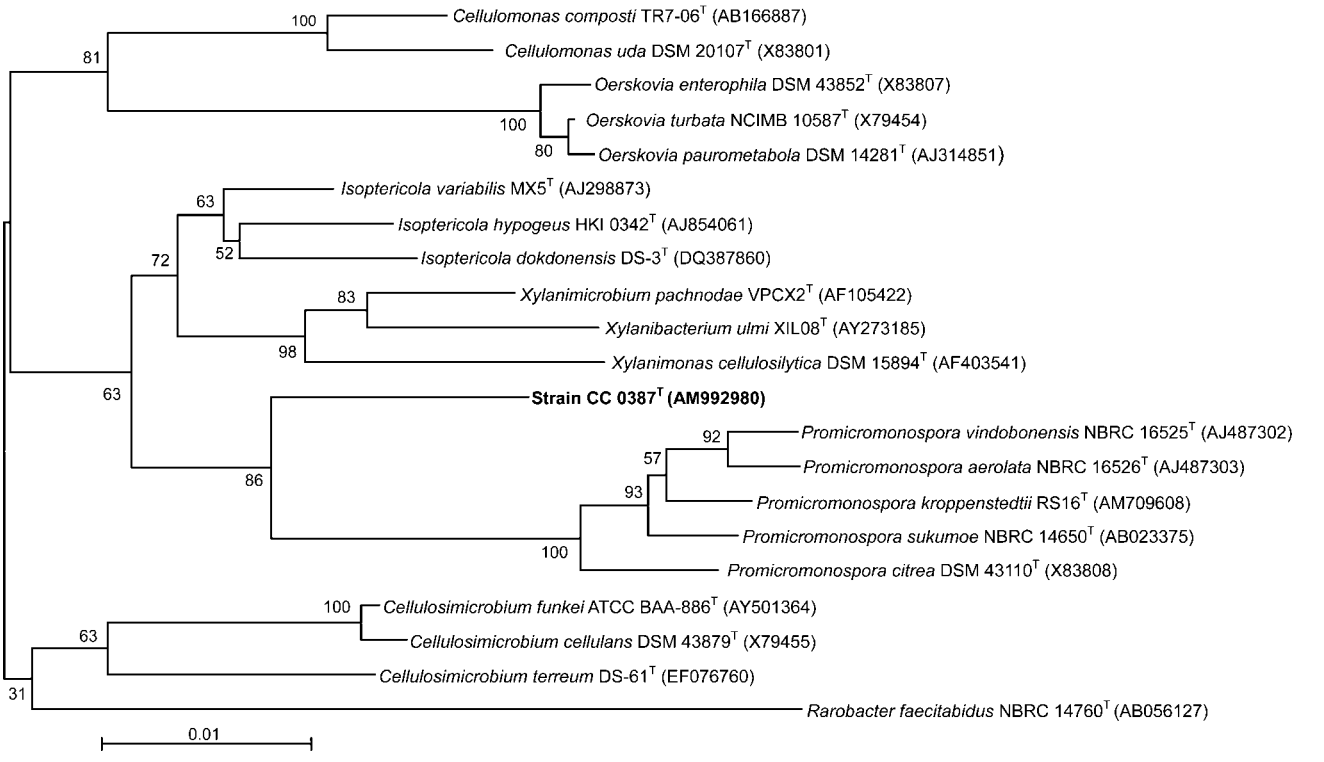

Fig. 1. Neighbour-joining phylogenetic tree derived from 16S rRNA gene sequences showing the relationship of strain CC 0387^T and species of related genera of the family *Promicromonosporaceae*. Numbers at branch nodes are bootstrap percentages (1000 replications). Bar, 1 % sequence divergence.

Description of *Promicromonospora flava* sp. nov.

Promicromonospora flava (fla′va. L. fem. adj. *flava* yellow, being a yellowish organism).

Aerobic, Gram-positive. No aerial mycelium is produced on all six media tested. Substrate mycelia fragment into non-motile, coccoid, Y-shaped, V-shaped or curved bacillary elements. No sessile spores or other spore-like elements are observed. Grows weakly on Czapek's agar but develops well on ISP 2, ISP 3, ISP 4, ISP 5 and nutrient agar. Substrate mycelium on different media is yellowish white to pale orange-yellow. Grows well in media containing 2–5 % (w/v) NaCl. Nitrate reduction is positive. Gelatin liquefaction, milk coagulation and peptonization, starch hydrolysis, growth on cellulose and production of H_2S and melanin are negative. D-Glucose, inositol, lactose, ribose, starch, sucrose, trehalose, xanthine and xylitol are utilized as sole carbon sources with no production of acid. D-Arabinose, dextrin, aesculin, erythritol, D-fructose, galactose, glycine, histidine, lysine, maltose, mannitol, D-mannose, melibiose, raffinose, rhamnose, sodium DL-malate, sorbose, sorbitol, succinic acid and xylose are not utilized. Cell wall contains alanine and lysine. Whole-cell hydrolysates contain galactose, glucose, rhamnose and ribose. Phospholipids consist of diphosphatidylglycerol, phosphatidylglycerol, an unknown phospholipid and an unknown glycolipid. The predominant menaquinone is MK-9(H_4). Major fatty acid components are anteiso-$C_{15:0}$ and iso-$C_{15:0}$. The genomic DNA G+C content of the type strain is 71.87 mol%.

The type strain CC 0387^T (=CCTCC AA208024^T=DSM 21481^T) was isolated from a sediment sample collected from the Baltic Sea, Germany.

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