Minicystis rosea gen. nov., sp. nov., a polyunsaturated fatty acid-rich and steroid-producing soil myxobacterium

Ronald Garcia,1,2,3 Katja Gemperlein1,2 and Rolf Müller1,2,3

1Department of Microbial Natural Products (MINS), Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) – Helmholtz Centre for Infection Research (HZI), Saarland University Campus Building C2.3, 66123 Saarbrücken, Germany
2Department of Pharmaceutical Biotechnology, Saarland University, Building C2.3, 66123 Saarbrücken, Germany
3German Center for Infection Research (DZIF), Partner site Hannover, 38124 Braunschweig, Germany

A bacterial strain designated SBNa008T was isolated from a Philippine soil sample. It exhibited the general characteristics associated with myxobacteria, such as swarming of Gram-negative vegetative rod cells, fruiting body and myxospore formation and predatory behaviour in lysing micro-organisms. The novel strain was characterized as mesophilic, chemoheterotrophic and aerobic. The major fatty acids were C20:4\(\text{cis}\)6,9,12,15 all\(\text{cis}\) arachidonic acid), iso-C15:0, C17:1\(\text{2-OH}\) and iso-C15:0 dimethylacetal. Interestingly, SBNa008T contained diverse fatty acids belonging to the commercially valuable polyunsaturated omega-6 and omega-3 families, and a highly conjugated dihydroxylated C28 steroid. The G+C content of the genomic DNA was 67.3 mol%. The 16S rRNA gene sequence revealed 95–96 % similarity to sequences derived from clones of uncultured bacteria and 94–95 % similarity to cultured members of the suborder Sorangiineae. Phylogenetic analysis revealed that strain SBNa008T formed a novel lineage in the suborder Sorangiineae. Based on a polyphasic taxonomic characterization, we propose that strain SBNa008T represents a novel genus and species, Minicystis rosea gen. nov., sp. nov. The type strain of Minicystis rosea is SBNa008T (=DSM 24000T=NCBB 100349T).

The search for novel myxobacteria is often motivated by their significant potential for exploitation. Myxobacteria are regarded as one of the few outstanding producers of novel secondary metabolites that are still considered an important source for drug discovery. More than 100 basic core structures have already been identified and elucidated from members of this order of bacteria (the Myxococcales), most of which were found to be novel and have a wide spectrum of biological activities (Weissman & Müller, 2010). The past three decades have seen success in the isolation of myxobacteria from diverse terrestrial sources, yielding thousands of isolates that have been classified into different taxonomic groups (Reichenbach, 2005; Dawid, 2000). The search for myxobacteria in marine, estuarine and anoxic conditions has led to the discovery of obligately halophilic, halotolerant and facultatively anaerobic isolates (Iizuka et al., 1998, 2003a, b; 2003b; Fudou et al., 2002; Sanford et al., 2002). These strains have contributed to the increasing diversity and number of myxobacteria isolated to date. Within the past 5 years, several taxa have been isolated successfully and described as representing novel families (Garcia et al., 2009b; Mohr et al., 2012), genera and species (Garcia et al., 2010; Iizuka et al., 2013; Sood et al., 2014). Since novel and unexplored myxobacteria often produce novel secondary metabolites (e.g. aetheramides, Plaza et al., 2012; jähnemannides, Plaza et al., 2013), we focus our strain-discovery programme towards potentially novel, rarely isolated and underexplored myxobacterial taxa. In line with this approach, we isolated strain SBNa008T, which is proposed here to represent a novel genus and species in the suborder Sorangiineae.

Strain SBNa008T was isolated in December 2007 from a dried Philippine soil sample containing decayed plant material taken from the Landsweiler-Reden collection, Saarland, Germany. More recently, several more strains, namely SBM002, SBM003 and SBM004, were isolated from various soils samples around the world. They were...
recognized as myxobacteria through their fruiting body and swarming characteristics on water agar baited with a spot of live *Escherichia coli*. Vegetative rod cells glided coherently on the surface of agar and, after several transfers of the swarm edge to the same medium, the strains were finally purified and isolated. The organisms were routinely cultivated and maintained in buffered VY/2 medium (Garcia et al., 2009a), and stored permanently as vegetative cells at −80 °C with an additional back-up with 20% glycerol as cryoprotectant. All characterizations were based with the first isolated strain SBNa008T. Phenotypic growth stages were studied and documented after cultivation of the strain on VY/2 (Reichenbach & Dworkin, 1992; Shimkets et al., 2006) and buffered VY/2 agars. In addition, the colour of vegetative cells was also recorded after cultivation in TG1 broth (w/v: 0.3% Bacto tryptone, 0.3% glucose, 0.025% CaCl2·2H2O, 0.05% MgSO4·7H2O, 10 mM HEPES; adjusted to pH 7.0 with KOH before autoclaving) and tryptone-starch (TS) broth [w/v: 0.2% Bacto tryptone, 0.2% soluble starch (Roth), 0.025% CaCl2·2H2O, 0.05% MgSO4·7H2O, 10 mM HEPES; adjusted to pH 7.0 with KOH before autoclaving].

Since myxobacteria are known for their growth development phases that culminate in the formation of multicellular fruiting bodies that bear myxospores, and these stages may change or be lost unexpectedly after subcultivation, phenotypic characteristics were documented early after isolation. Swarm and fruiting body stages were studied and photographed using a stereomicroscope (Zeiss Discovery V-20), while myxospores and vegetative cells were observed by phase-contrast microscopy (Zeiss Axiovert 200, Zeiss Axio-Star), photographed using an Axiocam MRC (Zeiss) camera and analysed by using the AxioVision LE software.

Reaction of the vegetative cells to Gram-staining was determined according to the established protocol (Gerhardt et al., 1981), while Congo red staining was performed according to the method described by McCurdy (1969). The catalase test was assessed on tryptone-based agar (w/v: 0.2% Bacto tryptone, 0.05% CaCl2·2H2O, 0.01% MgSO4·7H2O, 25 mM HEPES, 1.5% Bacto agar; adjusted to pH 7.0 with KOH before autoclaving) and a drop of cellulose or chitin powder solution (both from Sigma) on separate sections of the Petri dish. Hydrolysis of skimmed milk (Oxoid), milk casein (Sigma) and soluble starch (Roth) was assessed on tryptone-based agar (w/v: 0.2% Bacto tryptone, 0.05% CaCl2·2H2O, 0.01% MgSO4·7H2O, 25 mM HEPES; adjusted to pH 7.0 with KOH before autoclaving) supplemented with 0.2% (w/v) of each test substrate. Growth was evaluated by clearing of the medium, while the latter test was observed for the presence of a clear halo around the colony after flooding with an iodine solution. Nitrogen, peptone and sugar sources were tested by individual supplementation in 300 ml flasks containing 50 ml minimal medium (MM) (w/v: 0.05% CaCl2·2H2O, 0.01% MgSO4·7H2O, 25 mM HEPES; adjusted to pH 7.0 with KOH before autoclaving), a modification of previously described CM medium (Garcia et al., 2009b). Cultures were shaken at 200 r.p.m. during incubation. Nitrogen supplements were added to a final concentration of 5 mM and 0.2% (w/v) for peptone and sugar sources. The different peptone sources used were tryptone, peptone, neo-peptone, soytone, casitone, phytone, Casamino acids, polypeptone (Niho Seiyaku) and also complex organic sources such as yeast and beef extracts (all from Difco, unless specified). Carbohydrates tested were arabinose (Sigma), cellobiose (MP Biomedicals), fructose (Roth), galactose (Merck), glucose (Sigma), lactose (Fluka), maltose (Sigma), mannitol (Fluka), mannose (AppliChem), rhamnose (Sigma), soluble starch (Roth), sorbitol (Sigma), sucrose (MP Biomedicals), trehalose (Sigma) and xylose (Sigma). NaCl tolerance was assessed in buffered VY/2 agar at 0–3% (w/v), at intervals of 0.5% (w/v). MicrOBial predation was tested using Gram-negative *Escherichia coli* and *Pseudomonas stutzeri*, Gram-positive *Micrococcus luteus*.
and the yeast *Hansenula anomala*. An overnight culture of the test bacterial culture or a 36 h culture of the yeast culture were spotted (20 μl) on water agar and air-dried before inoculation on one side with the myxobacterium. Microbial lysis was evaluated by clearing of the bait organism. All tests described here were incubated for maximum of 10 days at 30 °C, except for temperature tolerance tests. Vegetative inocula came from a 3-day-old culture in TG1 medium that was washed and resuspended in MM. Cells were homogenized and adjusted to 1.0 McFarland (bioMérieux) prior to use as inoculum (50 μl).

Cellular fatty acid extraction was performed by the fatty acid methyl ester (FAME) method (Garcia *et al.*, 2011). A cell pellet from an actively growing culture was obtained from a 300 ml flask containing 50 ml TG1 medium, shaken at 200 r.p.m. and incubated at 30 °C for 5 days. GC-MS analysis and identification of fatty acids, including long-chain polyunsaturated fatty acids (PUFAs), were performed according to described methods and validated with commercially available fatty acid reference standards (Supelco 37 Component FAME Mix and LC-PUFAs; both from Sigma-Aldrich) (Gemperlein *et al.*, 2014). The DNA G+C content was determined by HPLC after nuclease P1 digestion of the genomic DNA (Shimelis & Giese, 2006; Li *et al.*, 2002), in addition to specifically designed primers Na08f (5′-CACACGTGCTACAATGGTCGG-3′) and Na8r (5′-CCTCGGAAATGCTGTGTCCTC-3′), which were also used for sequencing. Purification of the 16S rRNA gene was performed using a NucleoSpin PCR clean-up kit (Macherey-Nagel). Alignment of the 16S rRNA gene sequence of SBNa008T was performed using the BioEdit Sequence Alignment Editor version 7.1.3.0 software. The 16S rRNA gene sequence of SBNa008T was compared with sequences from the GenBank/EMBL/DDBJ databases using BLASTN version 2.2.29 + (Zhang *et al.*, 2000; Morgulis *et al.*, 2008). Other myxobacterial 16S rRNA gene sequences, mostly representing type strains in the suborder *Sorangineae*, were retrieved from GenBank. Sequence alignments were performed using the MUSCLE software (Edgar, 2004). Distance matrices between sequences were calculated using the Jukes–Cantor substitution model (Jukes & Cantor, 1969). A phylogenetic tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987), employing 1000-bootstrap resampling (Felsenstein, 1985), with *Desulfovibrio desulfuricans* MB chosen as the outgroup to root the tree. All these programs are packed in the Geneious Pro 5.6.5 software (Drummond *et al.*, 2010).

Colonies of strain SBNa008T were visible on yeast-based agar media (e.g. VY/2, buffered VY/2) with a film-like and thin appearance with no diffusing pigment or fluorescence. These characteristics differ from the long-vein swarms of strains of *Phaselicytis* and deeply penetrating pseudoplasmodial swarms from members of the *Polyangiumaceae*. Light-pink colonies were observed after 1–2 weeks of incubation under light, a trait that appears common to many myxobacteria. The colour appears to be associated with vegetative cells and becomes evident after scraping off the colony or after centrifugation of the culture broth. In some myxobacterial genera, carotenoids appear to be responsible for the pigmentation (Jensen *et al.*, 1995; Reichenbach & Kleinig, 1971), although it may also be attributed to secondary metabolites in some strains (Ohlendorf *et al.*, 2008; Trowitzsch-Kienast *et al.*, 1993). Unlike other myxobacteria, no radial veins or pseudoplasmodia were found in the colony. The cells swarmed in a circular pattern, but with more or less unstructured colony edges (Fig. 1a, b). In some rare cases, small flare-like patterns were produced at the colony border, which is reminiscent of some members of the *Myxococaceae*. Similar to members of the *Sorangineae* and *Nannocystineae*, SBNa008T and the three related strains penetrated into the agar and produced depressions but not deep agar holes, tunnels or excavations comparable to strains of *Nannocystis*. Fruiting bodies were composed of tiny spherical to ovoid sporangioles (4.0–12.0 μm diameter) and arranged singly or in clusters and mats (Fig. 1c, d). Dense clusters and chains commonly developed on agar surfaces, especially close to the centre of the colony, which could also be observed in other strains (Fig. S1, available in the online Supplementary Material). On water agar containing a streak of live *E. coli*, a thin layer of sporangioles was produced, matted on the lysed bait. Although strains of *Nannocystis* are able to form small fruiting bodies, large sporangioles (40.0 × 110.0 μm) could also be seen, while more or less uniformly tiny (8.0–15.0 mm) sporangioles could be found in *Nannocystis pusilla* (Reichenbach, 2005).

To date, SBNa008T bears the smallest sporangiole of any known myxobacterium, which may why it has gone undetected in past searches. In addition, the fruiting bodies of SBNa008T might in some instances be mistaken for encysted amoebae because of their size, shape and arrangement on the agar surface. The novel bacterium was remarkably different from *Nannocystis* in its ability to form fruiting bodies usually on the surface of a solid medium, while members of the latter genus commonly develop deep into corroded agar (Reichenbach, 2005). Unlike *Polyangium*, *Cystobacter* and *Kofleria*, which have tightly packed, large fruiting bodies held in slime, the novel isolate typically exhibited small sporangioles that were not encased in slime. The absence of a sorus enclosing the sporangioles distinguishes SBNa008T from *Sorangium* and *Byssovorax*.

All the isolates showed vegetative cells that were non-motile, phase-dark, long, slender rods with blunt ends (mean size 1.2–1.3 × 3.0–8.0 μm), although some cells were as long as 26 μm (Fig. 1e). SBNa008T produced intense pigmentation, from rose pink to red, which could be observed around the rim of the flask and in the medium
itself as small flakes or granules in TG1 and TS broth. The myxospores of SBNa008\textsuperscript{T} were desiccation resistant, phase-dark, slender, fat rods, 1.2 to 2.0–3.0 μm, with dark granules at the poles (Fig. 1f), features common for members of the Sorangiineae. Although the genus Nannocystis appears to be the nearest relative in terms of morphology, their myxospores and vegetative cells differ significantly. Strains of Nannocystis are distinct for their almost round myxospores.

**Fig. 1.** Photomicrographs of growth stages of SBNa008\textsuperscript{T}, obtained by stereomicroscopy (a–c) and phase-contrast microscopy (d–f). (a) Swarm pattern, showing a thin swarm colony with no clearly defined structure at the colony edge. Fruiting bodies may also be seen behind the swarm, towards the centre of the colony. (b) Increased magnification photomicrograph of the colony edge, showing the arrangement of swarming cells. (c) Tiny sporangioles commonly arranged in clusters on the agar surface. (d) Slide mount of sporangioles, showing their dense arrangement despite the absence of a sorus to enclose the whole cluster. (e) Slide mount of actively growing vegetative cells scraped from the colony edge in buffered VY/2 agar. (f) Myxospores released from partially pressed sporangioles (encircled by dotted lines). Intact sporangioles are also shown. Bars, 1000 μm (a), 200 μm (b), 500 μm (c), 10 μm (d), 20 μm (e) and 5 μm (f).
and close to cuboidal vegetative cells without clearly distinct dark granules at their poles.

The novel bacterium was negative in Gram and Congo red staining and showed catalase-positive and oxidase-negative reactions. Optimal growth was observed at 30 °C, while minimal growth was observed at 18 and 37 °C, with 37 °C showing the most evident agar depression. SBNa008T exhibited wide pH tolerance, from pH 5.0 to 8.5, but optimal growth, represented by colony diameter, was found at pH 7.0–8.0 on VY/2 agar. No growth was observed at pH 4.0 or beyond pH 9.0. Growth was seen on the surface of the tube appearing as a thin film layer, while no growth was found in an anaerobic jar, which clearly suggests its aerobic nature. Filter paper, cellulose and chitin powder solution were not degraded, indicating a lack of cellulolytic and chitinolytic activities, although skimmed milk, milk casein and soluble starch were hydrolysed. Unlike Nannocystis, the novel isolate commonly exhibited slight depressions on solid media, often found close to the centre of the colony, which indicates agar degradation. The predatory lytic response of the novel isolate against live microbial bait was observed only with Gram-negative E. coli. At later stages of growth, cell aggregation and fruiting bodies developed on the lysed bait. Pseudomonas stutzeri, Micrococcus luteus and Hansenula anomala were not cleared, implying the selectivity of strain SBNa008T to lyse and out-compete them.

Inorganic nitrogen sources such as urea, potassium nitrate and ammonium sulfate did not support growth of the novel myxobacterium. Optimal growth was determined on tryptone, polypeptone and peptone, while moderate growth was observed with neopeptone and soytone. The novel bacterium barely grew with casitone and phytone, and no growth was observed with Casamino acids and ammonium sulfate. Yeast and beef extracts also did not support growth. SBNa008T grew in the presence of all carbohydrates tested, but the most striking growth was observed with soluble starch and cellobiose. Comparable growth could be observed in the presence of all other tested carbohydrates, which supports the results obtained with an API ID32GN kit for sugar utilization (Table S1). Growth was observed only in the absence of NaCl, suggesting that salt is not required for growth. The novel isolate was resistant to ampicillin, neomycin, bacitracin, polymyxin, gentamicin, trimetoprim, spectinomycin and hygromycin, but was sensitive to ampicillin, carbenicillin, kanamycin, oxytetracycline, tetracycline, streptomycin, rifampicin, kasugamycin, fusidic acid, cephalosporin and thiostrepton.

Major fatty acids of SBNa008T present at 10% or more of the total were iso-C15:0, C17:1 2-OH, C20:4ω6,9,12,15 all cis (AA; arachidonic acid) and iso-C15:0 dimethylacetal (DMA) (Table 1). In addition to AA, fatty acids belonging to the omega-3 and omega-6 families were also discovered and identified as C20:5ω3,6,9,12,15 all cis (EPA; eicosapentaenoic acid), C20:3ω6,9,12 all cis (DHGLA; dihomo-γ-linolenic acid) and C18:3ω6,9,12 all cis (GLA; γ-linolenic acid), and trace amounts of C22:4ω6,9,12,15 all cis (DTA; docosatetraenoic acid/adrenic acid) and C20:3ω6,9,12,15 all cis (eicosatrienoic acid) were detected in TG1 medium (Fig. 2). PUFAs appear to be rare in prokaryotes (Nichols et al., 1999; Nichols & McMeekin, 2002): the large amount of PUFAs in SBNa008T, which can represent nearly 30% of the total cellular fatty acids, is uncommon for a terrestrial bacterium. However, the occurrence of these fatty acids in SBNa008T (Table 1) was not surprising, since they have been reported previously in some other novel myxobacteria.

### Table 1. Cellular fatty acid profile of SBNa008T determined after cultivation in TG1 medium in comparison with that of Phasellicystis flava SBKo001T

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>SBNa008T</th>
<th>P. flava SBKo001T</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Straight-chain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>C16:0</td>
<td>0.4</td>
<td>6.0</td>
</tr>
<tr>
<td>C17:0</td>
<td>1.5</td>
<td>5.5</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.4</td>
<td>3.2</td>
</tr>
<tr>
<td>C16:0 2-OH</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>C18:1 2-OH</td>
<td>0.5</td>
<td>1.7</td>
</tr>
<tr>
<td>C17:0 2-OH</td>
<td>-</td>
<td>6.6</td>
</tr>
<tr>
<td>C17:1 2-OH</td>
<td>17.0</td>
<td>25.2</td>
</tr>
<tr>
<td>C18:0 2-OH</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td>C18:1 2-OH</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td>C14:0 DMA</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td>C15:0 DMA</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>C14:0 OAG</td>
<td>-</td>
<td>0.8</td>
</tr>
<tr>
<td>C15:0 OAG</td>
<td>0.8</td>
<td>1.4</td>
</tr>
<tr>
<td>C16:0 OAG</td>
<td>1.0</td>
<td>2.9</td>
</tr>
<tr>
<td>C16:1 OAG</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td><strong>PUFAs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:3ω6,9,12 all cis (GLA)</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>C20:4ω6,9,12,15 all cis (AA)</td>
<td>22.4</td>
<td>12.4</td>
</tr>
<tr>
<td>C20:5ω3,6,9,12,15 all cis (EPA)</td>
<td>6.0</td>
<td>-</td>
</tr>
<tr>
<td>C20:3ω6,9,12 all cis (DHGLA)</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>C22:4ω6,9,12,15 all cis (DTA)</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Total PUFAs</td>
<td>29.4</td>
<td>12.4</td>
</tr>
<tr>
<td>Total straight-chain fatty acids</td>
<td>55.6</td>
<td>68.6</td>
</tr>
<tr>
<td><strong>Branched-chain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C13:0</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>iso-C14:0</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>18.2</td>
<td>25.5</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>2.7</td>
<td>-</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>5.9</td>
<td>3.7</td>
</tr>
<tr>
<td>iso-C17:1 2-OH</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>iso-C17:1 2-OH</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>iso-C14:0 DMA</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>iso-C15:0 DMA</td>
<td>9.7</td>
<td>-</td>
</tr>
<tr>
<td>iso-C15:0 OAG</td>
<td>4.5</td>
<td>-</td>
</tr>
<tr>
<td>anteiso-C17:0</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Total branched-chain fatty acids</td>
<td>44.4</td>
<td>31.3</td>
</tr>
</tbody>
</table>
and some marine genera (Iizuka et al., 2003a, b; Fudou et al., 2002; Garcia et al., 2011). A study on PUFA biosynthesis revealed that myxobacteria synthesize these fatty acids differently from other micro-organisms (Gemperlein et al., 2014), and this could perhaps be a link to the diversity and quantity of PUFA produced. To date, the production of EPA as well as AA has only been observed in a few genera of myxobacteria in the suborders Sorangiineae and Nannocystineae (Garcia et al., 2011; Stadler et al., 2010).

The presence of C_{17:1} 2-OH and the predominance of straight-chain over branched-chain fatty acids supports the clustering of the novel isolate into the suborder Sorangiineae based on a previous study (Garcia et al., 2011). The novel bacterium differs from Phaselicystis flava SBK001\(^{T}\) in 19 fatty acids including one of its major types, iso-C_{15:0} DMA (Table 1). In addition, the amounts of the major fatty acids varied significantly between SBNa008\(^{T}\) and P. flava SBK001\(^{T}\). Anteiso-branched fatty acids have been thought to be associated with marine myxobacteria (Iizuka et al., 2003a, b; Fudou et al., 2002); however, recent findings regarding the myxobacteria have shown that these fatty acids can also be produced by terrestrial strains (Garcia et al., 2011), including the novel myxobacterium SBNa008\(^{T}\).

The steroid produced by this organism was isolated by fermentation and identified to be a novel ergostane derivative, 9\(x\),11\(x\)-dihydroxyergosta-4,6,8(14),22-tetraen-3-one, with cytotoxic activity against human colon adenocarcinoma SW480 cells (Gawas et al., 2011). Unlike eukaryotes, bacteria rarely produce steroids; however, this is not the first report of steroid production in myxobacteria. Steroid production has been described in all suborders, including strains of Nannocystis (Nannocystineae), Polyangium (Sorangiineae), Cystobacter, Angiococcus, Archangium and Stigmatella (Cystobacterineae) (Zeggel, 1993; Kohl et al., 1983; Bode et al., 2003).

**Fig. 2.** GC-MS chromatograms from (a) strain SBNa008\(^{T}\), showing the production of different PUFAs after cultivation in TG1 medium, and (b) a Supelco 37 Component FAME Mix reference standard. Identified PUFAs: 1, \(\gamma\)-linolenic acid methyl ester (GLA; C_{18:3}ω6,9,12 all cis); 2, arachidonic acid methyl ester (AA; C_{20:4}ω6,9,12,15 all cis); 3, eicosapentaenoic acid methyl ester (EPA; C_{20:5}ω3,ω6,ω9,ω12,ω15 all cis); 4, eicosatrienoic acid (C_{20:3}ω6,ω9,ω12 all cis); 5, dihomoeicosatrienoic acid methyl ester (DHGLA; C_{20:3}ω6,ω9,ω12 all cis); 6, docosatetraenoic acid methyl ester (DTA; C_{22:4}ω6,ω9,ω12,ω15 all cis). PUFAs 4 and 6 were confirmed based on fragmentation patterns in comparison with the NIST 08 compound library.
The complete (1550 bp) 16S rRNA gene sequence of strain SBNa008\(^{\top}\) showed 95 % similarity to the sequences of *Byssvorax cruenta* By c2\(^{\top}\) (GenBank accession no. NR_042341) and *P. flava* SBKo001\(^{\top}\) (NR_044523), and 94 % similarity to both *Chondromyces apiculatus* Cm a14\(^{\top}\) (NR_025344) and *Sorangium cellulosum* DSM 14627\(^{\top}\) (NR_044443). The highest similarity (95–97 %) was found among sequences derived from clones of uncultured environmental bacteria (GenBank accession nos EU104167, FJ479473, EU662572 and AM490752). It is worth mentioning that the latter two sequences were obtained from microbial mats of sulfidic cave water samples from two different countries. The sequence represented by GenBank accession no. EU662572 was derived from Movile cave in Romania, while the sequence derived from clones of uncultured environmental bacteria (GenBank accession nos EU104167, FJ479473, EU662572 and AM490752). It is worth mentioning that the latter two sequences were obtained from microbial mats of sulfidic cave water samples from two different countries. The sequence represented by GenBank accession no. EU662572 was derived from Movile cave in Romania, while the sequence represented by GenBank accession no. AM490752 was obtained from a sample collected in Lower Kane Cave, in Wyoming, USA. Strain SBNa008\(^{\top}\) can tolerate pH 5.0, which implies a possible adaptation to acidic conditions, and similar strains could potentially be isolated from such an environment. The two other sequences were derived from samples obtained from activated sludge (New Zealand) and from the upper 5 cm soil layer of undisturbed tall prairie grass vegetation. This suggests that the taxon represented by strain SBNa008\(^{\top}\) may also be found in similar unexplored environments, which are not commonly searched when isolating myxobacteria.

Phylogenetic analysis revealed that SBNa008\(^{\top}\) belongs to the suborder *Sorangiineae* (Fig. 3). This finding agreed with previous studies showing its phylogenetic position in the *Myxococcales* based on the 16S rRNA gene sequence (Garcia et al., 2010). Morphological growth-stage features of strain SBNa008\(^{\top}\) are characterized by rod-shaped vegetative cells and myxospores with blunted ends, supporting the affiliation of the novel strain with the suborder. These characteristics are only known from and appear to be exclusive to the suborder *Sorangiineae*.

Since SBNa008\(^{\top}\) and the three other isolated strains formed a distinct novel cluster but with low bootstrap support (42.1 %) and produced different topology using different treeing methods (Figs S2 and S3), we refrained from designating the family affiliation of the novel taxon at this time. However, taking the neighbour-joining tree into consideration, and correlating the abundance of major fatty acids, including the large amount of AA, the results suggest that strain SBNa008\(^{\top}\) is more closely related to the genus *Phaselicystis* than to any members of the *Polyangiaceae*. To date, no myxobacteria are known to produce as large an

![Desulfovibrio desulfuricans MB (NR_036778)](http://ijs.sgmjournals.org)

**Fig. 3.** Neighbour-joining tree of myxobacteria based on 16S rRNA gene sequences showing the position of the novel isolate SBNa008\(^{\top}\) in the suborder *Sorangiineae*. GenBank accession numbers are shown in parentheses. Values at branch points indicate bootstrap support as percentages based on 1000 resamplings. *Desulfovibrio desulfuricans* MB was used as an outgroup to root the tree. Bar, 0.05 substitutions per nucleotide position.
amount of AA as SBNa008T except for *P. flava* and *San-
daracinus amylolyticus*. In addition, we have never found any members of the *Polyangiaceae* or *Phaseicystidaceae* that
synthesize diverse PUFAs in the way that strain SBNa008T
does. The straight-chain fatty acid C_{16:1}ω9c, a major bio-
marker fatty acid for most members of the *Polyangiaceae*
(Garcia et al., 2010), is absent from both *P. flava* SBKo001T
and SBNa008T, suggesting that these two taxa have dif-
f erent cellular chemical compositions. The high 16S rRNA
gene sequence difference from the closest related strains
(5.6 % from *P. flava* SBKo001T, 5 % from *Byssvorax
cruenta* By c2T) and its position on a novel branch in the
*Sorangiineae* clearly indicate that strain SBNa008T repre-
sents a novel genus and species.

**Description of Minicystis gen. nov.**

*Minicystis* (Mi.ni.cys’tis. L. comp. minor -us less, smaller,
inferior; N.L. n. cystis from Gr. fem. n. kysts the bladder, a
bag; N.L. fem. n. Minicystis small bladder, intended to
mean that the sporangiole size is smaller than those of
*Nannocystis*).

Vegetative cells are long, cylindrical rods with blunted ends
and non-flexuous type. Movement occurs by gliding on the
agar surface. Swarms appear film-like, thin and transparent
and is not stained with Congo red. Colony edges may
appear unstructured, with loose migrating cells sometimes
with tiny flares. Myxospores are non-refractive, phase-
dark, cylindrical, slender rods, shorter than vegetative cells,
and enclosed in a distinct sporangial wall. Fruiting bodies
appear as small, ovoid sporangioles. Lyse bacteria and lack
cellulolytic and chitinolytic activities. Catalase-positive
and oxidase-negative. Overall cellular fatty acid profile is
dominated by straight-chain types. Polysaturated omega-
3 and omega-6 fatty acids are present, with arachidonic acid
and eicosapentaenoic acid representing the major PUFAs
types. Contain hydroxy fatty acid C_{17:1} 2-OH and branched-
chain acids iso-C_{15:0} DMA and iso-C_{15:0} O-alkylglycerol
(OAG). The type species is *Minicystis rosea* sp. nov.

**Description of Minicystis rosea sp. nov.**

*Minicystis rosea* (ro’se.a. L. fem. adj. rosea rose-coloured,
rosy).

Exhibits all the characteristics of the genus, and the
following additional characteristics. Terrestrial soil myx-
obacterium. Vegetative cells have a mean size of 1.2–
1.3 × 3.0–8.0 μm and are phase-dark and pink to reddish.
Swarms are composed of scattered loose cells and produce
shallow agar depressions. Fruiting bodies are composed of
tiny sporangioles, 4.0–12.0 μm in diameter, and often appear
as monolayered clusters (32.0–86.0 × 52.0–193.0 μm) or as
mats on the agar surface. Myxospores are 1.0–1.2 × 3.2–
4.0 μm with rounded ends. Bacteriolytic nutritional type.
Mesophilic, aerobic and grows within a slightly acidic to
slightly alkaline pH range. Growth is inhibited by supple-
mentation with NaCl. Hydrolyses milk casein, skimmed milk
and soluble starch. Good growth in polypeptide, peptone
and tryptone. Soluble starch and cellobiose represent the best
tested carbohydrate sources, whereas trehalose, arabinose,
maltose, rhamnose, xylose, lactose, sucrose, fructose, galac-
tose, glucose, mannitol, mannose and sorbitol provide
satisfactory growth. Can utilize N-acetylgalcosamine, inosi-
tol, itaconic acid, suberic acid, sodium malonate, lactic acid,
l-serine, l-alanine, l-histidine, potassium 2-ketoglu-tarate,
potassium 5-ketoglu-tarate, l-fucose, propionic acid, valeric
acid, trisodium citrate, 3-hydroxybutyric acid and 4-hydro-
xybenzoic acid. Shows positive reactions in the API ZYM kit
for alkaline phosphatase, leucine arylamidase, valine aryla-
idase, cystine arylamidase, trypsin, α-chymotrypsin, acid
phosphatase, naphthol-AS-BI-phosphohydrolase, β-galacto-
sidase and β-glucosidase, weakly positive reactions for C4
esterase, C8 esterase lipase and α-glucosidase and negative
reactions for C14 lipase, α-galactosidase, β-glucuronidase,
N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.
Resistant to apramycin, neomycin, bacitracin, polymyxin,
gentamicin, trimetoprim, spectinomycin and hygromycin.
Sensitive to ampicillin, carbenicillin, kanamycin, oxytetra-
ycline, tetracycline, streptomycin, rifampicin, kasugamycin,
fusidic acid, cephalosporin and thiostrepton. Major cellular
fatty acids are iso-C_{15:0} 3-methylarachidic acid, C_{17:1} 2-OH
and iso-C_{15:0} DMA. Antise-o-type fatty acids are present.
Contains the PUFAs γ-linolenic acid, arachidonic acid,
eicosapentaenoic acid, dihomo-γ-linolenic acid, eicosatrie-
noic acid and docosatetraenoic acid.

The type strain is SBNa008T (=DSM 24000T = NCCB
100349T), isolated in December 2007 from a Philippine soil
taken sample from the Landsweiler-Reden collection,
Germany. The DNA G+C content of the type strain is
67.3 mol%.

**Acknowledgements**

We thank J. P. Euzéby for help with the nomenclature of the strain, Ms Irene Kochems for excellent technical assistance, Dr Kevin Sours for reading of this manuscript and the Landsweiler-Reden collection for generously providing us with sampling materials for the isolation of myxobacteria.

**References**

identification of myxobacterial steroids and cloning of the first bacterial 2,3(S)-oxidosqualene cyclase from the myxobacterium


Drummond, A. J., Ashton, B., Buxton, S., Cheung, M., Heled, J.,
Kearse, M., Moor, R., Stones-Havas, S., Sturrock, S. & other authors


Trowitzsch Kienast, W., Gerth, K., Reichenbach, H. & Höfle, G. (1993). Myxochromid A: ein hochungesättigtes Lipopentadialox aus...

