Saonia flava gen. nov., sp. nov., a marine bacterium of the family Flavobacteriaceae isolated from coastal seawater

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Abstract

A Gram-stain-negative, aerobic, yellow-pigmented, straight rod-shaped bacterium, strain MOLA117T, was isolated from a coastal water sample from the north-western Mediterranean Sea, near Banyuls-sur-Mer, France. On the basis of phylogenetic analysis of the 16S rRNA gene sequence, strain MOLA117T was placed within the family Flavobacteriaceae, but showed less than 93 % 16S rRNA gene sequence similarity to other recognized species within the family. The most closely related genera included Arenibacter, Cellulophaga, Marinibacter and Zobellia. The only isoprenoid quinone was menaquinone MK-6 and the predominant fatty acid was iso-C17:0 3-OH, representing over 33 % of the total fatty acids. The DNA G+C content was 36.9 mol%. Strain MOLA117T required NaCl for growth, and did not exhibit gliding motility or produce flexirubin. Based on the phenotypic and phylogenetic data, strain MOLA117T should be considered to represent a novel species of a new genus, for which the name Saonia flava gen. nov., sp. nov. is proposed. The type strain of Saonia flava is MOLA117T (=CIP 110873T=DSM 29762T).

The marine environment continues to be a major source of uncharacterized micro-organisms with potentially interesting activities. These activities can be of academic interest but can also result in biotechnological applications. At the Oceanological Observatory in Banyuls, we aim to isolate prokaryotes that are environmentally ubiquitous in marine coastal ecosystems to study their biodiversity and function as well as to explore their use in biotechnology. Each isolated strain is added to the Banyuls Bacterial Culture Collection (WDCM911). During such an effort we isolated a Gram-stain-negative, aerobic, yellow-pigmented bacterium, strain MOLA117T, from a water sample taken off the coast of Banyuls-sur-Mer, France. The closest cultured relatives, based on their 16S rRNA gene sequence, were species within the genus Zobellia, and the closest described species was Zobellia uliginosa ATCC 14397T with 92.7 % similarity (blast [1]). Some species within the genera Arenibacter, Cellulophaga and Marinibacter also showed similarity (close to 92 %) to strain MOLA117T. The highest similarities to environmental sequences were 94–93 % to three 16S rRNA gene sequences retrieved from the gut of a wood-feeding gastropod [2]. Thus, we suggest that MOLA117T represents a member of a novel genus within the family Flavobacteriaceae. Our aim was to investigate the exact phylogenetic position and to phenotypically describe strain MOLA117T.

Strain MOLA117T was isolated from a seawater sample collected in February 2004 from 3 m depth at station SOLA located in the bay of Banyuls-sur-Mer (42° 29’ N 3° 08’ E). The isolation and bacterial stock procedures were performed as described previously [3] using modified R2A agar with 75 % filtered Mediterranean seawater. Subsequent growth experiments were realized using marine agar (MA) or marine broth (MB) in the dark at 25 °C. Cellular morphology and size were determined using liquid culture incubated for 4 days, which corresponds to the end of log phase/beginning of stationary phase (data not shown), and a Model H-7500 transmission electron microscope (Hitachi) as previously described [4] (see Fig. S1, available in the online Supplementary Material).

Motility was tested using the hanging drop technique combined with light microscopy (model AX70 microscope; Olympus) with liquid cultures incubated for 4 days. Most of the tests for phenotypic characterization were performed as previously described [3]. Briefly, this included Gram staining using the Ryu KOH reaction [5], determination of oxidase activity using the oxidase reagent kit (bioMérieux) and testing for catalase activity by determination of gas production upon exposure to a 3 % hydrogen peroxide solution. Formation of H2S was tested with Hydrogen Sulfide Test strips (Sigma Aldrich) with cultures grown in MB for

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Abbreviations: EPS, extracellular polymeric substances; MA, marine agar; MB, marine broth; TLC, thin layer chromatography.

The GenBank/EMBL/DDJB accession number of the 16S rRNA gene sequence of strain MOLA117T is AM990891.

Two supplementary figures are available with the online Supplementary Material.
3 days. The requirement for and tolerance of strain MOLA117T to NaCl was determined at 25 °C for 6 days in MB with NaCl concentrations ranging from 0 to 15 % (w/v) (0, 0.5, 1, 2, 3, 4, 5, 6, 7, 10, 15 and 17 %). To verify the optimal and tolerated pH levels for growth, strain MOLA117T was incubated in MB at 25 °C for 6 days amended with different buffers to stabilize the pH as follows: pH 4, 5 and 6 (MES), pH 7 and 8 (HEPES) and pH 9 and 10 (AMPSO). Growth temperatures were determined on MA after 4 days of growth at temperatures ranging from 4 to 44 °C (4, 10, 15, 20, 25, 30, 37 and 44 °C). Antibiotic resistance was determined using antibiotic-impregnated paper discs on MA with a lawn of strain MOLA117T that was incubated at 25 °C for 4 days. Production of flexirubin was evaluated by flooding a 4-day-old plate with 20 % (w/v) KOH (positive for flexirubin if the colour changed from yellow to red, purple or brown). Hydrolysis of starch, aesculin and Tween 80 was tested according to Lányi [6]. Growth on different single carbon sources was verified with media supplemented with 1 % of each carbon source [7]. The carbon sources tested included acetate, arabinose, cellobiose, citrate, dextran, galactose, glucose, glutamic acid, glycerol, formate, fumarate, lactate, lactose, malate, maltose, pyruvate, succinate, sucrose, sorbitol, trehalose and xylene. To determine whether strain MOLA117T was able to degrade cellulose we utilized both filter paper dissolved in agar and filter paper in carbohydrate-free broth [8], as well as CM-cellulose [9].

Additional carbohydrate metabolism was tested using the API 20NE and API 50CH test systems (bioMérieux). Enzymatic activities were investigated using the API ZYM system (bioMérieux). For these tests, the medium utilized had the following composition (per litre): 25 g NaCl, 8 g MgCl₂, 0.5 g KCl and the cultures were incubated at 30 °C for a week in the dark [9].

Biomass for fatty acid analysis of strain MOLA117T was harvested from MA plates grown at 25 °C for 4 days. Biomass for analyses of polar lipids and respiratory quinones was harvested from cells grown in MB at 25 °C for 4 days and cell pellets from 3 litres of culture were lyophilized and sequenced with an AB3130xl genetic analyser (Life Technologies).

Sequence similarities of strain MOLA117T to sequences available in the NCBI GenBank were analysed using the program BLAST [1]. Phylogenetic analysis was performed as previously described [3], using the ARB software package [13] and the RAXML Webserver (http://www.phylo.org) for bootstrap analysis (450 replicates) [14]. This analysis revealed that strain MOLA117T was phylogenetically related to species within the genera Arenibacter and Cellulophaga. However, MOLA117T clearly does not group with either of these genera since the bootstrap values clustering species within the genus Arenibacter together were high, as were those clustering species within the genus Cellulophaga. Nevertheless, the exact phylogenetic position of strain MOLA117T is uncertain since the bootstrap values clustering MOLA117T with either of the genera Arenibacter or Cellulophaga are low (Fig. 1). Also, it is clear from the phylogenetic analysis that strain MOLA117T does not belong to any of the other described genera in the family Flavobacteriaceae.

Morphological, physiological and biochemical properties of strain MOLA117T are listed in Table 1 and in the genus and species descriptions. The major respiratory lipophilic quinone was menaquinone MK-6, which is also the major respiratory quinone in Flavobacteriaceae [15].

Pigmentation, salinity requirements for growth and gliding motility are some important characteristics for the description of genera within the family Flavobacteriaceae. All the closest described genera and strain MOLA117T are pigmented, but only Zobellia galactanivorans Dsii [9] is positive for flexirubin. This strain also exhibits gliding motility, as do Cellulophaga lytica 23178 [16] and Maribacter sedimenticola KMM 3903T [17], but not strain MOLA117T or Arenibacter latericius KMM 426T [18, 19]. Indeed, Arenibacter latericius KMM 426T and strain MOLA117T are similar in many characteristics, but they differ in that strain MOLA117T can hydrolyse starch while Arenibacter latericius KMM 426T cannot and that strain MOLA117T is susceptible to kanamycin. They also exhibit different ranges of growth temperature and pH tolerance; strain MOLA117T is the only one that is negative for growth at both 4, 37 and 42 °C. Furthermore, even though all compared strains require NaCl for growth (Table 1), strain MOLA117T is slightly less halotolerant than related genera (Table 1), as indicated by the more narrow range of NaCl concentrations required for growth. MOLA117T is the only strain, of those compared, that does not reduce nitrate.

The polar lipids that were extracted from strain MOLA117T could not be identified in detail by TLC and subsequent staining (Fig. S2). Acid production was observed with only a few substrates using single carbon sources with the API 50CH system, and the API 20NE test showed a relatively narrow range of carbon source utilization. However, we did see a somewhat wider range when the carbon sources were tested with more specialized media and a 1 % carbon source [7], indicating that the carbon utilization patterns from only the API 50CH and the API 20NE systems probably


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underestimate the range of substrates that strain MOLA117\textsuperscript{T} can utilize.

The most predominant fatty acid found in strain MOLA117\textsuperscript{T} was iso-C\textsubscript{17:0} 3-OH at 33.21\%, and this fatty acid is found in related genera, such as Zobellia, Cellulophaga and Maribacter, in relatively high amounts (14.9–20.8\%), but was not found in the genus Arenibacter. Other major fatty acids found in strain MOLA117 were: C\textsubscript{15:0} (1.17\%), C\textsubscript{16:0}ω5 (1.41\%), iso-C\textsubscript{15:0} (11.44\%), iso-C\textsubscript{15:1}ω9c (7.12\%), anteiso-C\textsubscript{15:0} (1.26\%), iso-C\textsubscript{17:1}ω9c (2.62\%), C\textsubscript{16:0} 3-OH (2.08\%), iso-C\textsubscript{15:0} 3-OH (7.41\%), summed feature 3 (4.14\%) and an unknown fatty acid at 14.90\%.

Generally, all the genera we compared directly contained C\textsubscript{15:0ω9c}, iso-C\textsubscript{15:0ω9c}, iso-C\textsubscript{15:1ω9c} and anteiso-C\textsubscript{15:0ω9c} in varying amounts. The main difference between strain MOLA117\textsuperscript{T} and the related genera with respect to fatty acids is that strain MOLA117\textsuperscript{T} contains much more iso-C\textsubscript{17:0} 3-OH than strains in related genera.

In summary, strain MOLA117\textsuperscript{T} is different from related genera in numerous metabolic capabilities listed in Table 1 and discussed above. Thus, both the phenotypic and the phylogenetic data suggest that strain MOLA117\textsuperscript{T} should be...
placed in a novel species of a new genus, *Saonia flava* gen. nov., sp. nov.

**DESCRIPTION OF *SAONIA GEN. NOV.*

*Saonia* (Sa.o’ni.a L. fem. n. Sao the Nereid Sao; N.L. fem. n. *Saonia* named after Sao, the Nereid of ‘safe’ passage, or the rescue of sailors, referring to the marine origin).

Gram-stain-negative, non-motile, aerobic and chemo-organoheterotrophic. Positive for catalase, oxidase and alkaline phosphatase. Requires NaCl for growth. Produces yellow pigment but no flexirubin. The most predominant fatty acid is iso-C₁₇:0 3-OH and the only respiratory lipoquinone is MK-6. Based upon the 16S rRNA gene sequence, the genus forms a separate branch within the family *Flavobacteriaceae* close to the genera *Arenibacter* and *Cellulophaga*. The type species is *Saonia flava*.

**DESCRIPTION OF *SAONIA FLAVA* SP. NOV.

*Saonia flava* (fla’va. L. fem. adj. flava yellow).

In addition to properties given in the genus description the species is characterized as follows. Cells are 1.5–3.4 µm long and 0.5–0.6 µm wide, non-spore forming, straight rods. Cells have an extracellular polymeric substances (EPS)-like structure and they form aggregates in liquid culture. Colonies are yellow, smooth and slightly convex on MA. Growth occurs at temperatures between 15 and 30 °C. Optimal pH for growth is 7, and growth occurs at pH 7–8. Growth occurs in the presence of 1–4% (w/v) NaCl, but the optimal NaCl concentration is 3%. H₂S is not produced. Nitrate is not reduced under aerobic conditions. Using the API 20NE system, positive for urease activity, ascinulin hydrolysis and β-galactosidase utilization and negative for denitrification, gelatin hydrolysis, indole production, malic acid, trisodium citrate and phenylacetic acid. Acid production is observed only with a few substrates using the API 50CH system, namely fructose, ascinulin, sucrose, trehalose, inulin, raffinose and starch. Assimilates cellobiose, arabinose, maltose, trehalose, xylose, dextran, lactate.
glycerol, sucrose, galactose, glucose and sorbitol as single carbon sources but not lactose, fumarate, succinate, formate, pyruvate, citrate, glutamic acid or acetate. When assayed with the API ZYM system, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and N-acetyl-β-glucosaminidase are present, but esterase (C4), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-Bl-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase are absent. Susceptible to cefotaxime (30 µg), ciprofloxacin (30 µg), rifampicin (30 µg), chloramphenicol (50 µg) and ampicillin (10 µg), but resistant to erythromycin (15 µg), penicillin (20 U), poly-myxin (50 µg), streptomycin (10 µg) and tetracycline (30 µg).

The type strain, MOLA117\(^{T}\) (=CIP 110873\(^{T}\)=DSM 29762\(^{T}\)), was isolated from seawater from the north-western Mediterranean Sea off the coast of Banyuls-sur-Mer, France. The DNA G+C content of the type strain is 36.9 mol%.

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Conflicts of interest
The authors declare there are no conflicts of interest.

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