Luteivirga sdotyamensis gen. nov., sp. nov., a novel bacterium of the phylum Bacteroidetes isolated from the Mediterranean sponge Axinella polypoides

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A novel aerobic bacterium, designated strain PIII.02T, was isolated from a Mediterranean sponge (Axinella polypoides) collected off the Israeli coast near Sdot Yam. The non-motile cells were Gram-staining-negative, oxidase-positive and catalase-positive. The orange pigment of colonies growing on marine agar was neither diffusible nor flexirubin-like. Strain PIII.02T grew at 15–35 °C, at pH 6.0–9.0, with 2.0–7.0 % (w/v) NaCl, and with 1.0–8.0 % (w/v) sea salts. The predominant fatty acids were iso-C₁₅:₀, iso-C₁₆:₁ H, iso-C₁₆:₀, C₁₆:₀, anteiso-C₁₅:₀ and C₁₆:₁ω7c. The major respiratory quinone was MK-7. The genomic DNA G+C content of the novel strain was 38.1 mol%. Results from 16S rRNA gene sequence analysis indicated that strain PIII.02T was distinctly related to established members of the phylum Bacteroidetes. The established species found to be most closely related to the novel strain was Persicobacter diffilues NCIMB 1402T (87.6% 16S rRNA gene sequence similarity). Based on the phenotypic and chemotaxonomic data and the results of the phylogenetic analyses, strain PIII.02T represents a novel species of a new genus, for which the name Luteivirga sdotyamensis gen. nov., sp. nov. is proposed. The type strain is PIII.02T (=ATCC BAA-2393T =LMG 26723T).

Sponges host diverse and often unique bacterial communities (Taylor et al., 2007; Schmitt et al., 2012; Simister et al., 2012). Previous studies on the bacteria on the Mediterranean sponge Axinella polypoides led to the isolation of novel bacterial species from the phyla Verrucomicrobia (Scheuermayer et al., 2006) and Actinobacteria (Pimentel-Elardo et al., 2009). Here we report the isolation of strain PIII.02T, a novel member of the phylum Bacteroidetes, from the same species of sponge.

For the isolation and characterization of bacteria associated with the sponge A. polypoides, samples were collected by SCUBA diving at a depth of 30 m in the Mediterranean Sea off Sdot Yam, Israel. The samples were washed three times with sterile artificial seawater. Subsamples (cubes of 1 cm³) were each crushed in 9 ml sterile artificial seawater with a sterile mortar and pestle. Each resultant supernatant suspension was used for plating a dilution series on various media. The designated strain PIII.02T was obtained and subcultured on plates of marine agar 2216 (MA; BD) incubated at room temperature (20–25 °C). The novel strain was preserved at −80 °C in marine broth (MB) supplemented with 17% (v/v) glycerol.

For the 16S-rRNA-gene-based phylogenetic analysis of strain PIII.02T, part of a colony was transferred to 30 μl Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) in a 1.5 ml Eppendorf tube. The suspended cells were then lysed by three cycles of freezing at −80 °C for 1 h followed by heating to 95 °C for 10 min. For PCR, a sample of the lysate (1 μl) was added to a 0.2-ml tube containing 2.5 μl × PCR buffer, 2.0 μl 2.5 mM of each deoxyribonucleotide triphosphate, 0.25 μl of each 100 mM primer solution (63f and 1387r; Marchesi et al., 1998), 0.2 μl of 5 U μl⁻¹ Dream Taq polymerase solution (5 U μl⁻¹; Fermentas) and 19.8 μl molecular-grade water. The thermocycler was set to give 3 min at 94 °C, then 30 cycles, each of 1 min at 94 °C, 1 min 20 s at 54 °C and 2 min at 72 °C, followed by 5 min at 72 °C. The PCR product was purified with the Nucleospin kit (Macherey-Nagel) following the manufacturer’s instructions. Both DNA strands were sequenced, at the Sequencing Unit of the Inter-Departmental Research Facility Unit of Tel Aviv University, using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and an ABI PRISM 3100 genetic analyser (Applied Biosystems). The raw

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PIII.02T is JN699062.

Two supplementary figures and a supplementary table are available with the version online of this paper.
sequence data were visualized and manually edited using version 1.7.2 of the 4Peaks software package created by A. Griekspoor and T. Groothuis (www.mekentosj.com). The sequence from the novel strain was then compared with all 16S rRNA gene sequences in GenBank and with the 16S rRNA gene sequences of type strains by using the basic local alignment search tool (BLAST; Altschul et al., 1990), with the MEGABLAST algorithm, and the EzTaxon-e server (Kim et al., 2012), respectively. The sequences of related type strains were downloaded from the sequence database of the European Molecular Biology Laboratory and aligned using Infernal 1.0 (Nawrocki et al., 2009), as implemented in release 10 of the Ribosomal Database Project (Cole et al., 2009). The alignment was manually improved and adjusted so that it started and ended at conserved positions in the 16S rRNA gene sequence of strain PIII.02^T. Ambiguously aligned positions were identified and removed by using version 0.91b of the Gblock program (Castresana, 2000) on the Phylogeny.fr platform (Dereeper et al., 2008). For this, the default settings were used but gaps in the final blocks were allowed if they were present in <50% of the sequences. The resulting alignment, which contained 1230 of the 1279 original positions, was used for phylogenetic analyses based on the maximum-likelihood and neighbour-joining methods. Using the Akaike information criterion in Modeltest3.7 (Posada & Crandall, 1998), as implemented in PAUP*4.0b10 (Swofford, 2003), the best nucleotide substitution model for the maximum-likelihood analysis was identified as the general time-reversible (GTR) substitution model, with a proportion of invariable sites (+I) and a gamma-distributed variation of substitution rates among sites (+G). The maximum-likelihood tree was generated within PhyML 3.0 (Guindon et al., 2010), starting from a neighbour-joining tree and using NNI (nearest neighbour interchange) and SPR (subtree pruning and regrafting) for tree optimization. All model parameters were estimated by PhyML 3.0. Version 5 of the MEGA package (Tamura et al., 2011) was used to construct the neighbour-joining tree. Evolutionary distances between sequences were based on the Kimura two-parameter model of sequence evolution, with pairwise deletion of ambiguous positions. The robustness of each tree was assessed by bootstrap analysis, with 1000 replicates.

In the BLAST searches, two uncultured clones, obtained from coral-reef waters in Bolinao in the Philippines, appeared very similar to strain PIII.02^T, with 16S rRNA gene sequence similarities of 99% and 98%. After these two clones, sequence similarities dropped to 90%, for an uncultured clone obtained from haloalkaline soil, and then to 89%, for several other uncultured clones. The three established species that appeared to be most closely related to the novel strain, as identified over the 1279 nt that were compared by the EzTaxon-e server, were Persicobacter diffuens NCIMB 1402^T (Flammeovirgaceae; 87.6% sequence similarity), Belliella pelovolcani CC-SAL-25^T (Cyclobacteriaceae; 87.3%) and Roseivirga ehrenbergii KMM 6017^T (Flammeovirgaceae; 87.3%). In both the neighbour-joining and maximum-likelihood trees, the genera Roseivirga and Persicobacter each grouped in well-supported clades with other genera and apart from strain PIII.02^T (Fig. 1). All of the members of the family Cyclobacteriaceae, including Belliella pelovolcani CC-SAL-25^T, also grouped together (with 100% bootstrap support in both trees) and apart from the clade containing strain PIII.02^T (Fig. S1, available in IJSEM Online). Thus it seems unlikely that strain PIII.02^T is a member of the Cyclobacteriaceae. The exact phylogenetic position of strain PIII.02^T remains unclear due to differences in the phylogenetic relationships between the maximum-likelihood and neighbour-joining trees and low levels of bootstrap support (<50%) at the family level. The data indicate, however, that strain PIII.02^T represents a novel genus within the order Cytophagales.

The cellular morphology of strain PIII.02^T grown on MA was examined by light microscopy (Axioskop, Zeiss). Gram staining was performed with standard staining solutions. Cell lysis in 3% (w/v) KOH was investigated and L-alanine-p-nitroanilide solution was used as a substrate to test for aminopeptidase activity. Congo red adsorption was determined according to Bernardet et al. (2002). Motility of cells that had been incubated in MB for 2 days at 25 °C was observed using both wet mounts and the hanging-drop technique, as recommended by Bernardet et al. (2002). Anaerobic growth on MA was examined by incubation for 2 weeks at 25 °C in a sealed chamber with the GazPak EZ anaerobe container system (BD). The pH range for growth was determined, for 7 days at 25 °C, on MA that had been adjusted to pH 5–10 (in increments of 1 pH unit) with NaOH and HCl solutions. Tolerance to NaCl or sea salts (Reef Salt, Aqua Medic) was investigated, for 7 days at pH 7.5 and 25 °C, on peptone-yeast extract agar containing (1 g) 5 g peptone, 1 g yeast extract, 18 g agar and either 100–100 g NaCl (in increments of 10 g) or 0–100 g sea salts (in increments of 10 g). Growth at 4, 10, 15, 20, 25, 30, 35, 37 and 40 °C was determined on MA for 7 days. Growth inhibition by penicillin G, chloramphenicol, streptomycin sulfate, tetracycline, ampicillin, kanamycin A and nalidixic acid and the minimal inhibitory concentration of each of these antibiotics, was determined in MB, in 96-well plates. In each of these assays, the antibiotic was tested as a two-fold serial dilution ranging from 0.2 to 200 μg ml^-1 and growth was evaluated, as optical density at 620 nm, after incubation at 25 °C for 24 and 48 h, as recommended by Kelman et al. (2001). Cytochrome oxidase activity was investigated by using Bactident oxidase test strips (Merck) according to the manufacturer’s instructions, with Pseudomonas aeruginosa PA01 and Bacillus subtilis as the positive controls and Escherichia coli GM1655 as the negative control. Presence of catalase activity was tested by submerging a loopful of cells, obtained from colonies grown for 24 h on MA, into a 3% (v/v) hydrogen peroxide solution and observing bubble formation. Further enzymic activities were determined using APIZYM tests (bioMérieux) in duplicate and API20NE kits (bioMérieux) in triplicate, always following the manufacturer’s instructions except that sterile saline (3%, w/v, NaCl) was used for the APIZYM tests and the NaCl concentration
in the AUX medium supplied in the API20NE kits was increased to 3 % (w/v). The novel strain’s ability to metabolize additional carbon sources was investigated using GN2 MicroPlates (Biolog) according to the manufacturer’s instructions, except that artificial seawater (3.3 %, w/v, sea salts) was used as the inoculation fluid and the plates were incubated for 48 h at 25 °C. In duplicated tests conducted over 2 weeks at 25 °C, further carbon sources (peptone, yeast extract, malt extract, tryptone, casein, soluble starch, acetate, citrate, pyruvate, L-malic acid, oxalic acid, formate, glycerol, succinic acid, D-glucose, sucrose, D-galactose, D-sorbitol, polyethylene glycol, Tween 20, urea, 4-aminobenzoic acid, L-glutamic acid, glycine, L-alanine, L-leucine, L-proline, L-cysteine and L-tryptophan) were each tested in the AUX medium supplied in the API20NE kits. The novel strain’s ability to metabolize additional carbon sources was investigated using GN2 MicroPlates (Biolog) according to the manufacturer’s instructions, except that artificial seawater (3.3 %, w/v, sea salts) was used as the inoculation fluid and the plates were incubated for 48 h at 25 °C. In duplicated tests conducted over 2 weeks at 25 °C, further carbon sources (peptone, yeast extract, malt extract, tryptone, casein, soluble starch, acetate, citrate, pyruvate, L-malic acid, oxalic acid, formate, glycerol, succinic acid, D-glucose, sucrose, D-galactose, D-sorbitol, polyethylene glycol, Tween 20, urea, 4-aminobenzoic acid, L-glutamic acid, glycine, L-alanine, L-leucine, L-proline, L-cysteine and L-tryptophan) were each tested in the medium containing (l−1) 0.05 g yeast extract, 33 g sea salts, 20 g agar and 2 g of the test compound. Negative controls without any added carbon source and positive controls (on MA) were run in parallel. The results for each test compound were compared against those for the negative control. Hydrolysis of crystalline cellulose was tested by submerging filter paper in MB cultures for 2 weeks. Hydrolysis of starch (0.2 %, w/v) and casein (10 %, w/v) was tested by incorporation of the test substrate in MA and observing the occurrence of clear zones around positive colonies. For detection of starch hydrolysis, the culture plates were additionally flooded with Lugol’s solution.

The complete biochemical and physiological properties of strain PIII.02^T are given in the genus and species descriptions. The main differences between the novel strain and members of the two closest related genera, Persicobacter and Roseivirga, are given in Table 1. Strain PIII.02^T can be distinguished from current members of the genera Persicobacter and Roseivirga by its strong ability to hydrolyse casein (an ability that is absent from, or weak in, the closest related genera) and its inability to hydrolyse

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**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences, showing the relationship between strain PIII.02 and members of the genera Persicobacter and Roseivirga. Bootstrap values over 50 %, based on 1000 repetitions, are given at the nodes. Circles indicate nodes supported in the maximum-likelihood tree by bootstrap values of 50–69 % (open circles) or ≥ 70 % (full circles). The complete tree, which includes other members of the order Cytophagales and Chlorobium limicola DSM 245^T used as an outgroup, is given in Fig. S1. Bar, 0.02 substitution per nucleotide position.
gelatin. Several additional characteristics distinguish strain PIII.02T from Persicobacter species (e.g. oxidase activity, gliding motility, anaerobic growth, growth at 40 °C and pH values >9, hydrolysis of agar, aesculin and starch, and nitrate reduction). Other characteristics of the novel strain (e.g. cysteine arylamidase, trypsin, α-chymotrypsin and α-glucosidase activities) have not been seen in members of the genus Roseivirga.

Chemotaxonomic characteristics were determined using cells grown for 20 h (genomic DNA G+C content) or 48 h on MA at 25 °C. The presence of flexirubin-like pigments was determined by the KOH test, as described by Bernardet et al. (2002). Cells for the analysis of major respiratory quinones, DNA G+C content and cellular fatty acids were harvested by scraping from the agar surface, frozen at −80 °C and then sent, either frozen or freeze-dried, to the laboratories of the Consiglio Nazionale delle Ricerche in Pozzuoli, Italy. Fatty acid methyl esters (FAMEs) were prepared according to the instructions of the Microbial Identification System (MIDI, 1999) and analysed by GC/MS on a Polaris Q spectrometer (Thermo). The FAMES were identified by comparison with standards and by interpretation of the mass spectra. Analysis of polar lipids was performed according to Romano et al. (2001). Polar lipids were extracted from 0.6 g of freeze-dried bacterial cells twice, using 100 ml chloroform/methanol/water (65:25:4, by vol.) for each extraction. They were then analysed by TLC, with chloroform/methanol/water (65:25:4, by vol.) used as the eluent, and visualized by

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH range for growth</td>
<td>6–9</td>
<td>6–11</td>
<td>3–11</td>
<td>5–10</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Growth at 40 °C</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>–</td>
<td>Facultative</td>
<td>Facultative</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+/W</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>Gliding</td>
<td>Gliding</td>
<td>Gliding</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Enzymic activity (APIZYM):
- Lipase (C14): –, ND, ND, +, –, +
- Cysteine arylamidase: –, ND, ND, +, +, +
- Trypsin: –, ND, ND, +, +, +
- α-Chymotrypsin: –, ND, ND, +, +, +
- α-Glucosidase: –, ND, ND, +, +, +
- β-Glucosidase: –, ND, ND, +, +, –
- N-Acetyl-β-glucosaminidase: –, ND, ND, +, +, –

Utilization (GN2 MicroPlate):
- α-D-Glucose: –, +, +, +, –, –
- d-Galactose: –, +, +, +, –, –
- Dextrin: –, +, +, –, ND
- l-Alanine: +, +, +, –, –, +
- l-Asparagine: +, +, +, –, –, ND
- l-Aspartic acid: +, –, +/W, –, ND
- l-Glutamic acid: +, –, +, –, +
- l-Ornithine: +, –/W, +/W, –, +, ND
- l-Proline: +, –, –/W, –, +, ND
- Tween 40: +, –/W, +/W, –, –, –
- Tween 80: W, –, –, –, –, ND

Hydrolysis of:
- Agar: –, +, +, +, –, –
- Aesculin: –, +, +, +, –, ND
- Casein: +, –/W, –/W, –, –, –
- Gelatin: –, +, +, +, +, +
- Starch: –, W, W, –, –, –

Nitrate reduction: –, +, +, –, –, –

DNA G+C content (mol%): 38.1 42.6–43.8 42.0–42.7 43.7 41.3 40.2

Table 1. Phenotypic characteristics that distinguish strain PIII.02T from members of the closest related genera, Persicobacter and Roseivirga

<table>
<thead>
<tr>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>5–10</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Growth at 40 °C</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>–</td>
<td>Facultative</td>
<td>Facultative</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+/W</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>Gliding</td>
<td>Gliding</td>
<td>Gliding</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Enzymic activity (APIZYM):
- Lipase (C14): –, ND, ND, +, –, +
- Cysteine arylamidase: –, ND, ND, +, +, +
- Trypsin: –, ND, ND, +, +, +
- α-Chymotrypsin: –, ND, ND, +, +, +
- α-Glucosidase: –, ND, ND, +, +, +
- β-Glucosidase: –, ND, ND, +, +, –
- N-Acetyl-β-glucosaminidase: –, ND, ND, +, +, –

Utilization (GN2 MicroPlate):
- α-D-Glucose: –, +, +, +, –, –
- d-Galactose: –, +, +, +, –, –
- Dextrin: –, +, +, –, ND
- l-Alanine: +, +, +, –, –, +
- l-Asparagine: +, +, +, –, –, ND
- l-Aspartic acid: +, –, +/W, –, ND
- l-Glutamic acid: +, –, +, –, +
- l-Ornithine: +, –/W, +/W, –, +, ND
- l-Proline: +, –, –/W, –, +, ND
- Tween 40: +, –/W, +/W, –, –, –
- Tween 80: W, –, –, –, –, ND

Hydrolysis of:
- Agar: –, +, +, +, –, –
- Aesculin: –, +, +, +, –, ND
- Casein: +, –/W, –/W, –, –, –
- Gelatin: –, +, +, +, +, +
- Starch: –, W, W, –, –, –

Nitrate reduction: –, +, +, –, –, –

DNA G+C content (mol%): 38.1 42.6–43.8 42.0–42.7 43.7 41.3 40.2
spraying with Dittmer’s reagent, ninhydrin and α-
naphthol. Quinone purification was performed by extract-
ing 1 g of freeze-dried bacterial cells twice with hexane. The
filtered extracts were combined and concentrated under
reduced pressure. The resultant crude extract (2 mg) was
purified on silica gel (230–400 mesh; Merck), with hexane/ethyl
acetate (98:2, v/v) used as the eluent. The fractions
were spotted on TLC plates and observed under UV light.
The UV-visible fractions were pooled, concentrated under
reduced pressure and analysed by LC/MS on an ESI-Q-
TOF Micromass spectrometer (Waters) fitted with a reverse-phase C-18 column. For this analysis, methanol
was used as the eluent, at a flow rate of 1.2 ml min−1.
Genomic DNA G+C content was determined as previously
previously by Poli et al. (2009), using DNA extracted
from freeze-dried cells.

Flexirubin-like pigments were not detected in strain
PIII.02T. The cellular fatty acid profile of strain PIII.02T
is compared with those of representatives of the two most
closely related genera in Table 2 and with the correspond-
ing profiles of other, phylogenetically related members of the
order Cytophagales in Table S1. The main differences are in
the proportions of iso-C16:0 and iso-C16:1 H, which are
higher in strain PIII.02T (16.5 % and 18.1 %, respectively)
than in closely related members of the order Cytophagales
(0–3.2 % for both fatty acids) and the absence of iso-C17:0
3-OH (a fatty acid found in almost all closely related members
of the order Cytophagales) from strain PIII.02T.
The polar lipid profile of strain PIII.02T comprised a great
abundance of phosphorylethanolamine, a trace of an
unidentified phospholipid, and no detectable glycolipids
(Fig. S2). The major isoprenoid quinone of strain PIII.02T
was MK-7. The novel strain’s genomic DNA G+C content,
38.1 mol%, is lower than the range reported for members
of the closest related genera (40.2–43.8 mol%).

In conclusion, the low levels of 16S rRNA gene sequence
similarity (<90 %) to any established species, and the
groupings in the phylogenetic analyses, indicate that strain
PIII.02T represents as novel species of a new genus within the
order Cytophagales. The novel strain can be readily
differentiated from its closest phylogenetic neighbours by several properties (Tables 1 and 2). Based on the phenotypic,
chemotaxonomic and phylogenetic data, strain PIII.02T
represents a novel species of a new genus within the order
Cytophagales (phylum Bacteroidetes), for which the name
Luteivirga sdotyamensis gen. nov., sp. nov. is proposed.

**Description of Luteivirga gen. nov.**

*Luteivirga* (Lu.te.i.vir*ga*. L. adj. luteus yellow, orange; L.
fem. n. virga staff, rod; N.L. fem. n. Luteivirga orange rod).
Cells are non-motile, Gram-staining-negative rods. Flexirubin-like pigments are absent. Catalase- and oxidase-
positive. Aerobic. The major isoprenoid quinone is MK-7.
The predominant fatty acids are iso-C15:0, anteiso-C15:0,
C16:0 and iso-C16:1 H. The type species is
*Luteivirga sdotyamensis*.

**Description of Luteivirga sdotyamensis sp. nov.**

*Luteivirga sdotyamensis* (sdot.yam.en*sis*. N.L. fem. n.
sdotyamensis of or belonging to Sdot Yam, named after
Kibbutz Sdot Yam, the nearest village to the collection site).

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**Table 2. Fatty acid contents (%) of strain PIII.02T and the two
phylogenetically closest related genera, Persicobacter and
Roseivirga**

<table>
<thead>
<tr>
<th>Fatty acid contents (%)</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td><strong>Straight chain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>–</td>
<td>1.1–1.7</td>
<td>–</td>
</tr>
<tr>
<td>C15:0</td>
<td>1.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C16:0</td>
<td>14.0</td>
<td>4.6–5.6</td>
<td>–</td>
</tr>
<tr>
<td><strong>Branched</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C13:0</td>
<td>–</td>
<td>TR</td>
<td>0.7–2.9</td>
</tr>
<tr>
<td>iso-C14:0</td>
<td>0.5</td>
<td>–</td>
<td>0.0–1.9</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>23.7</td>
<td>54.0–55.6</td>
<td>18.6–24.0</td>
</tr>
<tr>
<td>iso-C15:0 3-OH</td>
<td>–</td>
<td>3.2–7.6</td>
<td>3.0–4.1</td>
</tr>
<tr>
<td>iso-C15:1</td>
<td>–</td>
<td>–</td>
<td>12.5–34.2</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>16.5</td>
<td>TR</td>
<td>1.1–2.0</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>–</td>
<td>1.3–2.1</td>
<td>1.2–4.2</td>
</tr>
<tr>
<td>iso-C18:1 H</td>
<td>18.1</td>
<td>–</td>
<td>0.0–2.0</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>1.4</td>
<td>TR</td>
<td>0.0–1.0</td>
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<tr>
<td>iso-C17:0 3-OH</td>
<td>–</td>
<td>7.2–12.2</td>
<td>7.7–18.3</td>
</tr>
<tr>
<td>iso-C17:1</td>
<td>1.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>12.4</td>
<td>4.3–7.2</td>
<td>4.5–13.1</td>
</tr>
<tr>
<td>anteiso-C15:1</td>
<td>–</td>
<td>–</td>
<td>0.0–2.4</td>
</tr>
<tr>
<td>anteiso-C17:1,9c</td>
<td>–</td>
<td>–</td>
<td>0.0–10.8</td>
</tr>
<tr>
<td><strong>Unsaturated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:1,65c</td>
<td>–</td>
<td>2.8–3.5</td>
<td>–</td>
</tr>
<tr>
<td>C16:1,67c</td>
<td>5.9</td>
<td>–</td>
<td>?*</td>
</tr>
<tr>
<td>C17:1,66c</td>
<td>0.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C20:4,6,9,12,15c</td>
<td>–</td>
<td>2.0–2.2</td>
<td>–</td>
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<tr>
<td><strong>Hydroxy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0 2-OH</td>
<td>0.6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C15:0 2-OH</td>
<td>–</td>
<td>–</td>
<td>0.0–3.2</td>
</tr>
<tr>
<td>C16:0 3-OH</td>
<td>1.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C18:0 3-OH</td>
<td>–</td>
<td>4.1–5.7</td>
<td>0.0–1.4</td>
</tr>
<tr>
<td>C17:0 2-OH</td>
<td>2.0</td>
<td>TR–1.1</td>
<td>0.0–10.1</td>
</tr>
<tr>
<td><strong>Summed feature 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECL 16.582</td>
<td>–</td>
<td>TR–1.5</td>
<td>–</td>
</tr>
<tr>
<td>Unknown</td>
<td>–</td>
<td>–</td>
<td>0.0–10.9</td>
</tr>
</tbody>
</table>

*May be present, as all or part of summed feature 3.
†Summed features are groups of two or three fatty acids that cannot
be separated by GLC using the MIDI system. Summed feature 3
contained C15:1,67c and/or iso-C15:0 2-OH.
Cells are generally 0.5 μm wide and 4–7 μm long but longer filaments, up to 10 μm in length, occur. Cells lyse in 3% (w/v) KOH and test positive for aminopeptidase activity. Colonies on marine agar are circular and convex. Colonies do not adsorb Congo red. Growth occurs at 15–35°C, with 1–8% (w/v) sea salts, with 2–7% (w/v) NaCl, and at pH 6–9, but not under anaerobic conditions. Minimal inhibitory concentrations of antibiotics after 48 h incubation are 0.4 μg mL⁻¹ for penicillin G, 3.1 μg mL⁻¹ for chloramphenicol, 25 μg mL⁻¹ for streptomycin sulfate, 1.6 μg mL⁻¹ for tetracycline, 12.5 μg mL⁻¹ for ampicillin, 3.1 μg mL⁻¹ for nalidixic acid, and 200 μg mL⁻¹ for kanamycin A. Positive, in APIZYM tests, for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase activities but negative for lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. Negative, in API20NE tests, for nitrate reduction, indole production, fermentation of D-glucose, hydrolysis of gelatin and aesculin, and arginine dihydrolase, urease and β-galactosidase activities. Does not assimilate any of the carbon sources used in the API20NE tests (i.e. D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid). Positive, in GN2 MicroPlates, for the utilization of TWEEN 40, TWEEN 80 (weakly), L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-ornithine and L-proline. Negative for the utilization of α-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabino, 2,3-butanediol, glycerol, DL-thymidine, phenylethylamine, putrescine, 2-aminoethanol, L-serine, L-threonine, DL-carnitine, -aminobutyric acid, urocanic acid, inosine, uridine, glycyl L-glutamic acid, L-ornithine and L-proline. Growth occurs on agar plates supplemented with peptone, yeast extract, malt extract, tryptone, casein, L-glutamic acid or Tween 20 but not on those supplemented with soluble starch, acetal, citrate, pyruvate, glycerol, succinic acid, α-D-glucose, succrose, D-galactose, D-sorbitol, L-malic acid, oxalic acid, formate, polyethylene glycol, urea, 4-amino benzoic acid, glycine, L-alanine, L-leucine, L-proline, L-cysteine or L-tryptophan. Casein is hydrolysed but crystalline cellulose, starch and agar are not. The predominant fatty acids are iso-C₁₅:₀, α₃-C₁₆:₀, H, iso-C₁₆:₀, C₁₆:₀, 2-OH iso-C₁₆:₀, anteiso-C₁₅:₀.

The type strain, PHL.02T (≡ ATCC BAA-2393T =LMG 26723T), was isolated from the Mediterranean sponge Axinella polypoides collected off the Israeli coast near Sdot Yam, at a depth of 30 m. The genomic DNA G+C content of the type strain is 38.1 mol%.

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**References**


