**Sphingomonas cynarae** sp. nov., a proteobacterium that produces an unusual type of sphingan

Adelfia Talà,1 Marcello Lenucci,1 Antonio Gaballo,2† Miriana Durante,3 Salvatore M. Tredici,1 Danisha A. Debowles,4 Graziano Pizzolante,1 Carlo Marcuccio,1 Elisabetta Carata,1 Gabriella Piro,1 Nicholas C. Carpita,4 Giovanni Mita3 and Pietro Alifano1

1Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, Via Monteroni, 73100 Lecce, Italy
2CNR – Institute of Biomembranes and Bioenergetics (IBBE), Via G. Amendola, 165/A, 70126 Bari, Italy
3CNR – Institute of Sciences of Food Production (ISPA), Operative Unit of Lecce, via Provinciale Lecce-Monteroni, 73100 Lecce, Italy
4Department of Botany and Plant Pathology, Purdue University, 915 West State Street, West Lafayette, IN 47907-2054, USA

Strain SPC-1T was isolated from the phyllosphere of *Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori (wild cardoon), a Mediterranean native plant considered to be the wild ancestor of the globe artichoke and cultivated cardoon. This Gram-stain-negative, catalase-positive, oxidase-negative, non-spore-forming, rod-shaped and non-motile strain secreted copious amounts of an exopolysaccharide, formed slimy, viscous, orange-pigmented colonies and grew optimally at around pH 6.0–6.5 and 26–30 °C in the presence of 0–0.5 % NaCl. Phylogenetic analysis based on comparisons of 16S rRNA gene sequences demonstrated that SPC-1T clustered together with species of the genus *Sphingomonas sensu stricto*. The G+C content of the DNA (66.1 mol%), the presence of Q-10 as the predominant ubiquinone, sym-homospermidine as the predominant polyamine, 2-hydroxymyristic acid (C14:0 2-OH) as the major hydroxylated fatty acid, the absence of 3-hydroxy fatty acids and the presence of sphingoglycolipid supported this taxonomic position. 16S rRNA gene sequence analysis showed that SPC-1T was most closely related to *Sphingomonas hankookensis* ODN7T, *Sphingomonas insulae* DS-28T and *Sphingomonas panni* C52T (98.19, 97.91 and 97.11 % sequence similarities, respectively). However, DNA–DNA hybridization analysis did not reveal any relatedness at the species level. Further differences were apparent in biochemical traits, and fatty acid, quinone and polyamine profiles leading us to conclude that strain SPC-1T represents a novel species of the genus *Sphingomonas*, for which the name *Sphingomonas cynarae* sp. nov. is proposed; the type strain is SPC-1T (＝JCM 17498T＝ITEM 13494T). A component analysis of the exopolysaccharide suggested that it represents a novel type of sphingan containing glucose, rhamnose, mannose and galactose, while glucuronic acid, which is commonly found in sphingans, was not detected.

Sphingomonads are a group of alphaproteobacteria that are widely distributed in nature, commonly isolated from many land and water habitats, as well as from plant phyllosphere and rhizosphere, clinical specimens, and other sources. Besides their ecological role, these micro-organisms have a great potential for biotechnological applications in the degradation, bioremediation and wastewater treatment of xenobiotic pollutants, as bacterial antagonists of phytopathogenic fungi and for the production of industrially useful polymers and enzymes (Fialho et al., 2008; Lal et al.,

---

†Present address: Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, Via Monteroni, 73100 Lecce, Italy.

**Abbreviations:** EPS, exopolysaccharide; FAME, fatty acid methyl ester; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; TFA, trifluoroacetic acid.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain SPC-1T is HQ439186.

Two supplementary tables, five supplementary figures and two supplementary files are available with the online version of this paper.

---

**Correspondence**
Pietro Alifano
pietro.alifano@unisalento.it
Here, we report the taxonomic characterization of the orange-pigmented strain SPC-1T, which was preliminarily identified as a member of the genus Sphingomonas. The strain was isolated from the phyllosphere of Cynara cardunculus L. var. sylvestris (Lamk) Fiori (wild cardoon), a non-domesticated robust perennial plant characterized by a rosette of large spiny leaves and branched flowering stems considered to be the wild ancestor of the globe artichoke and cultivated cardoon. In addition, evidence is provided that strain SPC-1T secretes copious amounts of an unusual type of sphingan that lacks uronic acids.

Strain SPC-1T was isolated in pure culture by means of the standard dilution plating technique on trypticase soy agar (TSA). Single orange-pigmented colonies were visible after cultivation at 28 °C for 48 h. The type strains of three recognized Sphingomonas species were used as reference strains for DNA–DNA hybridization and phenotypic characterization: Sphingomonas insulae DSM 21792T (original strain designation: DS-288; Sphingomonas panni DSM 15761T (original strain designation: CS2T) and Sphingomonas hankookensis DSM 23329T (original strain designation: ODN7T). These strains were obtained from the DSMZ, Braunschweig, Germany. Morphological, physiological and biochemical characteristics of strain SPC-1T with respect to reference strains, which were tested in parallel, were investigated using routine cultivation at 28 °C on TSA or trypticase soy broth (TSB). Cell morphology and motility were examined by light microscopy. Gram staining was done using the bioMérieux Gram stain kit according to the manufacturer’s instructions. Temperature tolerance (4–40 °C) was examined on TSA. Salt tolerance was tested on TSA supplemented with various NaCl concentrations (0–5 %); for this, the TSA was prepared according to the formula of the Difco medium, except that NaCl was excluded from the medium formula. The pH range for growth was determined on TSA that was adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units). The pH was adjusted prior to sterilization by the addition of HCl or Na2CO3. Catalase and oxidase activities were determined by using the ID colour Catalase (ID-ASE; bioMérieux) and the Oxidase reagent kits (bioMérieux), respectively. Utilization of various substrates, enzyme activities, and other physiological and biochemical properties were tested by using the API 20E, API 20NE and API 50CH systems (bioMérieux); utilization of various substrates was determined by inoculating the API 50CH strip with bacteria suspended in AUX medium (bioMérieux). Results were recorded after 72 h incubation at 28 °C under aerobic conditions.

High-molecular-mass genomic DNA extraction from bacteria grown in TSB to late exponential phase was carried out as described previously (Stabili et al., 2008). The 16S rRNA gene was amplified and sequenced using the primer pair 5′-AGAGTTTTGATCATAATCCGCGTCAG-3′ and 5′-ACGGCTACCTTGTACACGAC-3′. PCR conditions and nucleotide sequencing procedures were as reported previously (Vigliotta et al., 2007; Stabili et al., 2008). The sequence was compared with those of closely related reference organisms using the EzTaxon service (Kim et al., 2012). Multiple sequence alignments were performed with CLUSTAL W (version 2.1) (Thompson et al., 1994) at the Kyoto University Bioinformatic Center (http://www.genome.jp/tools/clustalw/) using the following default settings: weight matrix IUB (for DNA), gap open penalty 15, gap extension penalty 6.66. Almost complete 16S rRNA gene sequences from type strains of members of the genus Sphingomonas were used (Table S1 and File S1, available in IJSEM Online). The CLUSTAL W output file (File S2) was utilized to infer evolutionary trees with the PHYLO_WIN package (Galtier et al., 1996) according to the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Sobey, 1983) and maximum-likelihood (Felsenstein, 1981) methods. Evolutionary distances were calculated with the neighbour-joining method according to the algorithm of Kimura’s two-parameter model (Kimura, 1980). Bootstrap resampling (1000 replicates each) was used to assess robustness of phylogenetic trees (Brown, 1994).

The DNA G+C content was determined using an HPLC system (Agilent 1100 Series HPLC) equipped with a Phenomenex-luna 5 μm C18 (2) 100 Å column (250 × 4.6 mm). A gradient elution mode was used according to Gehrke & Kuo (1990). The elution buffer was as follows: eluent A (2.50 % methanol in 0.01 M NH4H2PO4; pH 5.3), eluent B (20.0 % methanol in NH4H2PO4; pH 5.1), eluent C (35 % acetonitrile in 0.01 M NH4H2PO4; pH 4.9) at flow rate of 1 ml min−1 at 25 °C. Each nucleoside was detected by its UV absorbance at 270 nm. DNA was hydrolysed by nuclease P1 (Sigma) and the resultant nucleotides were treated with alkaline phosphatase (2.4 units ml−1) and then analysed by reversed-phase HPLC.

DNA–DNA hybridization experiments were carried out by using the membrane filter method described by Ezaki et al. (1989) with modifications (Stabili et al., 2008). Total DNAs (5 μg) from the different bacterial strains were restricted with HaeIII, serially diluted in a buffer containing 2 × SSC (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 50 % (v/v) formamide, heated at 95 °C for 5 min and immobilized onto positively charged nylon filters by slow filtration in a slot-blot apparatus (Minifold I Slot-Blot System; Sigma-Aldrich) in duplicate. The filters were dried at room temperature and directly used for hybridization. DNA probes were obtained by labelling HaeIII-restricted genomic DNAs with DIG-High Prime mix (Roche) according to manufacturer’s instructions. Pre-hybridization was carried out in a buffer containing 5 × SSC, 5 × Denhardt’s solution, 0.1 % SDS, 50 mM sodium phosphate buffer (pH 6.5), 50 % (v/v) formamide, 500 μg denatured salmon sperm DNA ml−1. The hybridization buffer was similar to the pre-hybridization solution but containing 100 ng digoxigenin-labelled DNA per millilitre in place of salmon sperm DNA. Pre-hybridization and hybridization steps were carried out at 47 °C. This temperature represents stringent conditions for strain SPC-1T, for which the
optimal renaturation temperature (44.7 °C) is calculated as
[[0.51 × DNA G + C content] + 47]–36 (Gillis et al., 1970),
where 36 °C is the correction for the presence of 50 %
formamide (McConaughy et al., 1969). A DNA G + C
content of 66.1 mol% was determined for strain SPC-1T (see
below). After hybridization, filters were washed three times
with a solution containing 2 × SSC, 0.2 % SDS at 47 °C. The
filters were then subjected to immunological detection,
according to the manufacturer’s instructions. Semi-quantita-
tive analysis of the hybridization signals was performed by
densitometry using a Scanmaster 3 (Howtek), a high-
performance desktop flatbed colour scanner equipped with
an RFLPrint (Pdi) software package.

For analysis of respiratory quinones, polyamine pattern,
polar lipids and fatty acid methyl esters (FAMEs), strain SPC-
1T and reference strains were grown to late exponential phase
(to 1.5 at O.D. 600 nm) in 2 l Erlenmeyer flasks each
bottom phase was collected. A portion of the total lipid
methanol/water ratio of 2 : 2 : 1.8 (by vol.). After settling,
adding chloroform and water to obtain a final chloroform/
ant was transferred into a separating funnel, and phase
(2001) at 80
30 m length, 0.25 mm ID and 0.25 µm thickness). The
GC parameters were as follows. The temperature of the
column was 80 °C for 10 min. After cooling at 5 °C,
lyophilized cell material with chloroform/methanol (2 : 1, v/v)
and analysed using reversed-phase HPLC and a Phenomenex-
luna 5µ C18 (2) 100 Å column (250 × 4.6 mm) according to
the method of Moss & Guerrant (1983) with modifications.
Mobile phases consisted of methanol (A), isopropanol (B)
and water (C). The isocratic elution was as follows: 0 min,
75% A, 20% B and 5% C; 0 to 7 min, 75% A, 25% B; 7 to
32 min, 35% A, 65% B; 32 to 35 min, 65% A, 20% B and 5%
C. The column was re-equilibrated for 10 min between runs.
The flow rate was 1.0 ml min⁻¹ and the column temperature
was maintained at 25 °C. The injection volume was 10 µl.
Absorbance was registered by diode array at 290 nm.

Lipids were extracted using the modified method of Bligh &
Dyer (1959) with some modifications. Lyophilized powder
(100 mg) was mixed with a total of 114 ml solvent
added in this sequence: chloroform, methanol, water
to achieve a final chloroform/methanol/water ratio of
1 : 2 : 0.8 (by vol.). Samples were shaken for 15 s after
addition of each solvent, and incubated overnight at 4 °C.
After centrifugation at 6500 × g for 10 min, the supernatant
was transferred into a separating funnel, and phase separation
of the biomass-solvent mixtures was achieved by adding
chloroform and water to obtain a final chloroform/
methanol/water ratio of 2 : 2 : 1.8 (by vol.). After settling,
the bottom phase was collected. A portion of the total lipid
extract was trans-esterified according to Eguchi et al.
(2001) at 80 °C for 1 h using a solution of methanol/
hydrochloric acid/chloroform (10 : 1 : 1). After the addition
of 1 ml water, the mixture was extracted twice with 3 ml
hexane/chloroform 4 : 1 (v/v) to obtain FAMES, which
were analysed using GC-MS. The GC-MS system consisted
of a Shimadzu GC-17A ver. 3.0, with MS QP5050A.
Compounds were separated on a DB-5 capillary column
(30 m length, 0.25 mm ID and 0.25 µm thickness). The
GC parameters were as follows. The temperature of the
column was 80 °C after injection, then programmed at
10 °C·min⁻¹ to 150 °C, at 5 °C·min⁻¹ to 250 °C and
maintained at that temperature for 15 min. Split injection
was conducted with a split ratio of 50 : 1, the flow-rate was
1.0 ml min⁻¹, carrier gas used was 99.999 % pure helium,
the injector temperature was 250 °C and the column inlet
pressure was 74 kPa. The MS detection conditions were as
follows. Interface temperature was set to 250 °C; ionization
mode, EI+; electron energy, 70 eV; scanning method of
acquisition, ranging from 30 to 450, for mass/charge (m/z)
was optimized. Spectrum data were collected at 0.5 s
intervals. Solvent cut time was set at 2 min and 45 min
retention time, which was sufficient for separation of all
the fatty acids. Compounds were identified by using online
NIST98-library spectra and published MS data. Moreover,
bacterial FAME mix and PUFA-3 (from menhaden oil)
authentic standards (both from Sigma-Aldrich) were used
to confirm MS data and to discriminate between C₁₈:₁(ββC)
and C₁₈:₁(βγC). Polar lipids were extracted according to
the procedures described by Minnikin et al. (1984), resolved
by two-dimensional TLC [first dimension, chloroform/meth-
anol/water (65:25:4, by vol.); second dimension, chlor-
roform/acetic acid/methanol/water (80:15:12:4, by vol.)]
detected by spraying with 5 % ethanolic molybdo-
sulfuric acid followed by charring at 180 °C.

Polyamines were extracted and derivatized as described
by Scherer & Kneifel (1983) with some modifications. In brief,
40 mg freeze-dried samples were hydrolysed in 1 ml of
0.2 M HClO₄ at 100 °C for 30 min with shaking once after
15 min. Internal standard 1,8-diaminooctane (360 nmol per
40 mg cells) was added before heating and the samples
were centrifuged (4500 × g for 10 min). Supernatant samples
(0.2 ml) were incubated with 300 µl Na₂CO₃ solution
(100 mg ml⁻¹) and 800 µl dansyl chloride (7.5 mg ml⁻¹
in acetone). Proline solution (100 µl of 50 mg ml⁻¹
solution) was added to bind the excess dansyl chloride
during incubation at 60 °C for 10 min. After cooling at 5 °C,
polyamines were extracted with 100 µl toluene. Toluene
was removed under a slight stream of N₂. The volume
was adjusted to 100 µl with acetonitrile and 20 µl was loaded
into the HPLC system equipped with a Phenomenex-luna
5µ C18 (2) 100 Å column (250 × 4.6 mm). The mobile
phase was: eluent A (70 mM acetic acid, 25 mM triethyl-
amine, pH 4.82), eluent B (80 % acetonitrile), eluent C
(methanol). A gradient elution mode was used according to
the method of Shaw et al. (2010), at a flow rate of 1.2 ml
min⁻¹ at 35 °C. Detection was carried out at 254 nm. To
quantify spermidine and spermine, an internal standard and
a calibration curve (0.05–50 mM) were used.

For exopolysaccharide (EPS) production, strain SPC-1T
was cultivated for 5 days at 28 °C with rotary shaking
(250 r.p.m.) in 300 ml baffled Erlenmeyer flasks with 50 ml
medium containing 20 g glucose l⁻¹, 10 g peptone l⁻¹, 10 g
yeast extract l⁻¹ and 5 g NaCl l⁻¹. The secreted EPS material
was isolated from liquid culture as described previously by
West & Strohbus (1998) and Nampoothiria et al. (2003).
Total sugar determination was performed by the phenol-sulphuric acid

Downloaded from www.microbiologyresearch.org by
IP:  54.70.40.11
On: Thu, 07 Mar 2019 19:54:03
method (DuBois et al., 1956) indicated that 30–40 % of the dry weight of this material comprised carbohydrates. Uronic acids were not detectable by the Blumenkranz & Asboe-Hansen (1973) method. The dried material was hydrolysed with 2.0 M trifluoroacetic acid (TFA) in sealed tubes at 120 °C for 90 min. TFA-resistant material was precipitated by centrifugation at 8800 × g, for 10 min. The supernatant (hydrolysate) was dried in vacuo, dissolved in 1 ml distilled water and analysed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as described previously by Lenucci et al. (2008).

Strain SPC-1T was isolated serendipitously during investigations aimed to establish an in vitro propagation system for wild cardoon (Fig. S1). Morphological, physiological and biochemical characteristics of strain SPC-1T are given in the species description and shown in Table 1. The analysis of the near-complete (1426 nt) 16S rRNA gene sequence indicated that the closest relatives of the orange-pigmented strain SPC-1T were two members of the genus Sphingomonas, MK01 and PB163 (accession nos GQ339888.1 and GQ339895.1, respectively) isolated from a ginseng field and stream sediment, respectively (both showing 98.70 % sequence similarity). Among the type strains of species with validly published names, S. hankookensis ODN7T (Yoon et al., 2009), S. insulae DS-28T (Yoon et al., 2008) and S. panni C52T (Busse et al., 2005) showed the highest sequence similarities (98.19, 97.91 and 97.11 %, respectively). This finding was supported by phylogenetic analysis that confirmed that strain SPC-1T belonged to the genus Sphingomonas sensu stricto (Takeuchi et al., 2001). In the neighbour-joining phylogenetic tree, it grouped with S. insulae DS-28T, S. hankookensis ODN7T and S. panni C52T (Fig. 1). The relationship between SPC-1T, S. hankookensis ODN7T and S. panni C52T was also maintained in trees constructed via the maximum-parsimony and maximum-likelihood algorithms. The predominant isoprenoid quinone detected in strain SPC-1T was ubiquinone-10 (Q-10) at a peak area ratio of 83 %; minor amounts of Q-9 were also detected (Table 2, Fig. S2). This predominant quinone is typical for the members of the genus Sphingomonas (Yabuuchi et al., 1990; Takeuchi et al., 1995, 2001; Lee et al., 2001; Buonaurio et al., 2002; Ohta et al., 2004; Busse et al., 2005; Yoon et al., 2008). The polyamine pattern showed the predominance of sym-homospermidine, the key characteristic of Sphingomonas sensu stricto (Busse et al., 1999; Takeuchi et al., 2001), with minor amounts of spermine and spermidine (Table 3, Fig. S3). Quantitative differences in both quinone and polyamine profiles between strain SPC-1T and phylogenetically related members of the genus Sphingomonas could be observed (Tables 2 and 3, Figs S2 and S3).

The fatty acid profile of strain SPC-1T was composed of the following: unsaturated fatty acids C16:0t07c (19.4 %), C18:1t07c (40.2 %), C18:1t09c (0.5 %) and C22:1t09 (2.4 %); straight chain fatty acids C12:0 (0.5 %), C14:0 (0.8 %), C16:0

---

**Table 1.** Differential phenotypic characteristics of strain SPC-1T and phylogenetically related strains of other members of the genus *Sphingomonas*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony pigmentation*</td>
<td>O</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Oxidase</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Growth in/at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 % (w/v) NaCl</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>37 °C</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pH 8.0</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin hydrolysis</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>+</td>
<td>( + )</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>–</td>
<td>( + )</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Celllobiose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Citrate</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d-Fructose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>d-Fucose</td>
<td>–</td>
<td>( + )</td>
<td>( + )</td>
<td>+</td>
</tr>
<tr>
<td>l-Fucose</td>
<td>–</td>
<td>( + )</td>
<td>( + )</td>
<td>+</td>
</tr>
<tr>
<td>d-Galactose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Gentiobiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucuronate</td>
<td>( + )</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>l-Malate</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>( + )</td>
<td>( + )</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Melezitose</td>
<td>( + )</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Methyl α-D-glucopyranoside</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Raffinose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>l-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Salicin</td>
<td>( + )</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>( + )</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>( + )</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Oxidative acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>l-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Y, Yellow; O, orange.
(18.4%), C_{18:0} (8.1%); hydroxy fatty acid C_{14:0} 2-OH (5.4%); and C_{17:0} cyclo (2.0%) (Table 4, Fig. S4). The fatty acid profile confirmed the results of phylogenetic analysis. The profile was characterized by predominance of C_{18:1} \( \text{cis-7} \) and high levels of C_{16:0}, which are typical of the majority of members of the alphaproteobacteria, while the presence of 2-hydroxymyristic acid (C_{14:0} 2-OH) as the major hydroxylated fatty acid, and the absence of 3-hydroxy fatty acids are important markers of members of the family Sphingomonadaceae (Busse et al., 1999, 2003; Takeuchi & Hiraishi, 2001; Takeuchi et al., 2001). Quantitative differences in the fatty acid profiles between strain SPC-1\(^T\) and

**Table 2.** Isoprenoid quinones in strain SPC-1\(^T\) and phylogenetically related strains of other members of the genus Sphingomonas

<table>
<thead>
<tr>
<th>Strain</th>
<th>Q-9 (peak area, %)</th>
<th>Q-10 (peak area, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC-1(^T)</td>
<td>17.0</td>
<td>83.0</td>
</tr>
<tr>
<td>S. insulare DS-28(^T)</td>
<td>9.7</td>
<td>90.3</td>
</tr>
<tr>
<td>S. panni C52(^T)</td>
<td>20.2</td>
<td>79.8</td>
</tr>
<tr>
<td>S. hankookensis ODN7(^T)</td>
<td>8.9</td>
<td>91.1</td>
</tr>
</tbody>
</table>

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of Sphingomonas cynarae sp. nov. SPC-1\(^T\) with respect to other members of the genus Sphingomonas. Bootstrap values (expressed as percentages of 1000 replicates) of >50% are shown at branch points. Filled circles and ‘X’ indicate that the corresponding nodes were also recovered in trees generated with maximum-parsimony and maximum-likelihood algorithms, respectively. *Rhodospirillum rubrum* ATCC 11170\(^T\) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.
Table 3. Polyamines in strain SPC-1<sup>T</sup> and phylogenetically related strains of other members of the genus *Sphingomonas*

Polyamine concentrations are given as μmol (g dry weight)<sup>-1</sup>.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Spermidine</th>
<th>sym-Homospermidine</th>
<th>Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC-1&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.84</td>
<td>15.88</td>
<td>2.71</td>
</tr>
<tr>
<td><em>S. insulae</em> DS-28&lt;sup&gt;T&lt;/sup&gt;</td>
<td>18.63</td>
<td>11.81</td>
<td>1.04</td>
</tr>
<tr>
<td><em>S. panni</em> C52&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.70</td>
<td>30.62</td>
<td>1.62</td>
</tr>
<tr>
<td><em>S. hankookensis</em> ODN7&lt;sup&gt;T&lt;/sup&gt;</td>
<td>1.88</td>
<td>42.40</td>
<td>1.98</td>
</tr>
</tbody>
</table>

phylogenetically related members of the genus *Sphingomonas* could be also observed (Table 4). Major polar lipids identified in strain SPC-1<sup>T</sup> were sphingoglycolipid, phosphatidylycholine, phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol. Small amounts of phosphatidylinositol and phosphatidylcholine were also detected (Fig. S5). The DNA G+C content of strain SPC-1<sup>T</sup> was 66.1 mol%.

As SPC-1<sup>T</sup>, *S. insulae* DS-28<sup>T</sup>, *S. hankookensis* ODN7<sup>T</sup> and *S. panni* C52<sup>T</sup> shared high (>97%) 16S rRNA gene sequence similarity, quantitative DNA–DNA hybridization was used to discriminate between these taxa. Analysis of DNA relatedness between the pairs SPC-1<sup>T</sup>/*S. insulae* DS-28<sup>T</sup> (26.5%, reciprocal 29.6%), SPC-1<sup>T</sup>/*S. hankookensis* ODN7<sup>T</sup> (19.0%, reciprocal 23.0%) and SPC-1<sup>T</sup>/*S. panni* C52<sup>T</sup> (20.9%, reciprocal 23.1%) did not reveal any relatedness at the species level. This finding was consistent with the results of phenotypic analysis (Table 1). Many differential phenotypic properties could be observed between strain SPC-1<sup>T</sup> and its close relatives *S. insulae* DS-28<sup>T</sup>, *S. hankookensis* ODN7<sup>T</sup> and *S. panni* C52<sup>T</sup>, which were tested in parallel (Table 1). The highest degree of similarity in terms of physiological characteristics (about 73%) was found between SPC-1<sup>T</sup> and *S. insulae* DS-28<sup>T</sup>.

In liquid medium, strain SPC-1<sup>T</sup> produced a considerable amount of water-soluble EPS. Analytical methods showed that SPC-1<sup>T</sup> EPS glycosyl composition is largely different from that reported for sphingans (Fialho et al., 2008) and from that we obtained from Phytagel (Sigma-Aldrich), a commercial gellan we used for comparison. HPAPC-PAD analyses revealed mannose as the most abundant sugar with a molar ratio of 42.7 ± 4.7%, followed by rhamnose (27.5 ± 3.9 mol%), glucose (23.9 ± 3.3 mol%) and galactose (5.9 ± 1.1 mol%) (Table S2). Interestingly, glucuronic acid, the sugar conferring the anionic charge to the sphingan backbone, and galacturonic acid were not detected indicating a substantial structural diversity of the isolated SPC-1<sup>T</sup> EPS with respect to most sphingans produced by strains of the genus *Sphingomonas* (Fialho et al., 2008; White et al., 1996). To our knowledge, glucuronic acid is absent only in the EPS produced by *S. paucimobilis* P4, in which the trisaccharide structure D-glucose–D-glucose–L-rhamnose is a repeating unit structure (Lobas et al., 1994) and in the EPS produced by *Sphingomonas* sp. C51 in which glucose, mannose, fucose and rhamnose were detected in a molar ratio of 2.1:1.1:1:0.1 (Seo et al., 2004).

The phylogenetic distinctiveness, together with DNA–DNA relatedness data and differential phenotypic properties, was sufficient to allocate strain SPC-1<sup>T</sup> to a species that is distinct from recognized *Sphingomonas* species (Wayne et al., 1987; Stackebrandt & Goebel, 1994; Tindall et al., 2010). Therefore, on the basis of the presented data, strain SPC-1<sup>T</sup> is considered to represent a novel species of the genus *Sphingomonas*, for which the name *Sphingomonas cynarae* sp. nov. is proposed.

**Description of *Sphingomonas cynarae* sp. nov.**

*Sphingomonas cynarae* [cy.na’ra.e. N.L. gen. n. cynarae of *Cynara*, referring to the source of isolation *Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori (wild cardoon)].

Cells are Gram-stain-negative, non-spore-forming rods with rounded poles and are 0.4–0.5 × 1.5–3.0 μm in size. No
motility is observed by light microscopy. Colonies on TSA are convex, smooth, bright orange-pigmented and 1.5–2.0 mm in diameter after 5 days of incubation at 28 °C. Good growth occurs on TSA. Optimal temperature for growth is 28 °C. Growth does not occur at 4 °C or 37 °C. Optimal pH for growth is around 6.0–6.5. Poor growth occurs above and below this range. Growth occurs in the presence of 0–0.5% (w/v) NaCl. Anaerobic growth does not occur on TSA or nutrient agar supplemented with nitrate. Oxidase-negative, catalase-positive. L-Arabinose, arbutin, cellobiose, D-fructose, D-galactose, gentiobiose (weakly), D-glucose, 2-ketogluconate, 5-ketogluconate, lactose, maltose (weakly), D-mannose, melibiose, melezitose (weakly), L-rhamnose, sucrose, trehalose, turanose, potassium gluconate (weakly) and salicin (weakly) are utilized as sole carbon and energy sources, but N-acetylglucosamine, D-adenitol, D-arabinose, D- and L-arabitol, amygdalin, D-dulcitol, erythritol, D- and L-fucose, glyceral, glycogen, inositol, inulin, D-lyxose, D-mannitol, methyl D-D-glucopyranoside, methyl 2-D-mannopyranoside, methyl β-D-xlyopyranoside raffinose, D-ribose, D-sorbitol, L-sorbos, starch, D-tagatose, D- and L-xylose, xylitol, trisodium citrate, capric acid, adipic acid, malic acid and phenylacetic acid are not. Negative for indole production, L-arginine dihydroxylase, L-lysine decarboxylase, L-ornithine decarboxylase and urease activities, and nitrate reduction. Positive for gelatin and aesculin hydrolysis, ONPG and reduction. Positive for gelatin and aesculin hydrolysis, ONPG and reduction. Positive for gelatin and aesculin hydrolysis, ONPG and reduction. Positive for gelatin and aesculin hydrolysis, ONPG and reduction. Positive for gelatin and aesculin hydrolysis, ONPG and reduction. Positive for gelatin and aesculin hydrolysis, ONPG and reduction. Positive for gelatin and aesculin hydrolysis, ONPG and reduction. Positive for gelatin and aesculin hydrolysis, ONPG and reduction.

The type strain, SPC-1T (=JCM 17498T=ITEM 13494T), was isolated from the phyllosphere of Cynara cardunculus L. var. sylvestris (Lamk) Fiori (wild cardoon), a Mediterranean native plant that is considered to be the wild ancestor of the globe artichoke and cultivated cardoon. The DNA G+C content of the type strain is 66.1 mol% (determined by HPLC).

Acknowledgements

This work was partially supported by grants from Italian MIUR to P.A. (Project PRIN-COFIN 2008).

References


