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# Sporobacterium olearium gen. nov., sp. nov., a new methanethiol-producing bacterium that degrades aromatic compounds, isolated from an olive mill wastewater treatment digester

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A strictly chemo-organotrophic, anaerobic bacterium was isolated from an olive mill wastewater treatment digester on syringate and designated strain SR1$^T$. The cells were slightly curved rods, stained Gram-positive and possessed terminal spores. Strain SR1$^T$ utilized crotonate, methanol and a wide range of aromatic compounds including 3,4,5-trimethoxybenzoate (TMB), 3,4,5-trimethoxycinnamate (TMC), syringate, 3,4,5-trimethoxyphenylacetate (TMPA), 3,4,5-trimethoxyphenylpropionate (TMPP), ferulate, sinapate, vanillate, 3,4-dimethoxybenzoate, 2,3-dimethoxybenzoate, gallate, 2,4,6-trihydroxybenzoate (THB), pyrogallol, phloroglucinol and quercetin as carbon and energy sources. Acetate and butyrate were produced from aromatic compounds, methanol and crotonate whereas methanethiol (MT) was produced from methoxylated aromatic compounds and methanol. Strain SR1$^T$ had a G+C content of 38 mol% and grew optimally between 37 and 40 °C at pH 7·2 on a crotonate-containing medium. Phylogenetically, strain SR1$^T$ was a member of cluster XIVa of the Clostridiales group and shared a sequence similarity of 90% with Clostridium aminovalericum and Eubacterium fissicatena. Consequently, its precise neighbourliness to any one of them depended on the selection of strains of the cluster. On the basis of the phylogenetic and phenotypic evidence presented in this paper, the designation of strain SR1$^T$ as Sporobacterium olearium gen. nov., sp. nov. is proposed. The type strain is SR1$^T$ (= DSM 12504$^T$).

## INTRODUCTION

Anaerobic degradation of aromatic compounds by pure cultures under fermentative conditions is possible with highly oxidized compounds. These include polyhydroxylated aromatic compounds such as resorcinol, phloroglucinol and gallate, which can be degraded by Coprococcus spp. (Patel et al., 1981), Eubacterium oxidoreducens (Krumholz & Bryant, 1986), Pelobacter acidigallici (Schink & Pfennig, 1982) and Pelobacter massiliensis (Schnell et al., 1991). In the case of Eubacterium oxidoreducens, but not Pelobacter or Coprococcus species, formate or hydrogen is necessary for growth and aromatic compound degradation. In other cases, phenolic compounds which do not contain hydroxyl groups in the meta-position, e.g. hydroquinone (1,4-dihydroxybenzene), have been shown to be degraded by the fermentative bacterium Syntrophus gentianae (Szewzyk & Schink, 1989). In general monohydroxylated aromatic compounds are not degraded by pure cultures of fermentative bacteria, but recently a new isolate, designated Sporotomaculum hydroxybenzoicum (Brauman et al., 1998), was re
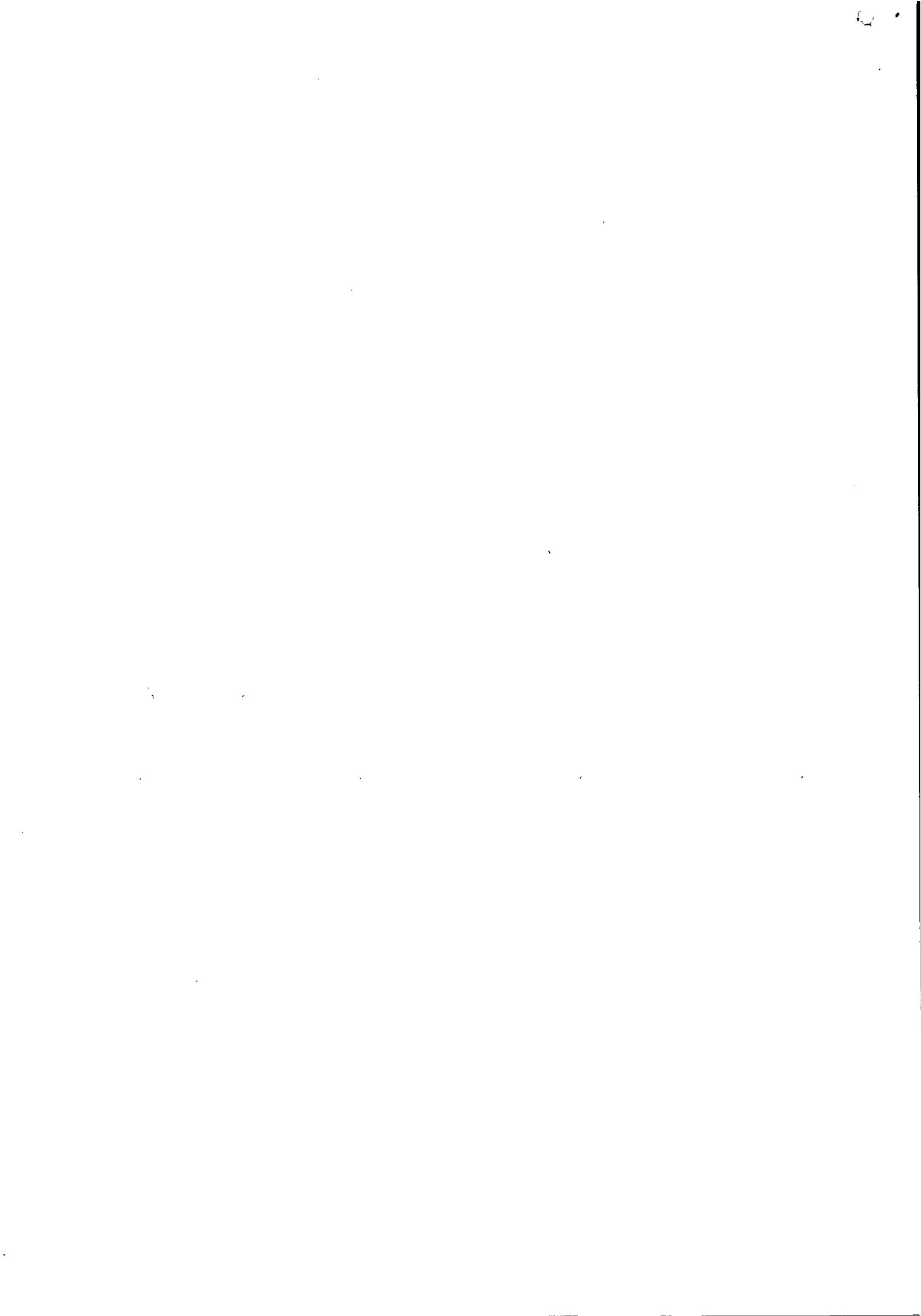
ported to use 3-hydroxybenzoate as the sole carbon source, suggesting that the degradation of the substrate was carried out via the benzoyl-CoA pathway subsequent to the removal of the hydroxyl group. *Holophaga foetida* (Liesack *et al.*, 1994) and *Sporobacter termitidis* (Grech-Mora *et al.*, 1996) represent yet another group of fermentative, aromatic compound-degrading bacteria which have been reported to transfer an ether-linked methyl group to sulfide, resulting in the formation of methanethiol (MT) and dimethylsulfide (DMS). The demethylated aromatic compound that is produced is further degraded to acetate, via the phloroglucinol pathway. The ability to degrade aromatic compounds appears to be widely distributed in different phylogenetic groups: for example, *H. foetida*, *P. acidigallici* and *P. massiliensis* are members of the *Proteobacteria*, whereas *Sporobacter termitidis* and *Coprococcus* spp. are members of the sub-branch of the low-G+C-content Gram-positive clostridial group. This paper reports the isolation and characterization of a new bacterium that ferments aromatic compounds to acetate and butyrate, with the production of MT from methyl groups and sulfide.

## METHODS

**Source of strains.** Strain SR1$^T$ was isolated from enrichment cultures that had been initiated with the liquid content of an anaerobic methanogenic digester fed with olive mill wastewater. The digester samples were collected anaerobically using N$_2$-flushed syringes. Strain SR1$^T$ was routinely cultured and maintained in the basal medium supplemented with 5 mM syringate, as described below.

**Culture media.** A basal medium used for enrichment, isolation and routine cultivation was prepared using the anaerobic techniques described by Hungate (1969), as modified for use with syringes (Macy *et al.*, 1972; Miller & Wollin, 1974). The basal medium contained (per litre of deionized water): 1 g NH$_4$Cl, 0·3 g K$_2$HPO$_4$, 0·3 g KH$_2$PO$_4$, 0·6 g NaCl, 0·1 g CaCl$_2$.2H$_2$O, 0·2 g MgCl$_2$.6H$_2$O, 0·1 g KCl, 0·5 g cysteine.HCl, 1 g yeast extract (Difco), 1·5 ml trace-element solution (Widdel & Pfennig, 1981) and 1 mg resazurin. The pH was adjusted to 7·0 with 10 M KOH solution and the medium boiled under a stream of O$_2$-free N$_2$ gas and cooled to room temperature; 5 ml aliquots were dispensed into Hungate tubes under N$_2$/CO$_2$ (80:20%, v/v) and subsequently sterilized by autoclaving at 110 °C for 45 min. Prior to use, 0·2 ml 5% (w/v) NaHCO$_3$ and 0·05 ml 2·5% (w/v) Na$_2$S.9H$_2$O were injected from sterile stock solutions into pre-sterilized basal medium.

**Enrichment and isolation.** A 0·5 ml sample of liquid from the digester was inoculated into 5 ml basal medium containing 5 mM syringate; this was followed by incubation at 37 °C. The enrichment culture was subcultured several times under the same conditions and then serial 10-fold dilutions were inoculated into roll tubes containing basal medium supplemented with 5 mM syringate and 1·6% (w/v) agar. Several single well-isolated colonies that developed were picked and resuspended; the purification procedure was then repeated several times. One of these cultures was designated strain SR1$^T$ and characterized further.

**Physiological tests.** Substrate- and electron-acceptor utilization, pH, temperature and NaCl growth-range determin-

ations were performed as described by Mechichi *et al.* (1999).

**Light microscopy and electron microscopy.** Light- and electron microscopy were performed as described previously (Fardeau *et al.*, 1997).

**Analytical methods.** Growth was measured at 580 nm by inserting anaerobic Hungate tubes directly into the cuvette holder of a Shimadzu model UV 160A spectrophotometer. Aromatic compounds were measured by HPLC with a model 1100 chromatograph equipped with a UV detector set at 240 nm and a 100-position autosampler/autoinjector (Hewlett Packard). Separation was achieved using a C$_{18}$ SYMMETRY (4·6 × 100 mm, 3·5 μm particle size) column (Waters Chromatography) maintained at 35 °C. The mobile phase, consisting of a mixture of two solvents (water in 0·01% acetic acid and methanol) was used at a flow rate of 0·75 ml min$^{-1}$. For separation of aromatic compounds, a 20% methanol solvent phase was initially held for 4 min, then the concentration was increased to 40% over a period of 24 min; this was followed by washing for 6 min, during which the concentration of methanol was increased to 100%. The column was re-equilibrated with 20% methanol for at least 5 min before the next injection. Quantification was performed using external standards.

Acetate and butyrate were measured by HPLC (Spectra Series 100 model; Thermo Separation Products) equipped with an Aminex HPX-87X column 300 × 7·8 mm (i.d.) (Bio-Rad) connected to a differential refractometer (RID-6A; Shimadzu). Analysis was performed using a CR-6A Shimadzu integrator. The mobile phase was 0·005 N H$_2$SO$_4$ at a flow rate of 0·6 ml min$^{-1}$, the temperature of the column was 35 °C, and the volume of the injection loop was 20 μl.

MT was detected on a Chromosorb WAW 80/100-mesh sp100 column (Alltech) using a Girdel gas chromatograph (Girdel) equipped with a flame-ionization detector and a C-R6A Chromatopac integrator (Shimadzu). N$_2$ was used as the carrier gas.

**Determination of G+C content.** DNA was isolated and purified and the G+C content was determined by using HPLC, as described by Mesbah *et al.* (1989), at the DSMZ–Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. Unmethylated λ DNA (Sigma) was used as the standard.

**DNA extraction and amplification of the 16S rRNA gene.** DNA was extracted from the isolate as described previously (Redburn & Patel, 1993; Andrews & Patel, 1996). The universal primers Fd1 and Rd1 were used to obtain a PCR product of approximately 1·5 kb corresponding to base positions 8–1542, on the basis of *Escherichia coli* numbering of the 16S rDNA (Winker & Woese, 1991). A 50 μl reaction contained 1–20 ng genomic DNA, 1 μM in the case of each primer, 5 μl 10 × buffer, 200 μM dNTP, 3·5 mM MgCl$_2$ and 2·5 U *Taq* polymerase (Promega). PCR was carried out using an initial denaturation at 94 °C for 7 min, then 29 cycles of annealing at 55 °C for 2 min, extension at 72 °C for 4 min, denaturation at 94 °C for 1 min and, finally, an extension cycle comprising 55 °C for 2 min and 72 °C for 20 min.

**Direct sequencing of PCR products.** PCR products were purified using the QIAquick Kit (Qiagen). The DNA concentration of the purified PCR product was estimated by comparison with the Low Mass Ladder (Gibco-BRL) on an agarose gel containing ethidium bromide. QIAquick-purified PCR products were sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Kit containing Ampli*Taq*

FS DNA polymerase and the ABI 373A sequencer. A 10 µl reaction contained 35 ng PCR product, 4 µl cycle sequencing reaction mix, 3·2 pmol primer and 2·5 µg BSA. Thermal cycling was carried out using a RapidCycler (Idaho Technology) at a temperature transition slope of 2, an initial denaturation at 94 °C for 15 s, then 25 cycles of denaturation at 94 °C for 0 s, annealing at 50 °C for 10 s and extension at 60 °C for 3 min.

**Sequence alignments and phylogenetic inferences.** The new sequence data generated were aligned and an almost full-length consensus 16S rRNA gene sequence was assembled and checked for accuracy manually using the alignment editor, ae2 (Maidak *et al.*, 1997). These sequence data were compared with other sequences in the GenBank database (Benson *et al.*, 1993) using BLAST (Altschul *et al.*, 1997) and, in the Ribosomal Database Project, version 7.0, using SIMILARITY_rank and SUGGEST_tree (Maidak *et al.*, 1997). Pairwise evolutionary distances based on 978 unambiguous nucleotides were computed using DNADIST (Jukes & Cantor, 1969) and neighbour-joining programs that form part of the PHYLIP suite of programs (Felsenstein, 1993). TREECON was used extensively for bootstrap analysis (Van de Peer & De Wachter, 1993).

## RESULTS

### Enrichment and isolation

An enrichment culture that was positive for growth on syringate after 2 weeks incubation at 37 °C was serially diluted and subcultured several times. A stable microbial consortium that developed degraded syringate with concomitant production of acetate and butyrate and consisted of a morphologically dominant bacterial population. Subsequently, this enrichment culture was serially diluted and used to inoculate roll tubes. Colonies developed within 4 weeks incubation, after which single, well-isolated colonies were picked; this procedure was repeated several times. A pure culture, designated as strain SR1$^T$, was selected for further characterization.

### Cellular morphology

Cells of strain SR1$^T$ were slightly curved rods (5–10 µm × 0·4–0·8 µm) (Fig. 1a). Some cells possessed terminal spherical spores that caused distension of the sporangium. The cells stained Gram-positive and electron micrographs of thin sections of the cells showed the presence of a thick, bilayered cell wall structure (Fig. 1b) resembling Gram-positive-type cell walls. Cells were motile: negatively stained cells showed evidence of peritrichous flagella (Fig. 1c).

### Effects of temperature, pH and NaCl

The temperature range for growth was 25–45 °C, the optimal temperature for growth being between 37 and 40 °C. The pH range for growth was between 6·5 and 8·5, with an optimum at 7·2. The NaCl concentration range for growth was between 0 and 30 g l$^{-1}$, the optimal concentration for growth being between 1 and 3 g l$^{-1}$
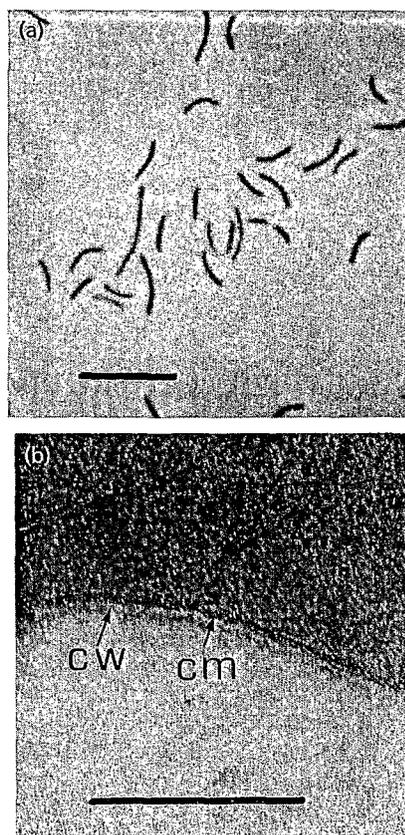


*Fig. 1.* (a) Phase photomicrograph of strain SR1$^T$ depicting curved cells. Bar, 10 µm. (b) Transmission electron micrograph of the cell wall ultrastructure of strain SR1$^T$ showing the presence of cytoplasmic membranes (cm) and a thick electron-dense cell wall (cw). Bar, 0·2 µm.

### Substrate utilization

Strain SR1$^T$ used crotonate (20 mM), methanol (20 mM) and some aromatic compounds (5 mM; 2,3-dimethoxybenzoate, 3,4-dimethoxybenzoate, TMB, TMC, TMPA, TMPP, syringate, sinapate, vanillate, ferulate, gallate, 2,4,6-trihydroxybenzoate, pyrogallol, phloroglucinol and quercetin) but was unable to utilize carbohydrates (20 mM; glucose, fructose, sucrose, xylose, sorbose, galactose, *myo*-inositol, lactose, ribose, mannitol and cellobiose), organic acids (20 mM; formate, fumarate, pyruvate, malonate and succinate), alcohols (20 mM; ethanol, propanol and butanol), H$_2$ + CO$_2$ (80:20%, at 200 kPa) or a few aromatic compounds (5 mM; benzoate, 2-, 3-, 4-methoxybenzoates and 2,4-, 2,5-, 2,6- and 3,5-dimethoxybenzoates). Yeast extract (0·2%) stimulated (but was not required for) growth.

### Fermentation end products

Acetate and butyrate were produced from gallate, THB, pyrogallol, phloroglucinol, quercetin and crotonate, whereas acetate, butyrate and MT were produced

**Table 1.** Fermentation end products from aromatic compound degradation

DHB, dihydroxybenzoate; DHC, dihydroxycinnamate; ND, no aromatic structure detected as the ring was cleaved; UI, unidentified product.

| Aromatic compounds tested (5 mM) | End products produced in presence of $H_2$ | | | End products produced in absence of $H_2$ | | |
|---|---|---|---|---|---|---|
| | Acetate (mM) | Butyrate (mM) | Aromatic structure detected in end products | Acetate (mM) | Butyrate (mM) | Aromatic structure detected in end products |
| 2,3-Dimethoxybenzoate* | 4·2 | <1 | Catechol and 2,3-DHB | 4 | <1 | Catechol and 2,3-DHB |
| 3,4-Dimethoxybenzoate* | 4·4 | <1 | Catechol and 3,4-DHB | 4·2 | <1 | Catechol and 3,4-DHB |
| TMB*† | 6·5 | 4·5 | ND | 8·2 | 2·5 | Pyrogallol |
| TMC*† | 6·4 | 4·6 | ND | 8 | 2·5 | Pyrogallol |
| TMPA*† | 6 | 3·8 | Pyrogallol | 7·6 | 2 | Pyrogallol |
| TMPP*†. | 6·2 | 4 | Pyrogallol | 7·8 | 2·2 | Pyrogallol |
| Syringate*† | 7 | 5 | ND | 10 | 3 | Pyrogallol |
| Vanillate* | 5 | <1 | Catechol and 3,4-DHB | 5 | <1 | Catechol and 3,4-DHB |
| Ferulate* | 4·8 | <1 | Catechol and 3,4-DHC | 4 | <1 | Catechol and 3,4-DHC |
| Gallate† | 5·6 | 4·3 | ND | 7 | <1 | Gallate |
| THB† | 6 | 4 | ND | 6·8 | <1 | THB |
| Pyrogallol† | 6 | 4·2 | ND | 8 | <1 | Pyrogallol |
| Phloroglucinol† | 6·2 | 4·4 | ND | 8·5 | <1 | Phloglucinol |
| Quercetin† | 10 | 6 | UI | 11 | <1 | UI |

* Compounds from which MT was produced.

† Compounds in which the aromatic rings were cleaved.

from 2,3-dimethoxybenzoate, 3,4-dimethoxybenzoate, TMC, TMB, TMPA, TMPP, syringate, sinapate, vanillate, ferulate and methanol (Table 1).

### Effect of the gas phase on aromatic-compound degradation by strain SR1ᵀ

Concentrations of syringate as high as 12 mM were completely transformed within 4 d incubation in the presence of $H_2$ but after 7 d in the absence of $H_2$. $H_2$ was also observed to accelerate the growth of strain SR1ᵀ on syringate and to shorten the growth lag time (4 d in the absence of $H_2$ but 1 d in the presence of $H_2$) (Fig. 2a). The ratio of acetate to butyrate produced was 10:9 when $H_2$ was present and 16:2 when $H_2$ was absent (Fig. 2b); the net carbon equivalent volatile fatty acids was also higher (60 mM) in the presence of hydrogen than in its absence (40 mM) (Fig. 2c).

### Electron acceptors

Sulfate, thiosulfate, sulfite, nitrate, elemental sulfur and fumarate were not used as electron acceptors

### G+C content

The G+C content of strain SR1ᵀ was 38 mol% as determined by the HPLC method.

### 16S rRNA sequence analysis

Using 12 primers, we determined an almost complete sequence consisting of 1511 bases of the 16S rRNA gene of strain SR1ᵀ (corresponding to *E. coli* positions 13–1532). Phylogenetic analysis revealed that strain SR1ᵀ was a member of cluster XIVa of the low G+C Gram-positive branch as defined by Collins *et al.* (1994). The closest relatives were *Eubacterium fissicatena* or *Clostridium aminovalericum* (with a sequence similarity of 90%) depending on the selection of representative members of the cluster in tree construction. Fig. 3 represents a dendrogram generated by the neighbour-joining method of the Jukes–Cantor evolutionary distance matrix (Jukes & Cantor, 1969) in which strain SR1ᵀ is located next to *Clostridium aminovalericum*.

### DISCUSSION

#### Ecology

Strain SR1ᵀ was isolated from an anaerobic digester fed with olive mill wastewater. It utilized a range of carbon and energy sources composed exclusively of aromatic compounds, methanol and crotonate. The presence of such a strain in the olive mill wastewater treatment digester is not surprising as the wastewater contains a wide range of aromatic compounds that are released from the olive cell walls during the oil-extraction process (Capasso *et al.*, 1995). Because the
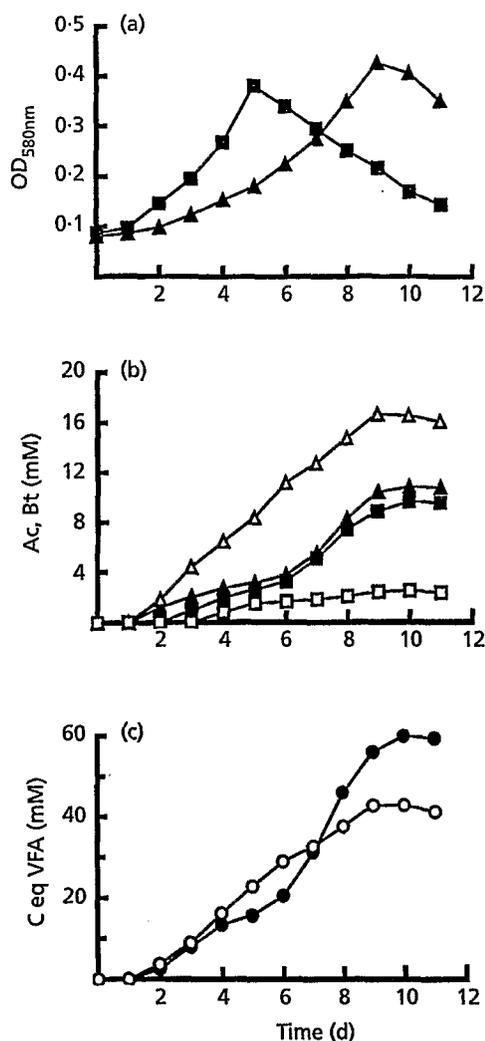
**Fig. 2.** (a) Growth of strain SR1^T in a basal medium containing 12 mM syringate (▲) and 12 mM syringate plus hydrogen (■). (b) End products from 12 mM syringate degradation by strain SR1^T in the presence of hydrogen [acetate (▲) and butyrate (■)] or in the absence of hydrogen [acetate (△) and butyrate (□)]. Ac, acetate; Bt, butyrate. (c) The total carbon transformed to acetate and butyrate by strain SR1^T during degradation of 12 mM syringate (represented as carbon equivalent volatile fatty acid, which is the sum of the carbon transformed to acetate and the carbon transformed to butyrate) in the presence of hydrogen (●) and in the absence of hydrogen, (○). C eq VFA, carbon equivalent volatile fatty acid.

aromatic ring of methoxylated aromatic compounds cannot be cleaved, demethoxylation is the initial key reaction for subsequent degradation of these compounds. Strain SR1^T and several other strains isolated from the same digester have been observed to demethoxylate aromatic compounds efficiently (unpublished results).

## Physiology

The cleavage of aromatic compounds such as methoxybenzoates (syringate, TMB) by strain SR1^T is a trait shared by *H. foetida* and *Sporobacter termitidis*.

However, there are numerous physiological differences that distinguish the former from the two latter species. Strain SR1^T and *H. foetida* have the ability to degrade a wide range of aromatic substrates but *Sporobacter termitidis* has a limited capacity; for example, it is unable to degrade trihydroxybenzenes and trihydroxybenzoates. Strain SR1^T produces acetate and butyrate from aromatic compound degradation and produces MT from sulfide and methoxyl groups of aromatic compounds. However, *H. foetida* and *Sporobacter termitidis* produce acetate as the only end product from aromatic compound degradation and MT and produce DMS from sulfide and methoxyl groups of aromatic compounds. DMS formation has been shown to proceed via MT (Bak *et al.*, 1992) but in the case of strain SR1^T DMS has never been detected, indicating that this strain is unable to transfer the methyl group to MT.

Both strain SR1^T and *Eubacterium oxidoreducens* have the ability to degrade trihydroxybenzenes (gallate, pyrogallol and phloroglucinol) and quercetin. However, *Eubacterium oxidoreducens* has an obligate requirement for hydrogen or formate as an external electron donor for this purpose. Strain SR1^T is able to degrade both trihydroxybenzenes and quercetin in the absence of hydrogen. In the presence of hydrogen it is able to degrade higher concentrations of trihydroxybenzenes and quercetin. The addition of formate as an electron donor has no effect on their degradation. A final difference is that *Eubacterium oxidoreducens* is unable to utilize methoxylated aromatic compounds and cannot produce MT or DMS, unlike strain SR1^T.

## Phylogeny and taxonomy

Phylogenetically, strain SR1^T is related to either *Eubacterium fissicatena* (Taylor, 1972) or *Clostridium aminovalericum* (Hardman & Stadtman, 1960) depending on which representative sequences are selected for use in tree construction. Therefore, the clustering of strain SR1^T with *Clostridium aminovalericum* depicted in Fig. 3 should be regarded as tentative until its position can be predicted with a greater degree of confidence. This would be likely to occur when other related members have been sequenced and added to this cluster.

The physiological and phenotypic data clearly show that strain SR1^T is different from both *Eubacterium fissicatena* and *Clostridium aminovalericum*. Strain SR1^T is a slightly curved, motile and spore-forming bacterium that utilizes aromatic compounds but not carbohydrates whereas *Eubacterium fissicatena* is a non-sporulating, rod-shaped bacterium that lacks motility and utilizes carbohydrates but not aromatic compounds. On the other hand, *Clostridium aminovalericum* is a motile spore-former like strain SR1^T but is rod-shaped, ferments carbohydrates and is not reported to utilize aromatic compounds. These and other major differences between the three strains are shown in Table 2.
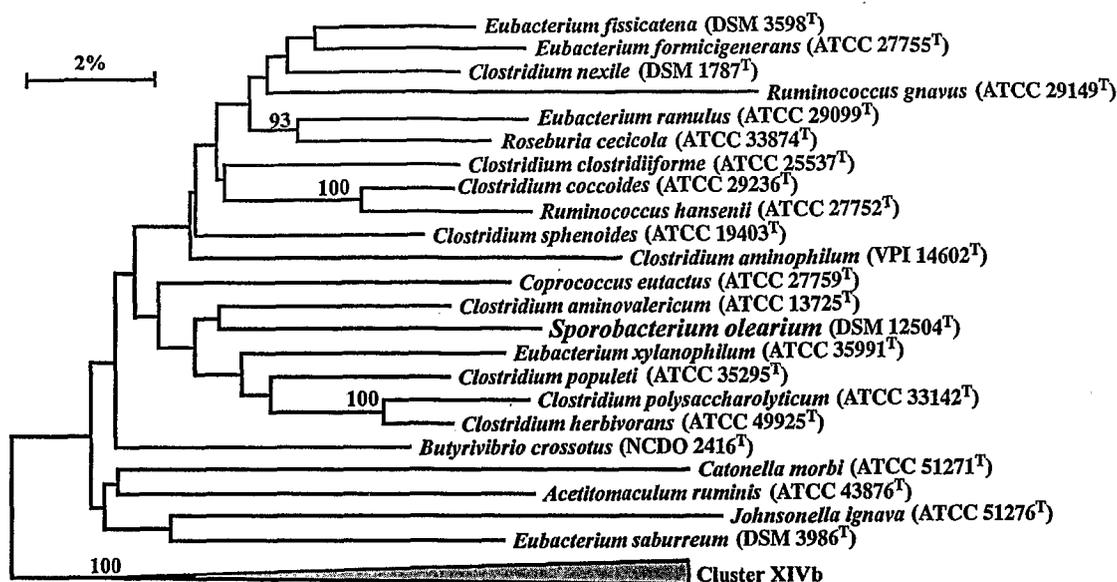
Fig. 3. Sporobacterium olearium (strain SR1$^T$ = DSM 12505$^T$) is a member of cluster XIVa of the Clostridiales group and is equidistant from Clostridium aminovalericum and Eubacterium fissicatena, its precise relationship to any one of them depending on the selection of strains of cluster XIVa. In this case the unrooted dendrogram depicted in the figure shows the nearest phylogenetic relative of Sporobacterium olearium to be Clostridium aminovalericum when members of the listed set of representatives of cluster XIVa are used in the analysis. The sequences represented in cluster XIVb (indicated by the shaded triangular region) were used as outgroups and include Clostridium lentocellum and Clostridium propionicum. The clusters are defined as suggested by Collins et al. (1994). All of the sequences used in the analysis were obtained from the Ribosomal Database Project, version 7.0 (Maidak et al., 1997). Bootstrap values, expressed as a percentage of 100 replications, are shown at the branching points. Only values above 90 % were considered significant and therefore reported. Bar, 2 nucleotide substitutions per 100 nucleotides.

**Table 2.** Differentiating characteristics for strain SR1$^T$, Eubacterium fissicatena and Clostridium aminovalericum

The characteristics of strain SR1$^T$ were determined in this study. Culturing was performed in basal medium (pH 7) supplemented with substrates; the results were recorded after 2 weeks incubation at 37 °C. Data for Eubacterium fissicatena and Clostridium aminovalericum were taken from Taylor (1972) and Hardman & Stadtman (1960), respectively. +, Positive reaction or present; −, negative reaction or absent; NR, not reported.

| Characteristic | Strain SR1$^T$ | E. fissicatena | C. aminovalericum |
| --- | --- | --- | --- |
| Cell shape | Curved rods | Straight rods | Straight rods |
| Cell size (μm) | 0·4–0·8 × 5–10 | 0·3–0·5 × 0·5–1 | 0·3–0·5 × 1·5–5·2 |
| Motility | + | − | + |
| Sporulation | + | − | + |
| pH optimum | 7·2 | NR | NR |
| Temperature optimum (°C) | 37–40 | 37 | 37 |
| Temperature range (°C) | 20–45 | 13–37 | 25–45 |
| G+C content (mol %) | 38 | 45 | 33 |
| Utilization of: | | | |
| Glucose | − | + | + |
| Aromatics* | + | − | − |
| Proteins† | − | + | + |
| Aminovalerate | − | NR | + |
| Yeast extract (0·2 %) | − | + | NR |

* Utilized at 5 mM: TMB, TMC, TMPA, TMPP, syringate, vanillate, ferulate, 3,4-dimethoxybenzoate, 2,3-dimethoxybenzoate, gallate, THB, pyrogallol, phloroglucinol and quercetin.

† Peptone, meat extract.

Strain SR1$^T$ is physiologically unique and is the first aromatic compound-degrading bacterium that is also capable of utilizing methanol and producing acetate, butyrate and MT as end products of degradation. This, together with the phylogenetic and physiological evidence presented above, leads us to propose the designation of strain SR1$^T$ as *Sporobacterium olearium* gen. nov., sp. nov.

## Description of *Sporobacterium* gen. nov.

*Sporobacterium* (Spo.ro.bac.ter'ium. M.L. n. *spora* spore; Gr. neut. n. *baktron* rod; M.L. neut. n. *Sporobacterium* spore-forming rod).

A spore-forming slightly curved rod that stains Gram-positive. It is motile, strictly anaerobic and ferments aromatic compounds, methanol and crotonate to produce MT from methyl groups and sulfide. The type species is *Sporobacterium olearium*.

## Description of *Sporobacterium olearium* sp. nov.

*Sporobacterium olearium* (o.le.a'ri.um. L. adj. *olearius* -a -um of oil, related to olive oil, referring to the isolation of strain SR1$^T$ from an olive mill wastewater treatment digester).

A slightly curved rod with a terminal spore. Stains Gram-positive. Motile, with peritrichous flagella. Strictly anaerobic chemo-organotrophic. Grows on crotonate, methanol, TMB, TMC, syringate, TMPA, TMPP, ferulate, sinapate, vanillate, 3,4-dimethoxybenzoate, 2,3-dimethoxybenzoate, gallate, THB, pyrogallol, phloroglycinol and quercetin. No growth with benzoate, 2-, 3-, 4-methoxybenzoates, 2,4-, 2,5-, 2,6-, 3,5-dimethoxybenzoates, glucose, fructose, sucrose, xylose, sorbose, galactose, *myo*-inositol, lactose, ribose, mannitol, cellobiose, formate, fumarate, pyruvate, malonate, succinate, ethanol, propanol, butanol or H$_2$ + CO$_2$. Yeast extract stimulates (but is not required for) growth. TMB, TMC, TMPA, TMPP, syringate, sinapate, 2,3-dimethoxybenzoate, 3,4-dimethoxybenzoate, vanillate, ferulate and methanol are fermented to acetate, butyrate and MT. Gallate, THB, pyrogallol, phloroglucinol and crotonate are fermented to acetate and butyrate. H$_2$ is not required for growth but is required for complete degradation of gallate, THB, pyrogallol and phloroglucinol. Sulfate, thiosulfate, sulfite, nitrate, elemental sulfur and fumarate are not reduced. The optimal growth temperature is between 37 and 40 °C (the temperature growth range being 25–45 °C). The optimum pH for growth is 7·2 (the pH growth range being between 6·5 and 8·5). Grows with NaCl concentrations between 0 and 30 g l$^{-1}$ but not with 35 g l$^{-1}$. The DNA G + C content is 38 mol%. The habitat is an anaerobic methanogenic digester fed with olive mill wastewater (Tunisia). The type strain is SR1$^T$ ( = DSM 12504$^T$).

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