**TAXONOMY/SYSTEMATICS**

**Eubacterium aggregans** sp. nov., a New Homoacetogenic Bacterium from Olive Mill Wastewater Treatment Digestor

Tahar Mechichi¹, Marc Labat¹, Tony H. S. Woo², Pierre Thomas³, Jean-Louis Garcia¹ and Bharat K. C. Patel²

A strictly anaerobic, homoacetogenic, Gram-positive, non spore-forming bacterium, designated strain SR12T (T= type strain), was isolated from an anaerobic methanogenic digestor fed with olive mill wastewater. Yeast extract was required for growth but could also be used as sole carbon and energy source. Strain SR12T utilized a few carbohydrates (glucose, fructose and sucrose), organic compounds (lactate, crotonate, formate and betaine), alcohols (methanol), the methoxyl group of some methoxylated aromatic compounds, and H₂+CO₂. The end-products of carbohydrate fermentation were acetate, formate, butyrate, H₂ and CO₂. End-products from lactate and methoxylated aromatic compounds were acetate and butyrate. Strain SR12T was non-motile, formed aggregates, had a G+C content of 55 mol % and grew optimally at 35°C and pH 7.2 on a medium containing glucose. Phylogenetically, strain SR12T was related to *Eubacterium barkeri*, *E. callanderi*, and *E. limosum* with *E. barkeri* as the closest relative (similarity of 98%) with which it bears little phenotypic similarity or DNA homology (60%). On the basis of its phenotypic, genotypic, and phylogenetic characteristics, we propose to designate strain SR12T as *Eubacterium aggregans* sp. nov. The type strain is SR12T (= DSM 12183).

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**Introduction**

A large number of anaerobic acetogenic and sulfate-reducing bacteria are involved in the degradation of one-carbon compounds such as methoxyl groups from aromatic compounds, methanol, formate, CO, H₂, CO₂, betaine, sarcosine, and methylamine. Acetate is the main end product produced from these substrates. However, in some strains acetate and butyrate or acetate, butyrate, and formate can be formed. Methoxyl groups from aromatic compounds...
can serve as growth substrate for many acetogenic bacteria such as Acetobacterium carbinolicum [1], A. malicum [2], A. woodii [3], Butyribacterium methylotrophicum [4], Clostridium formicoaceticum [5], C. perfringii [6], Morella thermoacetica (formerly C. thermoacetica) [7], Eubacterium limosum [8], E. callanderi [9], Sporomusa termitida [10], and S. silvaecetica [11]. Most of these strains belong to the Gram-positive bacteria with low G+C content. Acetobacterium woodii, M. thermoacetica, E. limosum, and S. termitida use a wide range of one carbon compounds in contrast to E. callanderi, which is only able to use methoxyl groups from aromatic compounds (ferulate, sinapate, syringate, trimethoxycinnamate, and vanillate). We report on the isolation and the characterization of a new homoacetogenic bacterium, Eubacterium aggregans. sp. nov. which specifically degrades methoxylated aromatic compounds.

**Materials and Methods**

**Source of strains**

Strain SR12T was isolated from enrichment cultures from an anaerobic methanogenic digester fed with olive mill wastewater. Liquid samples were collected anaerobically from the digester using N2-flushed syringes and inoculated into the basal medium. *Eubacterium barkeri* (DSM 1223) used as a reference culture was purchased from the DSMZ-Deutsche Sammlung von Microorganismen und Zellkulturen GmbH, Braunschweig, Germany. The reference culture was routinely grown using glucose containing basal medium.

**Culture media**

The basal medium used for enrichment, isolation, and cultivation was prepared using anaerobic techniques [12–14]. It contained (per liter of deionized water): 1 g of NH4Cl, 0.3 g of K2HPO4, 0.3 g of KH2PO4, 0.6 g of NaCl, 0.1 g of CaCl2·2H2O, 0.2 g of MgCl2·6H2O, 0.1 g of KCl, 0.5 g of cysteine–HCl, 1 g of yeast extract (Difco Laboratories), 1.5 ml of trace element solution [15], and 1 mg of resazurin. pH was adjusted to 7 with 10 M KOH solution, the medium was then boiled under a stream of O2-free N2 gas and cooled to room temperature. Five-milliliters aliquots of basal medium were dispensed into Hungate tubes, degassed under N2–CO2 (80:20% v/v) and subsequently sterilized by autoclaving at 110°C for 45 min. Prior to culture inoculation, 0.2 ml of 5% (w/v) NaHCO3 and 0.05 ml of 2.5% (w/v) Na2S·9H2O were injected from sterile stock solutions into the tubes.

**Enrichment and Isolation**

A 0.5-ml aliquot of sample was inoculated into Hungate tubes containing 5 ml of basal medium and 5 mM syringic acid. Tubes were incubated at 37°C. To obtain pure cultures, the enrichment was subcultured several times under the same growth condition and subsequently serially diluted ten-fold in roll tubes containing basal medium, 5 mM syringic acid and 1.6% agar. Single colonies that developed were picked up and this procedure was repeated several times until only one type of colony was observed.

**Substrate utilization**

Experiments were performed in duplicate with inocula that had been subcultured under the same test conditions at least once. The following substrates were tested: 26 mM carbohydrates (glucose, fructose, xylose, sorbose, galactose, myo-inositol, sucrose, lactose, cellobiose, mannitol, and ribose) and 10 g/L xylan and cellulose, 20 mM organic acids (formate, fumarate, pyruvate, crotonate, malonate, and succinate), 5 mM monomethoxylated aromatic compounds (2-, 3- and 4-methoxybenzoates), 5 mM dimethoxylated aromatic compounds (2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dimethoxybenzaldehydes), 5 mM dimethoxylated aromatic compounds (2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dimethoxybenzoates), 5 mM trimethoxylated aromatic compounds (3,4,5-trimethoxycinnamate, 3,4,5-trimethoxybenzoate), 5 mM mixed methoxylated/ hydroxylated aromatic compounds (4-hydroxy-3,5-dimethoxybenzoate (syringate), 4-hydroxy-3,5-dimethoxycinnamate (sinapate), 3-hydroxy-4-methoxybenzoate, 4-hydroxy-3-methoxybenzoate (vanillate), 4-hydroxy-3-methoxycinnamate (ferulate), 3-hydroxy-4-methoxycinnamate, 3-hydroxy-4-methoxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde (vanillin), and 4-hydroxy-3-methoxycinnamylalcohol), 20 mM alcohols (methanol, ethanol, and glycerol), and 20 mM betaine. Substrate stock solutions (100 ×) were prepared, neutralized if required, sterilized by filtration (pore size 0.2 μm, Millipore), rendered anaerobic by gassing with O2-free N2, and injected into Hungate tubes that contained presterilized media. The N2 in Hungate tubes was replaced with H2+CO2 (20:80%) (final pressure of 2 bars gas phase) for some experiments after inoculation. An increase in optical density (OD) of 0.1 at 580 nm in tubes containing the added substrate over control tubes lacking the added substrate, was considered as positive for growth. Carbon source degradation and production of volatile fatty acid, as determined by high-performance liquid chromatography (HPLC), were also used as indicators of growth.

Titre original : *Eubacterium aggregans* sp. nov., a new homoacetogenic bacterium from olive mill wastewater treatment digestor.


Titre en Français : *Eubacterium aggregans* sp. nov., une nouvelle bactérie homoacétogène isolée d’un digesteur traitant des effluents d’huilerie d’olives.

Mots-clés matières : *Eubacterium aggregans*, phylogénie, taxonomie, anaérobiose, olives, aromatiques, o-déméthylation, homoacétogénèse

Résumé en Français :
(150 mots maximum)

Plan de classement : Monde végétal et Animal - Fermentations
Characterization of *Eubacterium aggregans* sp. nov.

**Electron acceptors**

Sulfate, thiosulfate, sulfite, nitrate, elemental sulfur, and fumarate were tested as electron acceptors at a final concentration of 10 mM in basal medium containing 5 mM syringate.

**pH, Temperature, and NaCl concentration ranges**

The pH of the pre-reduced anaerobic basal medium was adjusted by injecting 5% NaHCO₃, 5% Na₂CO₃, or 0.1 M HCl to give a pH range between 5 to 9.5. Appropriate amounts of NaCl was weighed directly into Hungate tubes prior to dispensing of 5 ml of the medium to give the desired concentration (range 0 to 40 g/L). The temperature range for growth was tested from 15 to 45°C.

**Light microscopy and electron microscopy**

Light microscopy and electron microscopy observations were performed as described previously [16].

**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 quantified using a Constametric 200 LDC-Analytical High Performance Liquid Chromatograph (HPLC) equipped with a C₁₈ Symmetry 5-μm particle size column 250 by 4.6 (inside diameter) mm (Waters Chromatography), a Shimadzu SPD-6A UV detector at 240 nm and a CR-6A Shimadzu integrator. The column temperature was maintained at 35°C. An isocratic mobile phase of 30:69.5:0.5 (v:v:v) acetonitrile/distilled water/acetic acid was used at a flow rate of 0.6 mL/min. The volume of the injection loop was 20 μL. Volatile fatty acids, ethanol, glycerol, and carbohydrates were measured using HPLC (Spectra Series 100 model, Thermo Separation Products) equipped with an Aminex HPX-87X 300 by 7.8-mm (inside diameter) column (Bio-Rad Laboratories) and connected to a differential refractometer (RID-6A Shimadzu). Analysis was performed using a CR-6A Shimadzu integrator. The mobile phase was 0.005 M H₂SO₄ at a flow rate of 0.5 mL/min, and the column temperature was 35°C. The volume of the injection loop was 20 μL.

**Determination of G+C content**

The G+C content of DNA was determined at DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. The DNA was isolated and purified by chromatography on hydroxyapatite, and the G+C content was determined by HPLC [17]. Non-methylated lambda DNA (Sigma) was used as the standard.

**DNA–DNA hybridization**

DNA was isolated by the method of Cashion et al. [19]. DNA–DNA hybridization was performed at the DSMZ and carried out as described by De Ley et al. [20], with the modification described by Escara and Hutton [21] and Huss et al. [22] using a Gilford System model 2600 equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program by Jahnke and Bahnweg [23] and Jahnke [24].

**DNA extraction and amplification of 16S rRNA genes**

DNA was extracted from the isolate as described previously [25]. The universal primers Fd1 and Rd1 were used to obtain a PCR product of approximately 1.5 kb. A 50-μL reaction contained 1–20 ng of genomic DNA, 1 μM of each primer, 5 μL of 10 × buffer, 200 μM of dNTP, 3.5 mM of MgCl₂, and 2.5 U of Taq polymerase (Promega). PCR was carried out by 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 of 55°C for 2 min, and 72°C for 20 min.

**Direct sequencing of PCR products**

PCR products were purified using QIAquick Kit (Qiagen). The DNA concentration of the purified PCR product was estimated by comparison with the Low Mass Ladder (Gibco BRL) on an ethidium bromide containing agarose gel. Sequencing was carried out on an ABI 373A sequencer using the ABI PRISM Dye Terminator Cycle Sequencing Kit containing AmpliTaq FS DNA polymerase using the following conditions. A 10-μL reaction containing 35 ng of PCR product, 4 μL of cycle sequencing reaction mix, 3.2 pmol of primer [25], and 2.5 μg of BSA. Thermal cycling was carried out using a Rapid Cycler (Idaho Technology) at a temperature transition slope of 2, an initial denaturation of 94°C for 15 s, followed by 25 cycles of denaturation at 94°C for 0 s, annealing at 50°C for 10 s, extension at 60°C for 3 min.
Sequence alignments and phylogenetic inferences

The new sequence data that was generated was assembled, aligned to an almost full length consensus 16S rRNA gene sequence assembled and checked for accuracy manually using the alignment editor ae2 [26]. The sequence was compared with other sequences in the GenBank database [27] using BLAST [28], and in the Ribosomal Database Project, version 5.0 using SIMILARITY_RANK and SUGGEST_TREE [26]. Reference sequences most related to our newly generated sequences were extracted from these databases and aligned. Sequences for Acetobacterium woodii, A. malicum, A. paludosum, A. fimetartum, A. bakii and E. callenderi (accession numbers X96954, X96957, X96958, X96959, X96960, and X96961, respectively), were extracted from EMBL. Positions of sequence and alignment uncertainty were omitted from the analysis. Pairwise evolutionary distances based on 1198 unambiguous nucleotides were computed using dnadist (Jukes and Cantor option) and neighbor-joining programs that form part of the PHYLIP suite of programs [29]. TREECON was used extensively for bootstrap analysis [30].

Results

Enrichment and isolation

After 2 weeks of incubation at 37°C, a positive growth was obtained in the enrichment culture as indicated by syringate degradation and acetate production. Pure cultures were checked microscopically for culture purity and for aromatic compound degradation. A culture designated strain SR12T was used for further characterization studies.

Cellular morphology

Cells of strain SR12T were rod shaped measuring 2 to 3 μm long by 0.4 and 0.8 μm wide. Spores were not seen in late exponential growth phase and for cultures grown under different conditions. However, growth occurred in fresh medium after inoculation of cultures that had been incubated for 10 min at 80°C, 90°C, but not at 100°C indicated cellular thermal resistance and the probability that resistant bodies were present. Cells were non-motile and no flagella were observed in negative stained preparations using electron microscopy (Fig. 1a). The cells stained Gram-positive and electron micrographs of thin sections of the cells showed the presence of a thick bilayered cell wall structure resembling Gram-positive type cell walls (Fig. 1b). Cells of strain SR12T formed extensive aggregates (Fig. 1c), but were freed by vigorous vortexing. Examination of ultrathin section of cells identified numerous projections from the cell wall surface resembling extracellular glucocalix matrix, which is most likely to be responsible for cell aggregation (Fig. 1d).

Temperature, pH, and NaCl concentration ranges

The temperature range for growth was 20 to 42°C, with an optimal growth occurring at 35°C. The pH range for growth was between pH 6.0 and 9.0 with an optimum of pH 7.2. NaCl concentration range for growth was between 0 to 35 g/L with no growth occurring at 40 g/L.

Substrate utilization

Yeast extract was required for growth and could be used as sole carbon and energy source. Strain SR12T used a limited number of carbohydrates including glucose (20 mM), fructose (20 mM), and sucrose (20 mM); fatty acids including lactate (20 mM), formate (20 mM), and crotonate (20 mM); one-carbon compounds including methanol (20 mM), H2CO2 (2 bar), and betaine (20 mM). Strain SR12T did not utilize xylose (20 mM), sorbose (20 mM), galactose (20 mM), myo-inositol (20 mM), lactose (20 mM), ribose (20 mM), mannitol (20 mM), cellobiose (20 mM), xylan 10 g/L, cellulose 10 g/L, fumarate (20 mM), pyruvate (20 mM), succinate (20 mM), malonate (20 mM), glycerol (20 mM), and ethanol (20 mM).

Specific o-demethylation of methoxylated aromatic compounds by strain SR12T

o-demethylation of methoxylated aromatic compounds by strain SR12T was specific for the ortho position but not to the meta position of the methoxyl group. Strain SR12T demethylated various methoxylated aromatic compounds with at least one ortho-positioned hydroxyl group (2-methoxyphenol, 4-hydroxy-3,5-dimethoxybenzoate, 4-hydroxy-3,5-dimethoxycinnamate, 3-hydroxy-4-methoxybenzoate, 4-hydroxy-3-methoxybenzoate, 4-hydroxy-3-methoxycinnamate, 3-hydroxy-4-methoxycinnamate, 3-hydroxy-4-methoxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3-methoxycinnamylalcohol), or methoxyl group (3,4-, 2,3-di-methoxymethoxybenzoates, 3,4-dimethoxybenzaldehyde, 3,4,5-trimethoxycinnamate, 3,4,5-trimethoxybenzoate). Methoxylated aromatic compounds with
Characterization of *Eubacterium aggregans* sp. nov.

Figure 1. (a) Negatively stained cells of strain SR12<sup>T</sup>, Bar = 2 μm; (b) transmission electron micrograph of the cell wall ultrastructure of strain SR12<sup>T</sup> showing the presence of cytoplasmic membrane (CM) and a thick electron dense cell wall, Bar = 0.2 μm; (c) phase contrast micrograph of aggregating cells at the exponential growth phase of strain SR12<sup>T</sup>, Bar = 0.2 μm; (d) transmission electron micrograph of a thin section of strain SR12<sup>T</sup> showing numerous projections from the cell wall surface resembling extracellular glucocalix matrix which would be responsible for the aggregation, Bar = 0.2 μm.

Carboxyl group ortho-positioned (2-methoxybenzoate, 2,6-dimethoxybenzoate, 2,5-dimethoxybenzoate), or with meta-positioned methoxyl group (3,5-dimethoxybenzoate), or without any ortho-positioned methoxyl or hydroxyl group (3- or 4-methoxybenzoates and 3- or 4-methoxybenzaldehydes) were not used.

Fermentation end products

Fermentation end products from glucose utilization were H<sub>2</sub>, CO<sub>2</sub>, formate and butyrate; from fructose were H<sub>2</sub>, CO<sub>2</sub>, acetate and butyrate; from methanol and betaine were acetate; from lactate and crotonate were formate, acetate and butyrate; and from H<sub>2</sub>+CO<sub>2</sub> were formate and acetate (Table 1). Acetate, butyrate, and the corresponding hydroxylated aromatic compounds were the end products from the fermentation of 2-methoxyphenol, 3,4-, 2,3-dimethoxybenzoates, 3,4-dimethoxybenzaldehyde, 3,4,5-trimethoxycinnamate, 3,4,5-trimethoxybenzoate, 4-hydroxy 3,5-dimethoxybenzoate, 4-hydroxy-3,5-dimethoxycinnamate, 3-hydroxy-4-methoxybenzoate, 4-hydroxy-3-methoxybenzoate, 4-hydroxy-3-methoxycinnamate, 3-hydroxy-4-methoxycinnamate,
Table 1. Major end products from different carbon sources fermentation by strain SR12<sup>T</sup>

<table>
<thead>
<tr>
<th>Substrates</th>
<th>CO&lt;sub&gt;2&lt;/sub&gt;</th>
<th>H&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Formate</th>
<th>Acetate</th>
<th>Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Methanol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Lactate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Crotonate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Formate</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Betaine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Methoxyl groups&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+: compound is produced; -: compound is not produced.
<sup>a</sup>2-methoxyphenol, 3,4-dimethoxybenzoates, 3,4-dimethoxybenzaldehyde, 3,4,5-trimethoxycinnamate, 3,4,5-trimethoxybenzoate, 4-hydroxy-3,5-dimethoxybenzoate, 4-hydroxy-3,5-dimethoxycinnamate, 4-hydroxy-3-methoxybenzoate, 4-hydroxy-3-methoxycinnamate, 3-hydroxy-4-methoxybenzaldehyde, 4-hydroxy-3-methoxycinnamate, 3-hydroxy-4-methoxybenzaldehyde, and 4-hydroxy-3-methoxycinnamylalcohol.

Using 12 primers, we determined an almost complete sequence consisting of 1509 bases of the 16S rRNA gene of strain SR12<sup>T</sup> (corresponding to <i>E. coli</i> position 14 to 1539 [31]). Phylogenetic analysis revealed that strain SR12<sup>T</sup> was a member of cluster XV, the low G+C containing Gram-positive branch [32]. The closest relative were <i>Eubacterium barkeri</i> (similarity average of 98%) and <i>E. callanderi</i> and <i>E. limosum</i> (similarity average of 95%). Figure 2

**Electron acceptors utilization**

Sulfate, thiosulfate, sulfate, nitrate, elemental sulfur, or fumarate could not be used as electron acceptors in basal medium containing 5 mM syringate as the electron donor.

**G+C content and DNA–DNA relatedness.** The G+C content of strain SR12<sup>T</sup> was 55 mol% (HPLC). The level of DNA–DNA relatedness between strain SR12<sup>T</sup> and <i>Eubacterium barkeri</i> was 59.5%.

**16S rRNA sequence analysis**

Figure 2. Phylogenetic dendrogram based on 16s rRNA sequence data indicating the position of <i>Eubacterium aggregans</i> strain SR12<sup>T</sup> within the radiation of representatives of the low G+C containing Gram-positive bacteria. The clusters are defined based on the guidelines described by Collins et al. [32]. Sequences for <i>Acetobacterium woodii</i>, <i>A. malicium</i>, <i>A. paludosum</i>, <i>A. fimetarium</i>, <i>A. baiii</i>, and <i>Eubacterium callanderi</i> (accession numbers X96954, X96957, X96958, X96959, X96960, and X96961, respectively) were extracted from EMBL. Other sequences were obtained from the Ribosomal Database Project, version 5.0 [26]. Bootstrap values, expressed as a percentage of 100 replications, are shown at branching points. Only values above 90% were considered significant and reported. Scale bar indicates 10 nucleotide substitution per 100 nucleotides.
represents a dendrogram generated by the neighbor-joining method [29] from the Jukes & Cantor evolutionary distance matrix [33].

**Nucleotide sequence accession number**

The 16S rRNA gene sequence of strain SR12T has been deposited in the GenBank database under accession number AF013898.

**Discussion**

Strain SR12T is a Gram-positive obligate anaerobe that is rod-shaped, non-motile, and saccharolytic. The main end products of glucose fermentation are butyrate, formate, H2 and CO2. Phylogenetically strain SR12T is a member of cluster XV of the low G+C containing Gram-positive Clostridium and related genera [32]. The closest relative is *E. barkeri* (similarity value of 98%), with *E. limosum* and *E. callanderi* equidistant from strain SR12T at a similarity value of 95%. However, strain SR12T differs significantly both phenotypically and genotypically from its closest relative, *E. barkeri* (Table 2). The DNA–DNA relatedness value between the two strains was 59.5%. *Eubacterium barkeri* has a G+C content of 48% (determined by DSMZ by HPLC), and optimum growth temperature of 30°C, forms terminal spores, and uses mannitol, pyruvate, glycerol, and ribose. In contrast, strain SR12T has a G+C content of 55 mol%, an optimum growth temperature of 35°C, and does not utilize mannitol, pyruvate, glycerol, and ribose. In addition, strain SR12T uses sucrose and methoxylated aromatics, *E. barkeri* does not. The DNA homology between the two strains is also low (60%). Although strain SR12T is an aromatic o-demethylating bacterium and similar to *E. callanderi* and *E. limosum*, there are several differentiating phenotypic and genotypic characteristics (Table 2). The most significant difference is the G+C content of the DNA of the latter which is 47 mol% as opposed to strain SR12T which is 55 mol%. Strain SR12T also differs from *E. callanderi* in its ability to use one carbon compounds and in the fermentation end products produced from methoxyl groups, which are acetate and butyrate for SR12T but

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Strain SR12T</th>
<th><em>E. barkeri</em> b</th>
<th><em>E. callanderi</em> c</th>
<th><em>E. limosum</em> d</th>
</tr>
</thead>
<tbody>
<tr>
<td>G+C%</td>
<td>55</td>
<td>48</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>pH optimum</td>
<td>7.2</td>
<td>nr</td>
<td>7</td>
<td>nr</td>
</tr>
<tr>
<td>pH range</td>
<td>6-9</td>
<td>nr</td>
<td>6-8.5</td>
<td>nr</td>
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<tr>
<td>Temperature optimum °C</td>
<td>35</td>
<td>30</td>
<td>37</td>
<td>37</td>
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<tr>
<td>Temperature range °C</td>
<td>20-42</td>
<td>25-45</td>
<td>25-43</td>
<td>30-45</td>
</tr>
<tr>
<td>NaCl range</td>
<td>0-4%</td>
<td>0.6-5%</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>Morphology</td>
<td>Rods, Gram +, non spore forming</td>
<td>Rods, Gram +, spore forming</td>
<td>Rods, Gram +, non spore forming</td>
<td>Rods, Gram +, non spore forming</td>
</tr>
<tr>
<td>Habitat</td>
<td>Methanogenic digestor fed with olive mill wastewater</td>
<td>River mud, human feces</td>
<td>Anaerobic digestor fed with contents wood fiber-to-alcohol fermentation plant</td>
<td>Human feces, rumen, intestinal contents of man, rat, poultry, fish</td>
</tr>
<tr>
<td>Substrate utilization</td>
<td></td>
<td></td>
<td>Methoxyl groups</td>
<td>Methoxyl groups</td>
</tr>
<tr>
<td>one carbon compounds</td>
<td>Methanol, formate, betaine, methoxyl groups, H2 + CO2</td>
<td>nr</td>
<td>Methanol, formate, betaine, methoxyl groups, choline, CO, H2+CO2</td>
<td></td>
</tr>
<tr>
<td>other substrates</td>
<td>Glucose, fructose, sucrose, lactate, crotonate</td>
<td>Nicotinate, glucose, fructose, ribose, mannitol, sorbitol, pyruvate</td>
<td>Glucose in presence of acetate</td>
<td>Adonitol, arabinol, erythritol, fructose, glucose, isoleucine, lactate, mannitol, methoxyl groups</td>
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<tr>
<td>non utilized substrates</td>
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<td>Sucrose, sorbose, galactose, mannose, arabinose, lactose, cellobiose</td>
<td>Cellulose, maltoose, cellulose, galactose, xylose, mannose, lactose</td>
<td>Arabinose, galactose, mannose, cellobiose, sucrose, maltose, lactose</td>
</tr>
<tr>
<td>End products from fermentation of methoxyl groups carbohydrates</td>
<td>Acetate, butyrate, H2, CO2</td>
<td>not utilized a</td>
<td>Formate, acetate, butyrate, H2, CO2</td>
<td>Acetate, butyrate, H2, CO2</td>
</tr>
</tbody>
</table>

a This work.  
b From Cato et al. [36].  
c From Montfort et al. [39].  
d From Bache et al. [31].  

nr, not reported.
The pH range for growth is 6-8, and growth occurs with xylose, sorbose, galactose, myo-inositol, lactose, cellobiose, xylan, and cellulose. Isolated from a digestor loaded with olive mill wastewater. The type strain is SR12T and has been deposited in the German culture collection as strain DSM 12183.

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References


Description of Eubacterium aggregans sp. nov.

Eubacterium aggregans (a`ggreg`a.gans. L. v. aggregare, to flock or band together; L. pres. part. aggregans, assembling, aggregating). Cells are non-sphere forming, 2–3 μm by 0.4–0.8 μm, non-motile and stained Gram positive. The temperature range for growth is 20 to 42°C and the optimum temperature is 35°C. The pH range for growth is 6 to 9 and the optimum is 7.2. Growth occurs with NaCl concentration of 0–3.5%. Utilizes glucose, fructose, sucrose, lactate, formate, methanol, H2+CO2, betaine, crotonate, and methoxyl groups from the following compounds: 2-methoxyphenol, 3,4-, 3,5-dimethoxybenzoates, 3,4-dimethoxybenzaldehyde, 3,4,5-trimethoxycinnamate, 3,4,5-trimethoxybenzoate, 4-hydroxy-3,5- dimethoxybenzoate, 4-hydroxy-3,5-dimethoxycinnamate, 3-hydroxy-4-methoxybenzoate, 4-hydroxy-3-methoxybenzoate, 3-hydroxy-3-methoxybenzaldehyde, and 4-hydroxy-3-methoxycinnamylalcohol. No growth occurs with xylose, sorbose, galactose, myo-inositol, lactose, cellobiose, xylan, and cellulose. Isolated from a digester loaded with olive mill wastewater. The type strain is SR12T and has been deposited in the German culture collection as strain DSM 12183.
Characterization of *Eubacterium aggregans* sp. nov.


29. Felsenstein J. (1993) PHYLIP (Phylogenetic Inference Package) version 3.51c. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA


