Isolation from a shea cake digester of a tannin-degrading *Streptococcus gallolyticus* strain that decarboxylates protocatechuic and hydroxycinnamic acids, and emendation of the species

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A facultatively anaerobic, mesophilic, non-motile, non-sporulating bacterium, designated strain B7, was isolated from an anaerobic digester fed with shea cake rich in tannins and aromatic compounds, after enrichment on tannic acid. The coccoid cells (less than 2 µm in diameter) occurred in pairs or short chains and stained Gram-positive. Strain B7 fermented a wide range of carbohydrates (cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, melibiose, raffinose and trehalose), grew optimally at pH 7.0 and had a G+C content of 40.4±0.3 mol %. Strain B7 was closely related to *Streptococcus gallolyticus* ACM 3611^T, a member of the *Streptococcus bovis* rRNA cluster, with a sequence similarity of 98 % and a DNA hybridization value of 86 mol %. Isolate B7 hydrolysed tannic acid and decarboxylated gallic acid to pyrogallol, traits also observed in *S. gallolyticus* ACM 3611^T. In addition, both strains decarboxylated protocatechuic acid to catechol, *p*-coumaric acid to 4-vinylphenol, caffeic acid to 4-vinylcatechol and ferulic acid to 4-vinylguaiacol. An unsubstituted *para*-hydroxyl group on the benzene ring was required for decarboxylation. Glucose addition markedly increased the conversion rate. As these traits were not described previously, emendation of the description of the species *Streptococcus gallolyticus* is proposed.

Keywords: *Streptococcus gallolyticus*, tannin hydrolysis, aromatic compounds, decarboxylation, emendation

INTRODUCTION

Tannins are a group of highly polar phenolic compounds found in plant extracts. These oligomeric compounds, with molecular weights of 500–3000 g mol⁻¹, form insoluble complexes with proteins, cellulose, gelatin and pectin (Swain & Bate-Smith, 1962). Tannins are regarded as environmental pollutants. They are toxic to aquatic organisms, inhibit micro-organisms and enzymes involved in the decomposition of organic pollution (Scalbert, 1991) and, in some cases, are recalcitrant to biodegradation (Field & Lettinga, 1992).

Aerobic degradation of tannins has been widely studied. Soil fungi belonging to the genera *Aspergillus* and *Penicillium*, yeasts of the genus *Pichia* and bacteria of the genera *Klebsiella*, *Bacillus*, *Corynebacterium* and *Achromobacter* are the most frequently observed degraders of hydrolysable tannins. The interest in anaerobic degradation of hydrolysable tannins has resulted from an increase in the application of anaerobic systems for treatment of tannin-containing wastewaters (Field & Lettinga, 1992).

A previous study of a continuous anaerobic digester fed with shea cake showed high tannin-removal rates and production of organic acids and methane (Ouattara, 1994). Isolation and characterization are reported of a strain of *Streptococcus gallolyticus* capable of hydrolysing tannic acid and decarboxyl-

The GenBank accession number for the 16S rRNA gene sequence of strain B7 is AF323911.

ating gallic acid to pyrogallol from the same digester. Both the novel isolate and the type strain, *S. gallolyticus* ACM 3611^T, decarboxylated protocatechuic acid to catechol and some *p*-hydroxycinnamic acid derivatives to their corresponding 4-vinyl derivatives. It is proposed to emend the description of *S. gallolyticus* to include these newly described traits.

METHODS

Source of strains. Strain B7 was isolated from a continuous methanogenic digester located in Burkina Faso. This digester (20 l, 30 °C) was initially inoculated with anaerobic sludge from the pit of a slaughterhouse and fed for 6 months with shea cake. *Streptococcus bovis* DSM 20480^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). *S. gallolyticus* ACM 3611^T was obtained from the Australian Collection of Microorganisms. *S. gallolyticus* CIP 107089, *S. gallolyticus* CIP 107090 and *S. gallolyticus* CIP 107091 were obtained from the Collection of the Pasteur Institute.

Culture media. The anaerobic techniques of Hungate (Hungate, 1969; Macy *et al.*, 1972; Miller & Wolin, 1974) were used throughout this work. The basal medium contained (l⁻¹): 0.4 g NH₄Cl, 0.5 g KH₂PO₄, 0.4 g NaCl, 0.33 g MgCl₂·6H₂O, 0.05 g CaCl₂·2H₂O, 0.25 g cysteine hydrochloride, 2 g yeast extract (Difco), 1 ml trace-element mineral solution (Widdel & Pfennig, 1981) and 1 mg resazurin. The pH was adjusted to 7 with 10 M KOH. The medium was then boiled under a stream of O₂-free N₂ gas and cooled to room temperature. Aliquots of 5 ml 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 inoculation, 0.05 ml 10% (w/v) NaHCO₃ and 0.05 ml 5% (w/v) Na₂S·9H₂O were injected from sterile stock solutions. Substrates were injected from concentrated anaerobic sterile stock solutions to obtain the desired final concentration.

Strain B7 and the four strains of *S. gallolyticus* were routinely grown on basal medium containing 5 mM glucose. The type strain of *S. bovis* was grown aerobically at 37 °C using a medium containing (l⁻¹): 10 g casein peptone-tryptic digest, 5 g yeast extract, 5 g glucose and 5 g NaCl. The pH was adjusted to 7.2–7.4 with 10 M KOH.

Enrichment and isolation procedure. The digester wastewater sample (0.5 ml) was inoculated into 5 ml basal medium containing 1 g tannic acid l⁻¹ (Aldrich) and incubated at 37 °C. The enrichment culture was subcultured several times under the same conditions prior to isolation. For isolation purposes, the culture was serially diluted tenfold and single well-isolated colonies that developed in roll tubes (basal medium containing 1 g tannic acid l⁻¹ and 1.6% agar) were picked and serially diluted in fresh media. This procedure was repeated until only one colony type was observed. Purity was checked by microscopic examination of cultures grown in basal medium amended with 10 mM glucose and 0.2% Biotrypcase (Difco). The isolate was maintained in basal medium containing 1 g tannic acid l⁻¹.

Morphology and sporulation test. Light and electron microscopy were performed as described by Fardeau *et al.* (1997). For testing heat resistance, cells grown in basal medium containing glucose were exposed to 80, 90 and 100 °C for 10 min. The cultures were cooled quickly to ambient temperature and inoculated into fresh glucose-containing medium and growth was recorded after 24 h

incubation at 37 °C. Conditions for sporulation that were tested included growth in the presence of glucose or tannic acid and without added carbon sources.

Growth parameters. For all experiments, basal medium containing 0.2% yeast extract and 10 mM glucose was used. The pH of the pre-reduced anaerobic medium was adjusted with 5% NaHCO₃, 5% Na₂CO₃ or 0.1 M HCl to obtain a pH range between 5.0 and 9.5. Different amounts of NaCl were weighed directly in Hungate tubes prior to dispensing 5 ml medium to obtain the desired NaCl concentration (range 0–50 g l⁻¹). The temperature range for growth was determined between 10 and 50 °C (5 °C intervals).

Electron acceptors. Sulfate, thiosulfate, sulfite, fumarate (all at 20 mM) and elemental sulfur (2%, w/v) were tested as electron acceptors in basal medium containing 10 mM glucose.

Substrate utilization. Experiments were performed in duplicate with an inoculum subcultured at least once under the same test conditions. The substrates tested for utilization were injected from pre-sterilized and concentrated stock solutions into Hungate tubes that contained 5 ml pre-sterilized basal medium. The following substrates were used: 20 mM carbohydrates (arabitol, cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, melibiose, melzitose, *myo*-inositol, raffinose, ribose, sorbitol, sorbose, trehalose and xylose); 20 mM organic acids (acetate, adipate, butyrate, citrate, crotonate, formate, fumarate, isobutyrate, lactate, malate, propionate, succinate and valerate); 20 mM alcohols (butanol, ethanol, glycerol, isobutanol, 2-propanol, methanol and 1-propanol); 10 g peptides l⁻¹ [Biotrypcase (Difco), Casamino acids (Sigma), gelatin (Sigma), peptone (Difco) and yeast extract (Difco)]; 20 mM phenylalanine; and 5 mM aromatic compounds. Concentrated stock solutions were prepared, neutralized if necessary, rendered anaerobic by gassing with O₂-free N₂ and sterilized by filtration (pore size 0.2 µm; Millipore). Aromatic compounds were tested with or without 5 mM glucose. Autotrophic growth was tested using H₂/CO₂ (20:80%, v/v) at a final pressure of 2 bar. An increase in OD₅₈₀ in tubes containing added substrates, compared with control tubes lacking substrate, was considered as positive growth.

Analytical techniques. Bacterial growth was monitored by measuring the OD₅₈₀ directly from anaerobic Hungate tubes inserted into the cuvette holder of a spectrophotometer (Shimadzu UV 160A). Aromatic compounds, carbohydrates, volatile fatty acids and alcohols were measured by HPLC as described by Mechichi *et al.* (1999). H₂ and CO₂ were measured as described by Fardeau *et al.* (1993).

Determination of the G+C content and DNA–DNA hybridization. The DNA G+C content was determined by the DSMZ. DNA was isolated and purified by chromatography on hydroxyapatite (Cashion *et al.*, 1977) and the G+C content was determined by HPLC as described by Mesbah *et al.* (1989). Non-methylated lambda DNA (Sigma) was used as the standard. DNA–DNA hybridization was performed at the DSMZ as described by De Ley *et al.* (1970), with the modifications described by Escara & Hutton (1980) and Huß *et al.* (1983), 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 of Jahnke & Bahnweg (1986) and Jahnke (1992).

DNA extraction and amplification of the 16S rRNA gene. DNA was extracted from the isolate as described by Woo *et al.* (1997). Amplification and sequencing of the 16S rRNA

was done as described by Redburn & Patel (1993) and Andrews & Patel (1996). The sequence data that were generated were aligned to an almost full-length consensus 16S rRNA gene sequence, assembled and checked manually for accuracy using the alignment editor ae2 (Maidak *et al.*, 2000). These were compared with other sequences in the GenBank database using BLAST (Altschul *et al.*, 1997) and in the Ribosomal Database Project, version 8.0, using SIMILARITY_RANK and SUGGEST_TREE (Maidak *et al.*, 2000). Pairwise evolutionary distances based on 1235 unambiguous nucleotides were computed using the programs DNADIST (Jukes & Cantor, 1969) and NEIGHBOR that form part of the PHYLIP suite of programs (Felsenstein, 1993). TREECON was used extensively for bootstrap analysis (Van de Peer & De Wachter, 1994).

RESULTS AND DISCUSSION

Enrichment and isolation

Enrichment cultures developed in medium containing 1 g tannic acid l⁻¹ within 3 weeks of incubation at 37 °C, as shown by growth and pyrogallol production. After several transfers in the liquid medium, the enrichment developed a stable microbial population that degraded tannic acid. Several isolates were obtained using the roll-tube method (Hungate, 1969) and one of these cultures, designated strain B7, was studied further.

Morphology and physiology

Cells of strain B7 were non-motile cocci (< 2 µm in diameter) that occurred in pairs or short chains and stained Gram-positive. Spores were not observed and cells did not survive heat treatment of 10 min at 80 °C, indicating an absence of heat-resistant cells. Strain B7 was a facultatively anaerobic bacterium. The temperature range for growth was 15–48 °C, with optimal growth at 40 °C. No growth occurred at 10 or 50 °C. The pH range for growth was pH 5.0–9.0, with an optimum at pH 7.0. The NaCl concentration range for growth was 0–40 g l⁻¹. Yeast extract stimulated growth, but was not required. Strain B7 fermented a wide range of carbohydrates including cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, melibiose, raffinose and trehalose, producing predominantly lactate and minor amounts of acetate, formate and ethanol, but no gas. Malate and yeast extract were fermented into lactate. Isolate B7 did not utilize arabinol, melezitose, *myo*-inositol, ribose, sorbitol, sorbose or xylose, any of the organic acids tested, any of the alcohols tested, peptides, including Biotrypcase, Casamino acids, gelatin or peptone, phenylalanine or H₂/CO₂. Sulfate, thiosulfate, sulfite, elemental sulfur and fumarate could not be used as electron acceptors.

G + C content, 16S rRNA gene sequence analysis and DNA–DNA relatedness

The G + C content of strain B7 was 40.4 ± 0.3 mol %, as determined by HPLC. An almost complete 16S rRNA gene sequence was obtained for strain B7,

Fig. 1. Phylogenetic position of strain B7 within the radiation of the *S. bovis* group. The *S. bovis* group is represented by *S. gallolyticus*, *S. macedonicus*, *S. equinus*, *S. bovis* and *S. alactolyticus*. The outgroup is represented by members of the *Streptococcus mutans* group, which includes *S. mutans* (AJ243965) and *Streptococcus rattus* (X58304). For phylogeny, 1235 unambiguous nucleotides were included in the analysis. GenBank accession numbers are provided in parentheses.

consisting of 1535 nt. Phylogenetic analysis revealed that strain B7 was closely associated with members of the genus *Streptococcus* (Hardie, 1986) and, in particular, with *S. gallolyticus* ACM 3611^T (similarity of 98%), *S. bovis* DSM 20480^T, *Streptococcus equinus* NCDO 1037^T, *Streptococcus alactolyticus* ATCC 43492^T, *Streptococcus macedonicus* ACA-DC 206^T and *Streptococcus caprinus* ACM 3970 (a synonym of *S. gallolyticus*; Sly *et al.*, 1997), with a mean similarity of 98% (Fig. 1). *S. gallolyticus*, *S. bovis*, *S. equinus*, *S. alactolyticus*, *S. macedonicus* and *S. caprinus* form a cohesive rRNA cluster within the genus *Streptococcus* that is referred to as the *S. bovis* cluster (Farrow *et al.*, 1984). DNA hybridization studies showed that *S. gallolyticus*, *S. bovis*, *S. equinus*, *S. alactolyticus* and *S. macedonicus* are distinct genospecies (Sly *et al.*, 1997; Tsakalidou *et al.*, 1998; Kawamura *et al.*, 1998). The level of DNA–DNA relatedness was 29% between strain B7 and *S. bovis* DSM 20480^T and 86% between strain B7 and *S. gallolyticus* ACM 3611^T. According to phylogenetic analysis, strain B7 is a member of the genus *Streptococcus* and, in particular, the *S. bovis* group, which forms a cohesive rRNA cluster, with a mean similarity of greater than 97%. The level of DNA relatedness between strain B7 and *S. gallolyticus* ACM 3611^T (86%) indicates that it should be regarded as a strain of *S. gallolyticus*. Phenotypically, strain B7 ferments mannitol, hydrolyses tannic acid to release gallic acid and decarboxylates gallic acid to pyrogallol, traits it possesses in common with *S. gallolyticus* isolates (Sly *et al.*, 1997; Brooker *et al.*, 1994; Osawa & Sly, 1992; Osawa *et al.*, 1993), but not with other members of the *S. bovis* group.

S. gallolyticus has so far been isolated from diverse habitats including the faeces of koalas, kangaroos, brushtail, possums, ringtail possums, cows, horses, pigs, dogs and guinea pigs, as well as animals with bovine mastitis, human clinical sources and the sheep rumen (Osawa *et al.*, 1995; Sly *et al.*, 1997). Nelson *et al.* (1998) reported that tolerance of and/or degradation of tannins by rumen bacteria was widespread. The isolation of strain B7 from an anaerobic digester fed with shea cake extends the known habitat for *S. gallolyticus*. The isolation of B7 from such an en-

Table 1. Metabolism of aromatic compounds by strain B7 and *S. gallolyticus* ACM 3611^T

Bacteria were grown in basal medium containing 0.2 % yeast extract; aromatic compounds were used at 5 mM, with or without 5 mM glucose. Results were recorded after 2 days anaerobic incubation at 37 °C. For strain B7, the ΔOD_{580} (difference between OD_{580} after incubation at 37 °C and OD_{580} prior to incubation) of the culture was 0.10–0.13 without added glucose and 0.54–0.66 with added glucose. For *S. gallolyticus* ACM 3611^T, the ΔOD_{580} of the culture was 0.11–0.13 without added glucose and 0.57–0.68 with added glucose.

Aromatic acid metabolized	Compound produced	Amount of product (mM)			
		Without glucose		With glucose	
		B7	ACM 3611 ^T	B7	ACM 3611 ^T
Tannic acid	Pyrogallol	3.4	3.1	3.3	3.4
Gallic acid	Pyrogallol	3.6	2.9	4.8	4.1
Protocatechuic acid	Catechol	1.8	2.2	4.9	4.8
<i>p</i> -Coumaric acid	Vinylphenol	4.5	4.8	4.8	4.7
Caffeic acid	Vinylcatechol	3.0	2.8	4.8	4.8
Ferulic acid	Vinylguaiaicol	2.9	2.3	4.2	4.3

vironment after enrichment on tannic acid is not surprising, as the digester was rich in tannins and aromatic compounds and had been inoculated with anaerobic sludge from the pit of a slaughterhouse.

Metabolism of aromatic compounds

Strain B7 hydrolysed tannic acid (hydrolysable tannin) at 1 g l⁻¹ to release gallic acid, which was subsequently decarboxylated to pyrogallol. Glucose supplementation enhanced cell growth and markedly accelerated this conversion (Table 1). Tannin concentrations greater than 17 g l⁻¹ inhibited growth. Gallic acid, which is a monomer of tannic acid, was decarboxylated to pyrogallol, with or without added glucose (Table 1). Pyrogallol was the end product and the aromatic ring was not degraded after 1 month of incubation.

The following benzoic acid derivatives tested in the presence or absence of glucose were not metabolized: benzoate, hydroxylated benzoic acids (4-hydroxybenzoate, 3,5-, 2,4- and 2,6-dihydroxybenzoates and 2,4,6-trihydroxybenzoate), methoxylated benzoic acids (2,4-, 2,6-, 3,4- and 3,5-dimethoxybenzoates and 3,4,5-trimethoxybenzoate), mixed hydroxylated/methoxylated benzoic acids (4-hydroxy-3-methoxybenzoate, 3-hydroxy-4-methoxybenzoate and 4-hydroxy-3,5-dimethoxybenzoate) and 3,4-dimethylbenzoate. However, protocatechuic acid (3,4-dihydroxybenzoate) was decarboxylated to catechol. Again, the addition of glucose accelerated this conversion (Table 1).

Of a number of cinnamic compounds tested, namely cinnamic, *o*-, *m*- and *p*-coumaric, caffeic, ferulic, isoferulic and 3,4,5-trimethoxycinnamic acids, with or without added glucose, only *p*-coumaric, caffeic and ferulic acids were metabolized to their corresponding 4-vinyl (styrene) derivatives, respectively 4-

vinylphenol (4-hydroxystyrene), 4-vinylcatechol (3,4-dihydroxystyrene) and 4-vinylguaiaicol (4-hydroxy-3-methoxystyrene) (Table 1). The mechanism involved decarboxylation of the carboxyl group in the unsaturated C₃-aliphatic side chain. Supplementation with glucose increased the conversion yield.

Strain B7 did not decarboxylate phenylpropionic acids (hydrocinnamate and hydrocaffeate) or phenylacetic acids (phenylacetate, *p*-hydroxyphenylacetate, 2,5- and 3,4-dihydroxyphenylacetates, *p*-methoxyphenylacetate and 3,4-dimethoxyphenylacetate), with or without added glucose, indicating a specific selection. In addition, pyrogallol and phloroglucinol were not metabolized.

The ratio of pyrogallol, catechol and 4-vinyl derivatives to their corresponding gallic, protocatechuic and 4-hydroxycinnamic acids in the medium increased during the course of the experiment until the utilization of the substrate was complete. Adding glucose increased cellular yield and markedly accelerated these conversions. With all aromatic compounds tested, and even after 1 month incubation, no ring cleavage was observed.

The metabolism of aromatic compounds by the type strain, *S. gallolyticus* ACM 3611^T, and *S. gallolyticus* CIP 107089, *S. gallolyticus* CIP 107090 and *S. gallolyticus* CIP 107091 was similar to that observed with strain B7. Of all the aromatic compounds tested above, these four strains of *S. gallolyticus* decarboxylated only gallic acid to pyrogallol, protocatechuic acid to catechol, *p*-coumaric acid to 4-vinylphenol, caffeic acid to 4-vinylcatechol and ferulic acid to 4-vinylguaiaicol. Supplementation with glucose increased the conversion yield. The aromatic ring was not degraded, even after 1 month of incubation under anaerobic conditions.

In addition to hydrolysis of tannic acid and decarboxylation of gallic acid to pyrogallol, the type strain of *S. gallolyticus*, strain B7 and three other strains of *S. gallolyticus* decarboxylated protocatechuic acid to catechol and three hydroxycinnamic acids to their corresponding styrene derivatives. These characteristics have not so far been reported for *S. gallolyticus*. From these studies, it can be deduced that (i) both hydroxyl groups in the *para* and *meta* positions on the aromatic ring of benzoic acid derivatives were essential for decarboxylation and (ii) cinnamate derivatives were metabolized by decarboxylation of the carboxyl group in the unsaturated C₃-aliphatic side chain when the ring was substituted with a *para*-hydroxyl group. In no case was the aromatic ring cleaved. Studies have also shown that the addition of glucose increased cellular yield and conversion yield, but not the range of metabolizable substrates. The physiological significance of these decarboxylations is not known. One possible effect may be to prevent acid accumulation in the medium; alternatively, the microorganisms may conserve energy (as membrane potential) from the decarboxylation or the antiport of a negatively charged substrate into the cell and a neutral product out of the cell (Heider & Fuchs, 1997).

It is proposed that the description of *S. gallolyticus* be emended to include the novel characteristics described in this report.

Emended description of *Streptococcus gallolyticus* (Osawa et al. 1995)

Streptococcus gallolyticus (gal.lo.ly'ti.cus. N.L. n. *gallatum* gallate; N.L. adj. *lyticus* able to loosen; N.L. adj. *gallolyticus* gallate-digesting).

Colonies on blood agar or brain/heart infusion agar are circular, smooth and entire. Non-pigmented. Non-haemolytic. Coccoid cells mostly in pairs or short chains. Non-motile. Facultatively anaerobic with some strains requiring CO₂. Catalase-negative. Growth between 10 and 45 °C; optimum 37–40 °C. No growth at 50 °C. Chemo-organotrophic. Majority of strains have tannase activity. Metabolism fermentative. Acid produced from a wide range of carbohydrates including cellobiose, fructose, galactose, glucose, lactose, maltose, melibiose and trehalose. Majority of the strains ferment raffinose and mannitol. Acid is not produced from ribose or sorbitol. Majority of strains hydrolyse aesculin. Hippurate is not hydrolysed. Voges–Proskauer-positive. Leucine arylamidase-positive. Majority of strains are α -galactosidase-positive. Arginine dihydrolase- and alkaline phosphatase-negative. Decarboxylates gallic acid to pyrogallol, protocatechuic acid to catechol, *p*-coumaric acid to 4-vinylphenol, caffeic acid to 4-vinylcatechol and ferulic acid to 4-vinylguaiacol, with or without added glucose. Supplementation with glucose markedly accelerates these conversions. Benzoate, 4-hydroxybenzoate, 3,5-, 2,4- and 2,6-dihydroxybenzoates, 2,4,6-trihydroxybenzoate, 2,4-, 2,6-, 3,4- and 3,5-dimethoxybenzoates,

3,4,5-trimethoxybenzoate, 4-hydroxy-3-methoxybenzoate, 3-hydroxy-4-methoxybenzoate, 4-hydroxy-3,5-dimethoxybenzoate, 3,4-dimethylbenzoate, pyrogallol, phloroglucinol, cinnamate, *o*- and *m*-hydroxycinnamates, 3-hydroxy-4-methoxycinnamate, 3,4,5-trimethoxycinnamate, 3-phenylpropionate, 3,4-dihydroxyphenylpropionate, phenylacetate, *p*-hydroxyphenylacetate, 2,5- and 3,4-dihydroxyphenylacetates, *p*-methoxyphenylacetate and 3,4-dimethoxyphenylacetate are not metabolized, with or without addition of glucose. The genomic DNA G + C content is 37–40 mol %. Isolated from alimentary tracts of various animals, human clinical cases, including septicæmia and endocarditis, and an anaerobic digester fed with shea cake, previously inoculated with anaerobic sludge from the pit of a slaughterhouse. The type strain is ACM 3611^T; reference strain B7 (= DSM 13808 = CIP 106781).

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