Papillibacter cinnamivorans gen. nov., sp. nov., a cinnamate-transforming bacterium from a shea cake digester

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A new, strictly anaerobic, Gram-positive, non-sporulating, mesophilic bacterium, designated strain CIN1^T (T = type strain) was isolated from an anaerobic digester fed with shea cake rich in tannins and aromatic compounds. Cells of strain CIN1^T were rod-shaped, had characteristically pointed ends (1·3–3·0 × 0·5–0·6 µm) and occurred singly, in pairs and sometimes in chains of up to six. The pH range for growth was 6·9–8·5 and the temperature growth range was 15–40 °C. Optimum growth occurred with yeast extract and cinnamate at 37 °C and a pH of 7·5. The isolate transformed cinnamate by degrading the aliphatic side chain to produce acetate and benzoate rather than by aromatic ring cleavage or demethoxylation. The position of the methoxyl group appears to be important in the degradation of the aliphatic side chain of cinnamate; consequently, 3-methoxycinnamate and 4-methoxycinnamate, but not 2-methoxycinnamate, are transformed to produce acetate and methoxybenzoates, namely 3-methoxybenzoate and 4-methoxybenzoate, respectively. Crotonate is degraded to acetate and butyrate. The G+C content of the DNA is 56 mol%. Phylogenetic analysis of the 16S rRNA gene of strain CIN1^T indicated that it was a member of the low-G+C-containing Gram-positive branch with a specific relationship to *Sporobacter termitidis* (sequence identity of 88%). The phylogenetic results concur with the phenotypic data which reveals that the isolate is a novel bacterium and, based on these findings, strain CIN1^T (=DSM 12816^T=ATCC 700879^T) has been designated *Papillibacter cinnamivorans* gen. nov., sp. nov.

INTRODUCTION

Lignin is the second most abundant natural product on earth and consists of a vast array of aromatic monomers including cinnamate (Collberg, 1988; Healy & Young, 1979; Healy *et al.*, 1980). It is now well established that monoaromatic compounds are attacked by a phylogenetically diverse range of anaerobic micro-organisms, some of which completely ferment the aromatic compounds and others that are only involved in transformation of such compounds. Pure cultures with the ability to ferment aromatic compounds include *Coprococcus* sp. (Patel *et al.*, 1981), a

member of the low-G+C-containing Gram-positive branch, *Pelobacter acidigallici* (Schink & Pfennig, 1982) and *Pelobacter massiliensis* (Schnell *et al.*, 1991), members of the *Proteobacteria*, which de-aromatize aromatic compounds such as gallate, resorcinol and phloroglucinol to produce acetate, *Holophaga foetida* (Liesack *et al.*, 1994), a member of the *Proteobacteria*, and *Sporobacter termitidis* (Grech-Mora *et al.*, 1996), a member of the low-G+C-containing Gram-positive branch, are unusual in that they first transfer the ether-linked methyl groups to sulfide-producing methanethiol or dimethylsulfide followed by de-aromatization of the aromatic ring structure. Until recently, mono-hydroxylated aromatic compounds were considered undegradable by anaerobes, but recently *Sporotomaculum hydroxybenzoicum* (Brauman *et al.*, 1998), a member of the low-G+C-containing Gram-positive

branch, has been shown to degrade 3-hydroxybenzoate via the benzoyl-CoA pathway subsequent to the removal of the hydroxyl group. In other cases, de-aromatization of the ring requires syntrophic co-culture, as is the case with *Syntrophus buswellii* strain GA, a member of the Gram-negative *Proteobacteria* that degrades cinnamate under anaerobic syntrophic conditions only (Auburger & Winter, 1995). The anaerobic members of *Proteobacteria* which transform aromatic compounds with ring cleavage include photosynthetic bacteria (some strains of *Rhodopseudomonas palustris*) (Harwood & Gibson, 1988), sulfate-reducing bacteria (*Desulfobacterium* and *Desulfococcus*), metal-reducing bacteria (*Geobacter*) and denitrifying bacteria (*Thauera*).

In this paper, the isolation and characterization of a new anaerobic bacterium, which transforms a limited range of aromatic compounds, namely cinnamate, 3-methoxycinnamate and 4-methoxycinnamate, without de-aromatization of the ring are described. Physiological studies including metabolism of the substrates and end-products are reported.

METHODS

Source of strains. Strain CIN1^T was isolated from an anaerobic digester fed with shea cake situated in Burkina Faso (Africa). The digester had previously been inoculated with anaerobic sludge from the pit of a slaughterhouse. Carbon and energy sources were composed exclusively of tannins and aromatic compounds.

Culture media. A basal medium described below was used for enrichment, isolation and routine cultivation. The medium was prepared using the anaerobic technique described by Hungate (1969) and modified for use with syringes (Macy *et al.*, 1972; Miller & Wolin, 1974). The basal medium contained (l^{-1} deionized water): 0·2 g K$_2$HPO$_4$; 1 g NaCl; 0·15 g CaCl$_2$.2H$_2$O; 0·4 g MgCl$_2$.6H$_2$O; 0·5 g KCl; 0·5 g cysteine–HCl; 0·5 g yeast extract; 1·5 ml trace element solution (Widdel & Pfennig, 1981); and 1 mg resazurin. The pH was adjusted to 7·0 with a 10 M NaOH solution. The medium was then boiled under a stream of O$_2$-free N$_2$ gas and cooled to room temperature. Unless otherwise indicated, 5 ml aliquots were dispensed into Hungate tubes under N$_2$–CO$_2$ (80:2) gas mixture 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% (w/v) Na$_2$S.9H$_2$O were injected from anaerobic sterile stock solutions into the pre-sterilized basal medium.

Enrichment and isolation. The liquid digester sample (0·5 ml) was inoculated into the basal medium emended with 5 mM cinnamate and growth was followed by incubation at 37 °C. The enrichment culture that developed was subcultured several times under the same conditions. Subsequently, serial tenfold dilutions were prepared and inoculated into roll-tubes containing the basal medium supplemented with 5 mM cinnamate and 1·6% (w/v) agar. Several single well-isolated colonies that developed were picked, serially diluted in the anaerobic medium and the procedure was repeated at least three times. Several pure cultures were obtained, one of which was selected, designated strain CIN1^T, and characterized further. Purity was checked by microscopy of cultures grown in basal medium amended with 0·5% glucose and

0·5% Biotrypcase; strain CIN1^T failed to grow under these conditions.

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

Growth studies. Substrates were added from sterile anaerobic stock solutions. All growth studies were conducted in duplicate unless otherwise indicated. The isolate was subcultured at least twice under the same experimental conditions. Growth studies were performed in basal medium containing 5 mM cinnamate and 0·5 g yeast extract l^{-1}. For pH studies, the medium was adjusted to the required pH by injecting appropriate volumes from anaerobically prepared stock solutions of 1 M HCl, 10% NaHCO$_3$ and 8% Na$_2$CO$_3$. Temperature range for growth was determined between 10 and 55 °C using the basal medium adjusted to the optimum pH. For studies on NaCl requirements, NaCl was weighed directly in the tubes to give the required concentration. For concentrations lower than 1%, different aliquots of 10% (w/v) NaCl were injected into predispensed basal medium to give the required concentration.

Sporulation test. Cells from various stages of the growth phase and from cultures grown on different substrates were observed microscopically for the presence of spores. In addition, the culture was tested for heat resistance. For this, cells grown in basal medium containing 5 mM cinnamate were heated for 10 min at temperatures of 80, 90 and 100 °C. The cells were cooled quickly to ambient temperature, inoculated into fresh cinnamate-containing basal medium and growth was checked after at least 1 week incubation at 37 °C.

Substrate utilization studies. For substrate utilization studies, the isolate was subcultured at least once under the same experimental conditions. Substrate utilization studies were performed in basal medium amended with one of the following substrates to give a final concentration as indicated: 5 mM for all aromatic compounds including mono-methoxylated aromatic compounds (2-, 3-, 4-methoxycinnamate), di-methoxylated aromatic compounds (2,3-, 2,4-, 2,5-, 2,6-, 3,5-dimethoxybenzoate, 3,5-dimethoxycinnamaldehyde), tri-methoxylated aromatic compounds (3,4,5-trimethoxybenzoate, 3,4,5-trimethoxycinnamate, 3,4,5-trimethoxyphenylacetate, 3,4,5-trimethoxyphenylpropionate), hydroxy-methoxylated aromatic compounds (ferulate, syringate and sinapate), mono-hydroxylated aromatic compounds (2-, 3-, 4- hydroxycinnamate, phenol and 4-hydroxybenzoate), polyhydroxylated aromatic compounds (caffeate, gallate, tyrosol, phloroglucinol, pyrogallol, catechol and hydroquinone), non-hydroxylated and non-methoxylated substituted cinnamyl derivatives (α-methylcinnamate, methyl *trans*-cinnamate, cinnamyl alcohol, *trans*-cinnamate methyl ester and coumarine) and non-substituted aromatic compounds (cinnamate, benzoate, hydrocinnamate and phenylacetate); 10 mM for organic acids (crotonate, pyruvate, succinate, malate, fumarate, propionate and butyrate); 10 mM for carbohydrates (glucose, fructose, saccharose, ribose, xylose, cellobiose, maltose, galactose, mannose, lactose and arabinose); 5 mM for alcohols (butanol, methanol and ethanol); and 0·5% (w/v) for extracts (Biotrypcase, peptone, Casamino acids, gelatin and casein).

The utilization of a mixture of 2-, 3- and 4-methoxycinnamate (3 mM and 5 mM each) by isolate CIN1^T was investigated. Utilization was evaluated by monitoring substrate disappearance and product accumulation.

Electron acceptors. Thiosulfate, sulfate, sulfite and nitrate were tested as electron acceptors at a final concentration of 10 mM, whereas elemental sulfur and sodium fumarate were tested at a final concentration of 2% and 20 mM, respectively.

Analytical methods. Turbidity, as a measure of 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 using HPLC model 1100 (Hewlett Packard) equipped with a UV detector set at 240 nm and a 100-position autosampler/autoinjector. Separation was achieved using a C_{18} SYMMETRY column (4·6 × 100 mm, 3·5 μm particle size; 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, 20% methanol solvent phase was initially held for 4 min, then the concentration was increased to 40% over a period of 24 min followed by a wash for 6 min, over which time 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 by 7·8 mm (i.d.) (Bio-Rad Laboratories) connected to a differential refractometer (RID-6A; Shimadzu). Analysis was performed using a CR-6A Shimadzu integrator. The mobile phase was 0·0025 M H_2SO_4 at a flow rate of 0·6 ml min^{-1} and the column temperature was 35 °C. The volume of the injection loop was 20 μl.

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, Braunschweig, Germany). Non-methylated lambda DNA (Sigma) was used as the standard.

DNA extraction and amplification of 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 8–1500 based on *Escherichia coli* numbering of the 16S rDNA (Winker & Woese, 1991). A 50 μl reaction mix contained 1–20 ng genomic DNA, 1 μM 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 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 the QIAquick kit (Qiagen). The DNA concentrations of purified PCR products were estimated by comparison with the Low Mass Ladder (Gibco-BRL) on an ethidium bromide-containing agarose gel. Sequencing was carried out using the ABI Dye Terminator Cycle Sequencing kit containing Ampli*Taq* FS DNA polymerase under the following conditions. A 10 μl reaction mix contained 35 ng PCR product, 4 μl cycle sequencing reaction mix, 3·2 pmol primer (Andrews & Patel, 1996) and 2·5 μg BSA. Thermal cycling was carried out using a Rapid Cycler (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, extension at 60 °C for 3 min. The samples were purified from the unused dye terminators following the manufacturer's recommended procedures and sequenced using an ABI377 automated DNA sequencer (Applied Biosystems) at the Griffith University Molecular Biology Centre.

Sequence alignments and phylogenetic inferences. The new sequence data that were generated were assembled to an almost full-length consensus 16S rRNA gene sequence and checked for accuracy manually using the alignment editor ae2 (Maidak *et al.*, 1999). The new consensus sequence generated was 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.*, 1999). Reference sequences most related to our newly generated sequence were extracted from the databases and aligned. Positions of sequence and alignment uncertainty were omitted from the analysis. Pairwise evolutionary distances based on 1392 unambiguous nucleotides were computed using DNADIST (Jukes & Cantor option) and NEIGHBOR-JOINING programs that form part of the PHYLIP suite of programs (Felsenstein, 1993).

RESULTS

Enrichment and isolation

A cinnamate-enrichment culture was obtained after 3 weeks incubation at 37 °C. The enrichment culture was serially diluted and subcultured several times and resulted in the establishment of a stable microbial consortium that degraded cinnamate with concomitant production of acetate and benzoate. This consortium exhibited a morphologically dominant population of rod-shaped organisms which possessed characteristic terminal nipple-shaped ends. The enrichment culture was serially diluted and used to inoculate roll-tubes. Single well-separated colonies that developed within incubation for 1 month were picked and the purification procedure was repeated several times until the cultures were deemed pure. A pure culture,
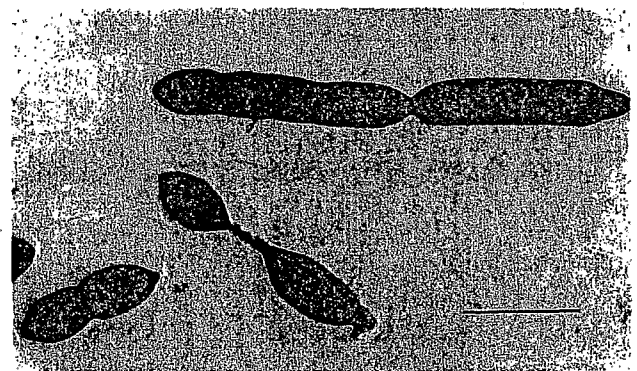

Fig. 1. Electron micrograph of cells of strain CIN1^T showing characteristic nipple-shaped pointed ends. Bar, 2 μm.
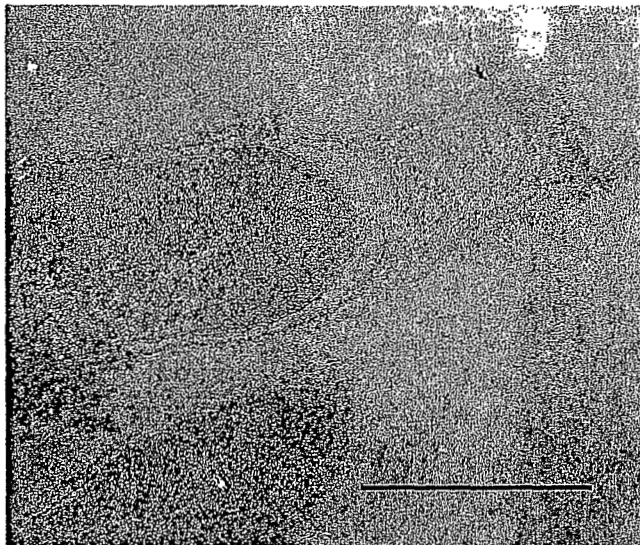
Fig. 2. Electron micrograph of a cell of strain CIN1^T showing terminal accumulation of an electron light surface layer (arrows). Bar, 0·5 µm.

designated strain CIN1^T, was selected for further characterization.

Cellular morphology

Cells of strain CIN1^T were rod-shaped ($1\cdot3$–$3\cdot0 \times 0\cdot5$–$0\cdot6$ µm) and had characteristic nipple-shaped pointed ends (Fig. 1a). The terminal nipple-shaped feature of strain CIN1^T lacked cytoplasmic material and appeared to be a part of the cell surface slime layer involved in the cell division process (Fig. 1b). The cells stained Gram-positive and ultrathin sections of strain CIN1^T showed a thin atypical Gram-positive-type cell wall with an external slime layer (Fig. 2). Motility was not observed and electron microscopic examination of negatively stained cells failed to reveal the presence of flagella. Spores were not observed. There was no cell growth after heat treatment indicating the lack of heat-resistant bodies such as spores.

Growth, physiological and metabolic properties

The optimum growth temperature for strain CIN1^T was 37 °C (growth was observed between 15 and 40 °C), with no growth occurring at 10 and 45 °C. The optimum pH for growth was pH 7·5 (pH growth range was 6·9–8·5) with no growth occurring at pH 6·0 and 9·0. Strain CIN1^T did not require NaCl for growth but grew better in the presence of 0·5–1·0 % NaCl, with no growth occurring in the presence of 2·0 % NaCl.

Yeast extract was required for growth on cinnamate. Biotrypcase and casein, but not gelatin, stimulated growth but were not essential for growth and could not replace yeast extract. The rate of transformation of cinnamate did not change when yeast extract or
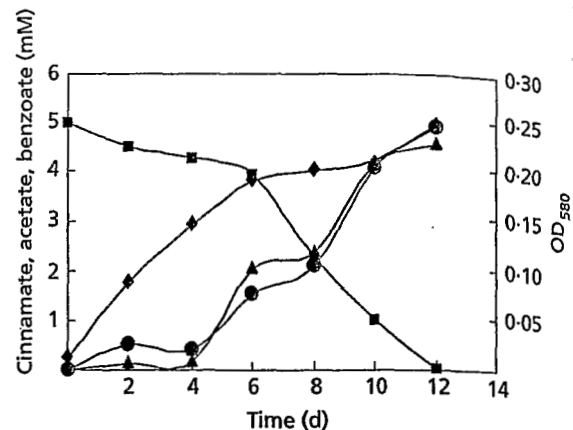

Fig. 3. Transformation of 5 mM cinnamate by strain CIN1^T. Growth is indicated by an increase in optical density (♦) and the transformation of cinnamate (■) to benzoate (●) and acetate (▲).

Biotrypcase concentrations were increased, although the cell yield increased.

Strain CIN1^T was unable to grow on any other substrates tested with the exception of cinnamate, 3-methoxycinnamate, 4-methoxycinnamate and crotonate, as listed in the Methods.

Acetate and benzoate were produced from cinnamate transformation (Fig. 3), acetate and 3-methoxybenzoate were produced from 3-methoxycinnamate transformation, and acetate and 4-methoxybenzoate were produced from 4-methoxycinnamate transformation. Acetate and butyrate were produced from crotonate degradation.

The transformation of cinnamate was concentration-dependent (Table 1). With 15 mM cinnamate, almost all the substrate was transformed. This is based on the observation that the ratios of benzoate:cinnamate and acetate:cinnamate were equal to 1 and are close to the calculated theoretical value of 1. At concentrations above 15 mM, cinnamate was incompletely transformed with no transformation occurring above 30 mM, which can be considered as threshold toxicity for the isolate.

The results from studies on a 3 mM mixture of each of 2-, 3- and 4-methoxycinnamate by strain CIN1^T indicated that the transformation of 3-methoxycinnamate had a shorter lag phase and occurred a bit faster than that of 4-methoxycinnamate (Fig. 4). The observation that the disappearance rates of the two isomers are different would suggest a positional selectivity for efficient transformation with regard to the methoxy group on the aromatic ring.

Electron acceptors

Sulfate, thiosulfate, sulfite, nitrate, elemental sulfur or fumarate were not utilized as electron acceptors.

Table 1. Transformation of cinnamate at different concentrations by strain CIN1^T

Basal medium supplemented with the various concentrations of cinnamate was used. Incubations were performed at 37 °C for 14 d, after which end products were analysed.

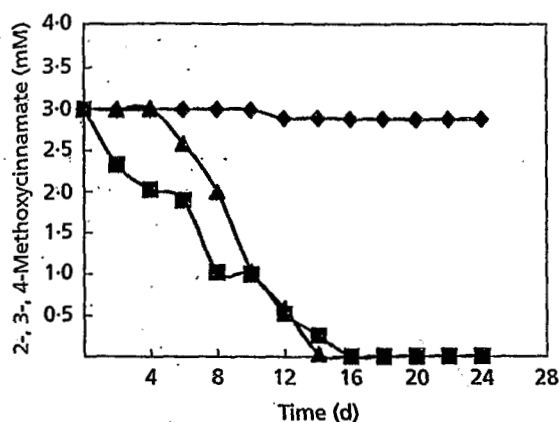| Cinnamate (mM)	Product formed (mM)		Ratio of:	
	Benzoate	Acetate	Benzoate:cinnamate	Acetate:cinnamate
5	5	4·94	1	0·98
10	10	9·98	1	0·99
15	15	14·98	1	0·99
20	15·36	14·23	0·76	0·71
25	13·25	13·15	0·53	0·52
30	8·95	7·56	0·29	0·25
35	0	0	0	0

Fig. 4. The rate of disappearance of three different methoxylated cinnamates in the presence of strain CIN1^T. The disappearance of 3-methoxycinnamate (■) is much faster than that of 4-methoxycinnamate (▲) whereas 2-methoxycinnamate (♦) is not utilized.

G+C content

The G+C content of the DNA of strain CIN1^T was 56 mol% as determined by HPLC.

Phylogeny

Using 12 primers, an almost complete sequence was determined consisting of 1492 nt of the 16S rRNA gene of strain CIN1^T (corresponding to *E. coli* position 8–1500). Phylogenetic analysis (Fig. 5) revealed that strain CIN1^T was a member of cluster IV of the low-G+C-containing Gram-positive branch as defined by Collins *et al.* (1994).

DISCUSSION

Physiologically, strain CIN1^T transforms aromatic compounds with a double bond in the C$_3$-aliphatic side chain. The anaerobe *Ruminococcus productus* (for-merly *Peptostreptococcus productus*) transforms the aromatic compound ferulate by (a) O-demethylation and (b) reduction of the double bond of the C$_3$-aliphatic side chain (Misoph *et al.*, 1996), and the anaerobe *Acetobacterium woodii* reduces the double bond of the C$_3$-aliphatic side chain of caffeate (Tschech *et al.*, 1984). However, strain CIN1^T does not trans-form ferulate and caffeate but instead transforms cinnamate, an aromatic compound similar to ferulate and caffeate (both contain a double bond in the C$_3$-aliphatic side chain) to benzoate and acetate. This trait of cinnamate transformation is also common to *Rhodopseudomonas palustris* and *Syntrophus buswellii* strain GA. However, strain CIN1^T transforms cin-namate to produce acetate and benzoate which accumulates without further degradation, whereas a syntrophic co-culture of *Syntrophus buswellii* strain GA and *Desulfovibrio vulgaris* degraded cinnamate to produce acetate and H$_2$S without accumulation of benzoate. On the other hand, *Rhodopseudomonas palustris* requires light for cinnamate degradation but strain CIN1^T does not. Again, these traits indicate that strain CIN1^T is physiologically distinct from these two isolates.

Strain CIN1^T utilizes a very limited range of aromatic compounds which include cinnamate, 3-methoxy-cinnamate and 4-methoxycinnamate. Such a charac-teristic has not been reported previously and hence can be regarded as a new physiological feature. The transformation of 3-methoxycinnamate and 4-methoxycinnamate but not 2-methoxycinnamate is also an interesting property of the new isolate and indicates stereospecific selection.

The closest phylogenetic neighbour to strain CIN1^T is *Sporobacter termitidis*, a member of cluster IV (Collins *et al.*, 1994) of the low-G+C-containing Gram-positive branch, with which it shares a sequence identity of 88%. Based on this alone, strain CIN1^T can be regarded as a member of a novel genus. In addition, there are numerous phenotypic differences which also
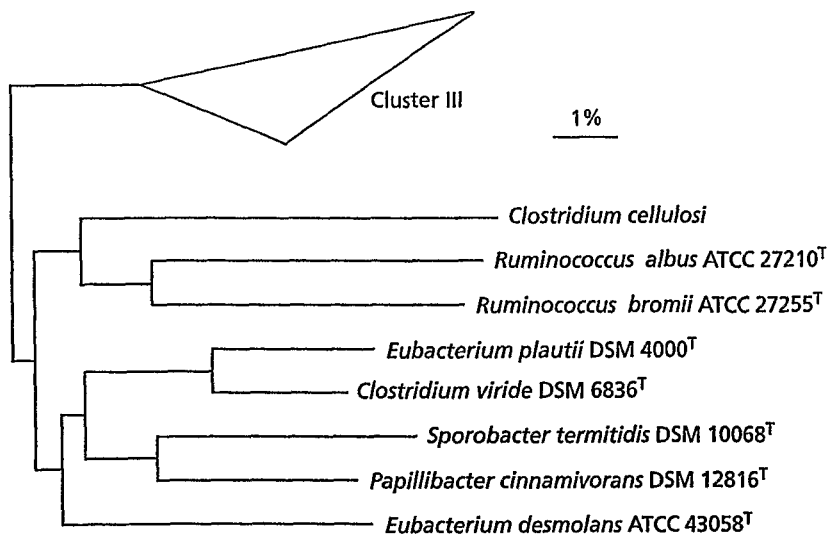
Fig. 5. Unrooted phylogenetic dendrogram based on 16S rRNA sequence data indicating the position of strain CIN1[T] within the radiation of representatives of members of cluster III (indicated as a triangle consisting of *Clostridium thermocellum, Clostridium termitidis, Clostridium cellulolyticum, Clostridium stercorarium, Clostridium thermolacticum* and *Acetivibrio cellulosolvens*) and cluster IV consisting of the genus *Clostridium* and relatives according to Collins et al. (1994). All sequences used in the analysis were obtained from the Ribosomal Database Project, version 7 (Maidak et al., 1999). Evolutionary distances (based on 1321 unambiguous nucleotides) were computed using programs that form part of the PHYLIP package (Felsenstein, 1993). Scale bar, 1 nucleotide substitution per 100 nucleotides.

Table 2. Comparison of characteristics of strain CIN1[T] and *Sporobacter termitidis*

Characteristic	Strain CIN1[T]	*Sporobacter termitidis**
Morphology	Rod shaped with pointed ends	Slightly curved
Size (μm)	0·5–0·6 × 1·3–3·0	0·3–0·4 × 1·2
Gram reaction	+	+
Spores	−	+
Motility	−	+
pH optimum	7·5	6·7–7·2
NaCl optimum (g l⁻¹)	5–10	0–5
Temperature optimum (°C)	37	32–35
Yeast extract requirement	+	+
Growth on aromatic compounds:†		.
Cinnamate	+	−
2-Methoxycinnamate	−	ND
3-Methoxycinnamate	+	ND
4-Methoxycinnamate	+	−
3,4,5-Trimethoxycinnamate	ND	+
Sinapate	−	+
3,4-Dimethoxycinnamate	ND	+
3,4,5-Trimethoxybenzoate	−	+
Ferulate	−	+
Syringate	−	+
Vanillate	−	+
G+C content (mol%)	56	57

* Data from Grech-Mora et al. (1996).

† Substrates tested but not used by both strains include the following: aromatic compounds (benzoate, phenylacetate, caffeate, gallate, phloroglucinol, pyrogallol, catechol, p-coumarate, phenol, 4-hydroxybenzoate), organic acids (pyruvate), carbohydrates (glucose, fructose, ribose, xylose, maltose, galactose, lactose) and alcohols (methanol, ethanol).

ND, Not determined.

set these two strains apart. Amongst these are the distinctly different morphologies and the range of substrates used (Table 2). Based on the evidence presented here, it is proposed that strain CIN1[T] be designated a new species of a new genus, *Papillibacter cinnamivorans* gen. nov., sp. nov.

Description of *Papillibacter* gen. nov.

Papillibacter (Pa.pil.li.bac'ter. L. fem. n. *papilla* teat; M.L. n. *bacter* masc. equivalent of Gr. neut. n. *bakterion* rod or staff; M.L. masc. n. *Papillibacter* a rod with ends looking like teat).

Cells are rod-shaped, occur singly, in pairs or in chains, and stain Gram-positive. No spores are formed and the cells are non-motile. Growth is strictly anaerobic. Growth is by degradation of a limited range of aromatic compounds and crotonate but not carbohydrates, organic acids or alcohols. *Papillibacter* represents a new line of descent in the low-G + C-containing, Gram-positive branch based on 16S rRNA sequence analysis. The type species is *Papillibacter cinnamivorans*.

Description of *Papillibacter cinnamivorans* sp. nov.

Papillibacter cinnamivorans (cin.na.mi.vo'rans. M.L. n. *cinnamum* cinnamic acid; L. part. adj. *vorans* devouring, digesting; M.L. part. adj. *cinnamivorans* cinnamic-acid-digesting).

Cells are non-sporulating anaerobes which stain Gram-positive and are non-motile. The isolate utilizes cinnamate, 3-methoxycinnamate, 4-methoxycinnamate and crotonate. No growth occurs with the following substrates: mono-methoxylated aromatic compound (2-methoxycinnamate), di-methoxylated aromatic compounds (2,3-, 2,4-, 2,5-, 2,6-, 3,5-dimethoxybenzoate, 3,5-dimethoxycinnamaldehyde), tri-methoxylated aromatic compounds (3,4,5-trimethoxybenzoate, 3,4,5-trimethoxycinnamate, 3,4,5-trimethoxyphenylacetate, 3,4,5-trimethoxyphenylpropionate), hydroxy-methoxylated aromatic compounds (ferulate, syringate and sinapate), mono-hydroxylated aromatic compounds (2-, 3-, 4-hydroxycinnamate, phenol and 4-hydroxybenzoate), poly-hydroxylated aromatic compounds (caffeate, gallate, tyrosol, phloroglucinol, pyrogallol, catechol and hydroquinone), non-hydroxylated and non-methoxylated substituted cinnamyl derivatives (α-methylcinnamate, methyl *trans*-cinnamate, cinnamyl alcohol, *trans*-cinnamate methyl ester and coumarine), non-substituted aromatic compounds (benzoate, hydrocinnamate and phenylacetate), organic acids (crotonate, pyruvate, succinate, malate, fumarate, propionate and butyrate), carbohydrates (glucose, fructose, saccharose, ribose, xylose, cellobiose, maltose, galactose, mannose, lactose and arabinose), alcohols (butanol, methanol and ethanol) or extracts (Biotrypcase, peptone, Casamino acids, gelatin and casein). Sulfate, thiosulfate, sulfite, nitrate, elemental sulfur or fumarate are not reduced. Optimum pH for growth is 7·5 (pH growth range 6·9–8·5). Optimal growth temperature is 37 °C (temperature growth range 15–40 °C). Optimum growth occurs in the presence of 0·5–1·0 % NaCl, with no growth occurring in the presence of 2·0 % NaCl. The G + C content of the DNA is 56 mol%. The type strain is CIN1^T (= DSM 12816^T = ATCC 700879^T). Isolated from an anaerobic digester fed with shea cake, Burkina Faso.

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