NOTE

Metabolism of cinnamic acids by some *Clostridiales* and emendation of the descriptions of *Clostridium aerotolerans*, *Clostridium celerecrescens* and *Clostridium xylanolyticum*

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The ability of *Clostridium aerotolerans* DSM 5434^T, *Clostridium celerecrescens* DSM 5628^T, *Clostridium methoxybenzovorans* DSM 12182^T, *Clostridium stercoarium* ATCC 35414^T, *Clostridium subterminale* DSM 2636, *Clostridium termitidis* DSM 5398^T, *Clostridium thermolacticum* DSM 2910^T, *Clostridium thermopalmarium* DSM 5974^T and *Clostridium xylanolyticum* DSM 6555^T to metabolize cinnamic acid and various derivatives, with or without glucose supplementation, was examined. Only *C. aerotolerans* DSM 5434^T and *C. xylanolyticum* DSM 6555^T, closely related species, transformed cinnamic acid to 3-phenylpropionic acid. Both species also reduced a wide range of cinnamic acid derivatives, including *o*-, *m*- and *p*-coumaric, *o*-, *m*- and *p*-methoxycinnamic, *p*-methylcinnamic, caffeic, ferulic, isoferulic and 3,4,5-trimethoxycinnamic acids to their corresponding 3-phenylpropionic acid derivatives. *C. aerotolerans* DSM 5434^T, however, also decarboxylated *p*-coumaric acid into 4-vinylphenol, which was then reduced to 4-ethylphenol. *C. celerecrescens* was grouped with *C. aerotolerans* and *C. xylanolyticum* in subcluster XIVa of the *Clostridiales*. *C. celerecrescens* DSM 5628^T only metabolized *m*- and *p*-methoxycinnamic and *p*-methylcinnamic acids to their corresponding 3-phenylpropionic acid derivatives, reducing the double bond in the C₃ aliphatic side chain. Addition of glucose markedly increased the yield of the biotransformations by these three species. An emendation of the descriptions of *C. aerotolerans*, *C. celerecrescens* and *C. xylanolyticum* is proposed, based on these observations.

Keywords: *Clostridium aerotolerans*, *Clostridium celerecrescens*, *Clostridium xylanolyticum*, cinnamic acids, emendation

Many anaerobic bacteria cannot completely degrade aromatic compounds, but catalyse oxygen-independent biotransformations. Other species attack the aromatic ring anaerobically. With anaerobic bacteria where the ring is not attacked, the conversions involve specific substitutions on the aromatic ring through different strategies. The effect of these reductive anaerobic biotransformations is to dispose of redox equivalents generated in oxidative reactions (Heider & Fuchs, 1997). Some cinnamic compounds are free, ester-linked to arabinoxylan and lignin, or cross-linked to lignin and polysaccharide through ether and ester bonds, respectively. A *Clostridium* sp., closely related

to *Clostridium xylanolyticum*, hydrolysed and then hydrogenated hydroxycinnamic acids (ferulic and *p*-coumaric acids), which are esterified to arabinoxylan in plant cell walls (McSweeney *et al.*, 1999). Two new strains of *Clostridium* have been isolated from olive mill wastewater after enrichment cultures on cinnamic acid: *Clostridium glycolicum* (Chamkha, *et al.*, 2001a) and *Clostridium bifermentans* (Chamkha, *et al.*, 2001b). Both strains were members of the low-G + C-containing Gram-positive branch and grouped with members of cluster XI (Collins *et al.*, 1994). *C. glycolicum* reduced 11 cinnamic acids to their corresponding phenylpropionic acids. *C. bifermentans*

reduced cinnamic acid to hydrocinnamic acid, but did not reduce any of the other cinnamic acid derivatives tested. Therefore, nine *Clostridium* spp. available in our laboratory were studied for their ability to metabolize cinnamic acid and various derivatives, with or without glucose supplementation. Only three species, *Clostridium aerotolerans* DSM 5434^T, *Clostridium celerecrescens* DSM 5628^T and *C. xylanolyticum* DSM 6555^T, could transform some cinnamic acid compounds. Considering the new metabolic information, emendation of the descriptions of these three species is proposed.

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.HCl; 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 solution; 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 culture inoculation, 0.05 ml 10% (w/v) NaHCO₃ and 0.05 ml 5% (w/v) Na₂S·9H₂O were injected into the 5 ml pre-sterilized medium from sterile stock solutions. Substrates were injected from concentrated anaerobic sterile stock solutions to obtain the desired final concentration. Reference cultures were routinely grown using glucose-containing basal medium at the optimum temperature for growth: 37 °C for *C. aerotolerans* X8A62^T (= DSM 5434^T), *C. celerecrescens* 18A^T (= DSM 5628^T), *Clostridium methoxybenzovorans* SR3^T (= DSM 12182^T), *Clostridium subterminale* DSM 2636, *Clostridium termitidis* CT1112^T (= DSM 5398^T) and *C. xylanolyticum* DSM 6555^T; 55 °C for *Clostridium thermopalmarium* BVP^T (= DSM 5974^T); and 65 °C for *Clostridium stercorarium* ATCC 35414^T and *Clostridium thermolacticum* TX41^T (= DSM 2910^T).

For aromatic substrate utilization, experiments were performed in duplicate with an inoculum that had been subcultured at least once under the same test conditions. Substrates tested were taken from pre-sterilized and concentrated stock solutions and injected at a final concentration of 5 mM into Hungate tubes containing 5 ml pre-sterilized basal medium. Substrates included: cinnamic acid; hydroxylated cinnamic acids [*o*-, *m*- and *p*-hydroxycinnamic acid (coumaric acid) and 3,4-dihydroxycinnamic acid (caffeic acid)]; methoxylated cinnamic acids [*o*-, *m*- and *p*-methoxycinnamic acid and 3,4,5-trimethoxycinnamic acid]; mixed hydroxylated/methoxylated cinnamic acids [4-hydroxy-3-methoxycinnamic acid (ferulic acid) and 3-hydroxy-4-methoxycinnamic acid (isoferulic acid)]; methylated cinnamic acid (*p*-methylcinnamic acid); cinnamyl alcohol; and phenylpropionic acids [3-phenylpropionic

acid (hydrocinnamic acid) and 3,4-dihydroxyphenylpropionic acid (hydrocaffeic acid)]. 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. An increase in OD₅₈₀ in tubes containing added substrates, compared to control tubes lacking substrate, was considered an index of positive growth. Strains able to metabolize cinnamic acid were tested for their ability to metabolize other cinnamic compounds, with or without 5 mM glucose. Bacterial growth was monitored by measuring the increase in turbidity at 580 nm by inserting anaerobic Hungate tubes into the cuvette holder of a spectrophotometer (Shimadzu UV 160A). Aromatic compounds and volatile fatty acids were measured by HPLC as described by Mechichi *et al.* (1999).

C. thermopalmarium DSM 5974^T (Soh *et al.*, 1991) and *C. subterminale* DSM 2636 (Hall & Whitehead, 1927) are members of cluster I of the *Clostridiales* (Collins *et al.*, 1994). *C. stercorarium* ATCC 35414^T (Madden, 1983), *C. termitidis* DSM 5398^T (Hethener *et al.*, 1992) and *C. thermolacticum* DSM 2910^T (Le Ruyet *et al.*, 1985) are members of group III of the *Clostridiales* (Collins *et al.*, 1994). These strains did not metabolize cinnamic acid, in the presence or absence of glucose.

C. aerotolerans (van Gylswyk & van der Toorn, 1987), *C. celerecrescens* (Palop *et al.*, 1989), *C. methoxybenzovorans* (Mechichi *et al.*, 1999) and *C. xylanolyticum* (Rogers & Baecker, 1991) are members of the low-G+C-containing Gram-positive branch, as defined by Collins *et al.* (1994), and grouped with members of subcluster XIVa. They exhibited high levels of 16S rRNA gene sequence relatedness (mean similarity of 98%) (Mechichi *et al.*, 1999). Our results showed that *C. methoxybenzovorans* DSM 12182^T did not metabolize cinnamic acid, with or without glucose supplementation, but demethoxylated some methoxycinnamic derivatives: 3,4,5-trimethoxycinnamic acid; 3,5-dimethoxycinnamic acid; ferulic acid; isoferulic acid; and 3-methoxycinnamyl alcohol (Mechichi *et al.*, 1999). Similarly, *C. celerecrescens* DSM 5628^T did not metabolize cinnamic acid, but reduced *m*- and *p*-methoxycinnamic and *p*-methylcinnamic acids to their corresponding 3-phenylpropionic acid derivatives (Table 1). *o*-, *m*- and *p*-Coumaric, *o*-methoxycinnamic, caffeic, ferulic, isoferulic and 3,4,5-trimethoxycinnamic acids were not metabolized by *C. celerecrescens* DSM 5628^T, indicating a stereospecific selection.

C. xylanolyticum is phylogenetically closely related to *C. aerotolerans* (> 99% similarity) (Collins *et al.*, 1994). The DNA G+C contents of both species were 40 mol% (Rogers & Baecker, 1991; van Gylswyk & van der Toorn, 1987). *C. xylanolyticum* DSM 6555^T and *C. aerotolerans* DSM 5434^T reduced a wide range of cinnamic acids, including cinnamic, *o*-, *m*- and *p*-

Table 1. Metabolism of cinnamic acids by *C. celerecrescens* DSM 5628^T

Cells were grown in basal medium containing 0.2 % yeast extract; cinnamic compounds were used at 5 mM, with or without 5 mM glucose. Results were recorded after 2 d, 4 d and 2 weeks anaerobic incubation at 37 °C. Cinnamic compounds tested were completely converted with supplementation of glucose, but not in the absence of glucose.

Cinnamic acid metabolized	Glucose	Product formed (mM) after:		
		2 d	4 d	2 weeks
<i>m</i> -Methoxycinnamic acid	—	0.86	1.74	2.82
	+	0.94	2.60	4.65
<i>p</i> -Methoxycinnamic acid	—	0.51	1.38	2.90
	+	1.86	4.01	4.77
<i>p</i> -Methylcinnamic acid	—	0.48	0.92	2.26
	+	0.90	4.89	4.90

Table 2. Metabolism of cinnamic acids by *C. xylanolyticum* DSM 6555^T

Cells were grown in basal medium containing 0.2 % yeast extract; cinnamic compounds were used at 5 mM, with or without 5 mM glucose. Results were recorded after 2 d, 4 d and 1 month anaerobic incubation at 37 °C. Cinnamic compounds tested were completely converted except for *o*-methoxycinnamic acid, with or without added glucose. Data were only recorded after 1 month when transformation of compounds was not complete after 4 d.

Cinnamic acid metabolized	Glucose	Product formed (mM) after:		
		2 d	4 d	1 month
Cinnamic acid	—	1.00	2.90	4.82
	+	2.36	3.08	4.76
<i>o</i> -Coumaric acid	—	3.28	4.60	
	+	4.53	4.53	
<i>m</i> -Coumaric acid	—	4.84	4.87	
	+	4.63	4.67	
<i>p</i> -Coumaric acid	—	5.22	5.15	
	+	5.18	5.11	
<i>o</i> -Methoxycinnamic acid	—	0.00	0.00	0.84
	+	0.00	0.00	1.34
<i>m</i> -Methoxycinnamic acid	—	2.90	3.54	4.66
	+	4.75	4.83	
<i>p</i> -Methoxycinnamic acid	—	3.00	4.03	4.85
	+	4.62	4.70	
<i>p</i> -Methylcinnamic acid	—	2.78	4.24	4.93
	+	5.10	5.12	
Caffeic acid	—	3.18	3.66	4.56
	+	3.81	4.43	
Ferulic acid	—	5.70	5.60	
	+	5.63	5.54	
Isoferulic acid	—	4.90	5.21	
	+	5.11	5.15	
3,4,5-Trimethoxycinnamic acid	—	1.85	4.90	
	+	3.17	4.87	

coumaric, *o*-, *m*- and *p*-methoxycinnamic, *p*-methylcinnamic, caffeic, ferulic, isoferulic and 3,4,5-trimethoxycinnamic acids to their corresponding 3-

phenylpropionic acids (Tables 2 and 3). However, *C. aerotolerans* showed a second metabolic pathway: decarboxylation of *p*-coumaric acid into 4-vinyl-

Table 3. Metabolism of cinnamic acids by *C. aerotolerans* DSM 5434^T

Cells were grown in basal medium containing 0.2% yeast extract; cinnamic compounds were used at 5 mM, with or without 5 mM glucose. Results were recorded after 2 d, 4 d and 1 month anaerobic incubation at 37 °C. Cinnamic compounds tested were completely converted except for *o*-methoxycinnamic acid, with or without added glucose, and *m*-methoxycinnamic acid, *p*-methoxycinnamic acid and *p*-methylcinnamic acid, all without addition of glucose. Data were only recorded after 1 month when transformation of compounds was not complete after 4 d.

Cinnamic acid metabolized	Glucose	Product formed (mM) after:		
		2 d	4 d	1 month
Cinnamic acid	—	1.80	4.65	
	+	3.95	4.44	
<i>o</i> -Coumaric acid	—	1.02	2.69	4.91
	+	4.55	4.62	
<i>m</i> -Coumaric acid	—	4.87	5.10	
	+	4.70	4.73	
<i>p</i> -Coumaric acid	—	4.57, 0.82, 0.36*	4.36, 1.18, 0.00*	
	+	4.31, 0.47, 0.00*	4.31, 0.46, 0.00*	
<i>o</i> -Methoxycinnamic acid	—	0.00	0.00	0.75
	+	0.00	0.00	2.13
<i>m</i> -Methoxycinnamic acid	—	1.30	1.69	3.90
	+	4.61	4.53	
<i>p</i> -Methoxycinnamic acid	—	1.41	1.63	4.08
	+	4.51	4.48	
<i>p</i> -Methylcinnamic acid	—	0.76	1.06	4.35
	+	4.82	4.91	
Caffeic acid	—	1.40	2.30	4.77
	+	3.62	4.57	
Ferulic acid	—	2.21	3.87	4.65
	+	4.74	4.98	
Isoferulic acid	—	3.49	4.00	4.80
	+	4.78	4.87	
3,4,5-Trimethoxycinnamic acid	—	1.47	4.75	
	+	2.92	4.68	

* Values given are concentrations of *p*-hydroxyhydrocinnamic acid, 4-ethylphenol and 4-vinylphenol, respectively.

phenol, which was then reduced to 4-ethylphenol (Fig. 1). There was no evidence for the occurrence of the decarboxylation of *p*-hydroxyhydrocinnamic acid in this study. Phenotypically, *C. xylanolyticum* differed from *C. aerotolerans*, which fermented lactose, ribose and trehalose (Rogers & Baecker, 1991). Results suggest that the metabolism of *p*-coumaric acid is another phenotypic difference, *C. aerotolerans* DSM 5434^T exhibiting two transformation pathways and *C. xylanolyticum* DSM 6555^T only one. The transformation of *o*-methoxycinnamic acid by *C. xylanolyticum* DSM 6555^T and *C. aerotolerans* DSM 5434^T was slower than that of other cinnamic acid derivatives tested. A different disappearance rate of *o*-methoxycinnamic acid (Tables 2 and 3) suggests an inhibition of growth rather than a positional selectivity for an efficient transformation with regard to the methoxy group on the aromatic ring. The ratio of phenylpropionic acids to their corresponding cinnamic acids in the medium increased during the course of the experiment until conversion of substrate was complete.

Addition of glucose always enhanced growth and markedly accelerated these conversions by *C. xylanolyticum* DSM 6555^T, *C. aerotolerans* DSM 5434^T and *C. celerecrescens* DSM 5628^T. The mechanism involves reduction of the double bond in the C₃ aliphatic side chain. The presence of cinnamic acids did not result in higher growth yields. With all aromatic compounds tested and even after one month incubation, no ring cleavage was observed. Cinnamyl alcohol and phenylpropionic acids, including 3-phenylpropionic acid and 3,4-dihydroxyphenylpropionic acid, were not metabolized by these strains, with or without the addition of glucose, indicating that these conversions occur only in the presence of an acrylic acid chain.

Reduction of the double bond in the side chain of some cinnamic acid derivatives was reported in rat intestinal microflora (Scheline, 1968), anaerobic microbial consortia (Nali *et al.*, 1985; Angeles de la Torre & Gomez-Alarcon, 1991), methanogenic consortia

Fig. 1. Metabolism of *p*-coumaric acid by *C. aerotolerans* DSM 5434^T. *p*-Coumaric acid (I) is reduced (A) to *p*-hydroxyhydrocinnamic acid [3-(4-hydroxyphenyl) propionic acid (II)]. In another pathway, *p*-coumaric acid can be decarboxylated (B) to yield 4-vinylphenol (III) which is then reduced (C) to 4-ethylphenol (IV).

(Grbic-Galic & Young, 1985), *Pseudomonas cepacia* (Andreoni *et al.*, 1984), *Pseudomonas stutzeri* and *Pseudomonas putida* (Andreoni & Bestetti, 1986), *Wolinella succinogenes* (Ohmiya *et al.*, 1986) and *Corynebacterium glutamicum* (Labuda *et al.*, 1993). Our results extend the mechanism to some *Clostridium* species. Decarboxylation of the propane side chain or degradation of the side chain of cinnamic acids are other metabolic patterns found in some microorganisms. Transformation of cinnamic acids to their corresponding 4-vinyl derivatives has been reported in fungi, yeast and bacteria (Cavin *et al.*, 1997; Edlin *et al.*, 1998). *Papillibacter cinnamivorans* (Defnoui *et al.*, 2000), a strictly anaerobic bacterium, transforms cinnamic acid to acetic and benzoic acids, which accumulate without further degradation. In contrast, some anaerobic bacteria cleave the aromatic ring. A syntrophic co-culture of *Syntrophus buswellii* strain GA and *Desulfovibrio vulgaris* degrades cinnamic acid to produce acetate and H₂S without accumulation of benzoic acid (Auburger & Winter, 1995). *Rhodospseudomonas palustris*, a photosynthetic bacterium, requires light to degrade cinnamic acid (Harwood & Gibson, 1988).

As these results highlight the bioconversion of several cinnamic acids by three species of *Clostridium*, *C. xylanolyticum* DSM 6555^T, *C. aerotolerans* X8A62^T (= DSM 5434^T) and *C. celerecrescens* 18A^T (= DSM 5628^T), it is proposed that the descriptions of these species should be emended.

Emended description of *Clostridium xylanolyticum* (Rogers and Baecker 1991)

Clostridium xylanolyticum (xy.lan.o.ly'ti.cum. Gr. n. *xylanosum* xylan; Gr. adj. *lyticus* dissolving, loosening; N.L. part. adj. *xylanolyticum* xylan-dissolving).

Cells are Gram-negative, obligately anaerobic and rod-shaped (1.8–3.0 × 0.5–0.8 µm). A single, terminal, round endospore is formed by each cell; the spore distends the cell wall. Cells are motile by means of peritrichous flagella. Thin sections reveal a five-layer cell wall structure. Xylan is a good substrate for growth. Amygdalin, arabinose, glycogen, inositol, lactose, mannitol, ribose, sorbitol and trehalose are not fermented. Gelatin is not liquefied. Urease, indole, lecithinase, lipase and catalase are not produced and nitrate is not reduced. Fermentation end products from PYG are formic, lactic and acetic acids. β-Haemolytic and resistant to clindamycin. Optimum temperature and pH for growth are 35 °C and 7.2, respectively. Reduces the double bond of the side chain of a wide range of cinnamic acids: cinnamic, *o*-, *m*-, *p*-coumaric, *o*-, *m*-, *p*-methoxycinnamic, *p*-methylcinnamic, caffeic, ferulic, isoferulic and 3,4,5-trimethoxycinnamic acids. End products from cinnamic acid metabolism are the corresponding phenylpropionic acids: 3-phenyl, 3-(2-hydroxyphenyl), 3-(3-hydroxyphenyl), 3-(4-hydroxyphenyl), 3-(2-methoxyphenyl), 3-(3-methoxyphenyl), 3-(4-methoxyphenyl), 3-(4-methylphenyl), 3-(3,4-dihydroxyphenyl), 3-(4-hydroxy-3-methoxyphenyl), 3-(3-hydroxy-4-methoxyphenyl) and 3-(3,4,5-trimethoxyphenyl) propionic acids. Addition of glucose markedly increases the rate of conversions. Cinnamyl alcohol and phenylpropionic acids, including 3-phenylpropionic acid and 3,4-dihydroxyphenylpropionic acid, are not transformed. Genomic DNA G+C content is 40 mol %. Phylogenetically, *C. xylanolyticum* is a member of the low-G+C-containing Gram-positive branch as defined by Collins *et al.* (1994) and is grouped with members of subcluster XIVa. Type strain is strain ATCC 49623^T (= DSM 6555^T). Isolated from a *Pinus patula* chip pile which had been exposed to environmental conditions for 10 months.

Emended description of *Clostridium aerotolerans* (van Gylswyk and van der Toorn 1987)

Clostridium aerotolerans (ae.ro.to'le.rans. Gr. masc. n. *aer* air, gas; L. v. *tolerare* to endure, to put up with; L. neut. part. adj. *aerotolerans* air-enduring).

Cells grow under strictly anaerobic conditions and also in agar or liquid medium free from added reducing agent. They also grow in a shallow layer of liquid medium free from added reducing agent but exposed to air. Vegetative cells are straight rods (0.4–0.6 × 1.5–3.0 µm) often with somewhat pointed ends. Usually occur singly. Cells are motile by peritrichous flagella. Spores are terminal and distend the cells where they are formed. They are slightly oval and measure about 0.6 × 0.8 µm. After incubation for 3 d on films of glucose (0.5%) agar medium in roll bottles, surface colonies are round with smooth or almost smooth edges. They are convex and appear translucent at the edges to semi-translucent and light brown at the centre. Colonies are up to 2.5 mm wide. Saccharoclastic and

mesophilic. Growth does not occur at 45 °C. Gelatin is not liquefied, casein is not digested and H₂S is not formed from peptone or from sulfate reduction. Neither indole nor α -methylindole is produced. There is no indication that cells possess urease, catalase or enzymes for reducing nitrate. Some lipase activity was exhibited by the four strains tested. A wide range of carbohydrate energy sources is fermented with the final pH higher than 5.2. Xylan is fermented to formic, acetic and lactic acids, ethanol, hydrogen and carbon dioxide. Acetylmethylcarbinol is not produced. Various cinnamic acids including cinnamic, *o*-, *m*-, *p*-coumaric, *o*-, *m*-, *p*-methoxycinnamic, *p*-methylcinnamic, caffeic, ferulic, isoferulic and 3,4,5-trimethoxycinnamic acids are reduced to their corresponding phenylpropionic acids: 3-phenyl, 3-(2-hydroxyphenyl), 3-(3-hydroxyphenyl), 3-(4-hydroxyphenyl), 3-(2-methoxyphenyl), 3-(3-methoxyphenyl), 3-(4-methoxyphenyl), 3-(4-methylphenyl), 3-(3,4-dihydroxyphenyl), 3-(4-hydroxy-3-methoxyphenyl), 3-(3-hydroxy-4-methoxyphenyl) and 3-(3,4,5-trimethoxyphenyl) propionic acids. In addition to the reduction of *p*-coumaric acid to 3-(4-hydroxyphenyl) propionic acid, *C. aerotolerans* involves another pathway: *p*-coumaric acid can be decarboxylated to yield 4-vinylphenol, which is reduced to 4-ethylphenol. Supplementation of glucose markedly accelerates all these conversions. Cinnamyl alcohol and phenylpropionic acids, including 3-phenylpropionic acid and 3,4-dihydroxyphenylpropionic acid, are not transformed. Genomic DNA G + C content is 40 mol %. Phylogenetically, *C. aerotolerans* is a member of the low-G + C-containing Gram-positive branch as defined by Collins *et al.* (1994) and is grouped with members of subcluster XIVa. Type strain is X8A62^T (= ATCC 43524^T = DSM 5434^T). Isolated from the rumen of a sheep.

Emended description of *Clostridium celerecrescens* (Palop *et al.* 1989)

Clostridium celerecrescens (ce'le.re.cres.cens. L. adv. *celere* fast; L. v. *crescere* to grow; M.L. adj. *celerecrescens* intended to reflect the fast growth of the organism).

Cells are straight to slightly curved rods, 2.0–4.0 × 0.5–0.8 µm. Vegetative cells stain Gram-positive, although they decolorize readily in older cultures. Endospores are spherical and terminal and produce swollen cells. Cells are motile by means of peritrichous flagella. On cellulose agar medium, colonies appear after 10–14 d incubation at 37 °C and exhibit haloes of cellulolysis. Deep colonies are smooth, circular, approximately 0.5 mm in diameter, translucent and unpigmented. Ferments cellulose, cellobiose, glucose, fructose, galactose, mannitol, maltose, adonitol, rhamnose, xylose, ribose, mannose, raffinose, trehalose, arabinose and aesculin. Weakly ferments lactose, melezitose, melibiose, sorbose and sucrose. Does not ferment dulcitol, erythritol, glycerol, inulin, sorbitol or starch. Fermentation products in cellobiose and cellulose

broth are ethanol, H₂ and CO₂, and acetic, formic, butyric, isobutyric, isovaleric, caproic, lactic and succinic acids. Gelatin is liquefied and indole is produced. Catalase, lipase, lecithinase and urease are negative. Nitrate reduction and casein hydrolysis are negative. Acetylmethylcarbinol is not produced. Milk digestion and curd formation are positive. Optimum growth temperature is 30–37 °C. Growth rate decreases substantially at 23 °C. Optimum pH is near 7.0. Growth rate decreases substantially below pH 7 and over pH 8. Reduces *m*-methoxycinnamic acid to 3-(3-methoxyphenyl) propionic acid, *p*-methoxycinnamic acid to 3-(4-methoxyphenyl) propionic acid and *p*-methylcinnamic acid to 3-(4-methylphenyl) propionic acid. Addition of glucose markedly increases the yield of conversions. Cinnamic, *o*-, *m*-, *p*-coumaric, *o*-methoxycinnamic, caffeic, ferulic, isoferulic and 3,4,5-trimethoxycinnamic acids, cinnamyl alcohol and phenylpropionic acids, including 3-phenylpropionic acid and 3,4-dihydroxyphenylpropionic acid, are not metabolized after 2 weeks incubation, with or without addition of glucose. DNA G + C composition is 38 mol %. Phylogenetically, *C. celerecrescens* is a member of the low-G + C-containing Gram-positive branch as defined by Collins *et al.* (1994) and is grouped with members of subcluster XIVa. Type strain is 18A^T (= CECT 954^T = DSM 5628^T). Isolated from a methanogenic culture started with a cow manure inoculum.

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