Clostridium carboxidivorans sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL1 as *Clostridium drakei* sp. nov.

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A novel solvent-producing, anaerobic clostridium, strain P7[T], was isolated from sediment from an agricultural settling lagoon after enrichment with CO as the substrate. The metabolism of this Gram-positive, motile, spore-forming rod was primarily acetogenic. Acetate, ethanol, butyrate and butanol were the end-products of metabolism. Strain P7[T] grew on CO, H_2/CO_2, glucose, galactose, fructose, xylose, mannose, cellobiose, trehalose, cellulose, starch, pectin, citrate, glycerol, ethanol, propanol, 2-propanol, butanol, glutamate, aspartate, alanine, histidine, asparagine, serine, betaine, choline and syringate as sole substrates. Growth was not supported by methanol, formate, D-arabinose, fucose, lactose, melibiose, amygdalin, gluconate, lactate, malate, arginine, glutamine or vanillate. Nitrate reduction, production of indole, gelatin hydrolysis and aesculin hydrolysis were not observed. Analysis of the 16S rRNA gene sequence of the isolate showed that it was closely related to *Clostridium scatologenes* ATCC 25775[T] (99·7 % sequence similarity) and clostridial strain SL1[T] (99·8 % sequence similarity). Strain SL1 had been classified as a strain of *C. scatologenes*. However, DNA–DNA reassociation analysis showed that both strain P7[T] and strain SL1 represented novel clostridial species. It is proposed that strain P7[T] (=ATCC BAA-624[T] =DSM 15243[T]) be classified as the type strain of *Clostridium carboxidivorans* sp. nov. and that strain SL1[T] (=ATCC BAA-623[T] =DSM 12750[T]) be reclassified as the type strain of *Clostridium drakei* sp. nov.

The classic presentation of the acetogenic phenotype is the anaerobic reduction of CO_2 to acetate, with the implication that the micro-organism is using the 'Wood–Ljungdahl' pathway (Drake, 1994). Other C_1 compounds, including formate, methanol and CO, are utilized by acetogens. Some acetogens can produce additional end-products of metabolism. *Acetobacterium woodii* (Buschhorn *et al.*, 1989) and *Clostridium* strain PETC (Vega *et al.*, 1989), later identified as *Clostridium ljungdahlii* (Tanner *et al.*, 1993), can produce ethanol from C_1 substrates. *Eubacterium limosum* produces the C_4 product butyrate from H_2/CO_2 (Genthner *et al.*,

1981) and the closely related '*Butyribacterium methylotrophicum*' produces butanol from CO (Grethlein *et al.*, 1991).

Other metabolic capabilities in addition to the reduction of a C_1 substrate to acetate were discovered by Kusel *et al.* (2000). A *Clostridium* strain, SL1, was isolated from an acidic sediment using H_2 as the energy source and presumptively identified as an acetogen. Analysis of the 16S rRNA gene sequence indicated that strain SL1 was a strain of *Clostridium scatologenes*, which is usually cultured on fermentable carbohydrates, forming acetate and butyrate as the main end-products of fermentation. *C. scatologenes* ATCC 25775[T] was also shown to utilize H_2/CO_2 or CO, and had key enzyme activities for the acetogenic pathway, leading to the conclusion that *C. scatologenes* ATCC 25775[T] is an acetogen (Kusel *et al.*, 2000).

A *Clostridium* strain, designated P7[T], was enriched from an agricultural settling lagoon, using CO as the substrate. This

was done in a search for bacteria with the potential to ferment synthesis gas (CO/CO$_2$/H$_2$) and produce ethanol as a biofuel (Worden et al., 1991). This strain, like strain SL1[T] (Kusel et al., 2000), was found to be closely related to C. scatologenes ATCC 25775[T]. Further investigation showed that both P7[T] and SL1[T] represented novel species of the genus Clostridium.

Clostridium strain P7[T] was isolated by Rossukon Lao-paiboon. Sediment from an agricultural settling lagoon at Oklahoma State University (Stillwater, OK, USA) was incubated with an acetogen medium (ATCC medium no. 1754; Tanner et al., 1993) at 37 °C with an initial pH of 5 and an atmosphere of CO/N$_2$/CO$_2$ (70 : 24 : 6) at a gauge pressure of 230 kPa. Enrichments were monitored by GC for ethanol and acetate production. Strain P7[T] was isolated from the enrichment using roll tubes (Hungate, 1969).

C. scatologenes ATCC 25775[T] was obtained from the American Type Culture Collection. C. scatologenes strain SL1 (Kusel et al., 2000) was obtained from the laboratory of Harold L. Drake at the University of Bayreuth (Bayreuth, Germany). The basal medium for routine culture contained the following (l^{-1}): 25 ml mineral solution (a source of sodium, ammonium, potassium, phosphate, magnesium, sulfate and calcium; Tanner, 2002); 10 ml vitamin solution (Tanner, 2002); 10 ml trace metal solution (Tanner, 2002); 1 g yeast extract (no. 0127-17-9; Difco); 10 g MES, with the pH adjusted to 6·1; and 6 ml cysteine.sulfide reducing agent (Tanner, 2002). Media were prepared using a strict anaerobic technique (Balch & Wolfe, 1976). Substrate-utilization cultures (5 g substrate l^{-1}) were grown under an atmosphere of N$_2$/CO$_2$ (80 : 20) at a gauge pressure of 70 kPa. For growth with H$_2$ or CO, the atmosphere was H$_2$/CO$_2$ (80 : 20) or CO/N$_2$/CO$_2$ (70 : 24 : 6) at a gauge pressure of 230 kPa.

The pH range and optimum for growth of strain P7[T], strain SL1[T] and C. scatologenes ATCC 25775[T] was examined from pH 4 to 8 with fructose as the substrate and a Good's buffer (20 g l^{-1}), HOMOPIPES (pK_a 4·6), MES (pK_a 6·0), TES (pK_a 7·2) or TAPS (pK_a 8·1) as appropriate (Tanner, 2002). The temperature range and optimum for growth was examined at 4–45 °C. The pH and temperature ranges for strain P7[T] were also examined with CO as the substrate. Further phenotypic characterization was performed using the procedures described by Holdeman et al. (1977) and Smibert & Krieg (1994).

Cells in exponential growth phase in a medium with yeast extract (5 g l^{-1}) were fixed with 1 % glutaraldehyde, spread onto carbon-coated Formvar grids and then stained with phosphotungstate (0·5 %). Transmission electron microscopy was performed using a JEOL JEM 2000 FX transmission electron microscope.

DNA was isolated from cells of strains P7[T], SL1[T] and C. scatologenes ATCC 25775[T] by chromatography on hydroxyapatite according to Cashion et al. (1977) or by using a modified Marmur procedure (Ludwig, 1991). The DNA

G + C content (mol%) was determined using the method of Mesbah et al. (1989). The G + C content (mol%) was also determined in the laboratory of Dr Peter Schumann at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany.

DNA was used as a template for PCR amplification of the 16S rRNA gene using the amplification primers fD1 and rP2 as described by Weisberg et al. (1991). The PCR amplification products were sequenced with an automated sequencer; the resulting sequence was assembled to produce 1015-base contiguous DNA sequences corresponding to Escherichia coli positions 23–1055 (Brosius et al., 1978). These contiguous sequences were aligned by hand, on the basis of the secondary structure of the 16S rRNA molecule (Gutell et al., 1994), to the most closely related sequences from both the Ribosomal Database Project and the GenBank/EMBL databases. The phylogenetic positions of strain P7[T] and SL1[T] were analysed using distance-matrix (Felsenstein, 1993), maximum-likelihood (Olsen et al., 1994) and parsimony (Swofford, 2000) methods. A heuristic search was conducted first (using the standard program defaults), followed by a bootstrap analysis (Felsenstein, 1985) to assess the branch points of the resulting phylogenetic trees. A consensus tree was generated by bootstrapping at the greater-than-50 % confidence limit, with 1000 replications.

A DNA–DNA reassociation analysis was performed in the laboratory of Dr Peter Schumann (DSMZ) as described by De Ley et al. (1970), with the modifications described by Escara & Hutton (1980) and Huß et al. (1983), using a model 2600 spectrophotometer equipped with a model 22527-R thermoprogrammer and plotter (Gilford Instrument Laboratories). Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992).

Repetitive DNA PCR fingerprinting was performed using the BOXA1R primer (Invitrogen) and the protocol of Versalovic et al. (1994). PCR mixtures contained 2·5 µl 10 × buffer B (500 mM KCl and 100 mM Tris/HCl), 2 µl MgCl$_2$.6H$_2$O (25 mM), 0·25 µl Taq polymerase (Fisher Scientific), 0·5 µl each dNTP (10 mM; Promega), 1 µl primer, and sample DNA in a final volume of 25 µl. The PCR was run on a Robocycler Gradient 40 temperature cycler (Stratagene) using a protocol consisting of an initial denaturation step (94 °C, 4 min), 30 reaction cycles (94 °C for 1 min, 50 °C for 1 min, 72 °C for 8 min) and a final extension step (72 °C for 8 min). The PCR product (10 µl) was run on a 5 % polyacrylamide vertical gel with a 100 bp ladder (Fisher Scientific) for 17 h at 26 °C and 120 mA. The ethidium-bromide-stained gel image was analysed using the NucleoCam digital image documentation system (Nucleo Tech).

Growth in liquid cultures was measured at 600 nm using a Spectronic 20D spectrophotometer (Milton Roy; Balch & Wolfe, 1976). Fructose was measured using the phenol/sulfuric acid carbohydrate assay (Dubois et al., 1956). Acetate, ethanol, butyrate and butanol were measured using

a 3400 Varian GC equipped with a flame ionization detector and a 2·0 m glass column (2·6 mm i.d., 5 mm o.d.) packed with Carbopack B DA 80/20 4 % Carbowax 20 M resin (Supelco). The concentrations of acetate and butyrate were confirmed using ion-exclusion HPLC on an Aminex HPX-87H organic acid analysis column (Bio-Rad), and the concentration of ethanol was confirmed using an alcohol dehydrogenase ethanol assay (Kit 333-B; Sigma-Aldrich). Gases were measured using a GC equipped with a thermal conductivity detector (Varian) and a Porapak Super Q column (Alltech) or a molecular sieve 5A column (Alltech).

Colonies of P7^T grown with CO appeared white and opaque, with lobate edges, and were 2–4 mm in diameter after 1–2 weeks incubation. Phase-contrast microscopy (CH-2 phase microscope; Olympus) and electron microscopy revealed that cells of strain P7^T were Gram-positive, rod-shaped (0·5 μm in diameter and, usually, 3 μm in length) bacteria occurring most often singly or in pairs (see Supplementary Fig. S1 available in IJSEM Online). Cells can be motile and peritrichously flagellated, but active motility is not always observed. Spores were rarely observed, but, when present, were subterminal to terminal with some cell swelling. This morphology differs from that reported for strain SL1^T with regard to the Gram reaction and the presence of spores (Kusel *et al.*, 2000).

The growth rate, temperature range and pH range for strain P7^T, strain SL1^T and *C. scatologenes* ATCC 25775^T are given in Table 1. All three bacteria had similar temperature and pH ranges and optima. The growth rates under optimal conditions with fructose or CO as substrate for the three clostridia were in the same range. At 38 °C, an initial culture pH of 6·2 and with fructose as the substrate, a doubling time of 2·4 h was observed for strain SL1^T. With fructose as the substrate and at 30 °C, the doubling time for SL1^T was 5·3 h (Kusel *et al.*, 2000). The doubling time of *C. scatologenes* ATCC 25775^T (17·3 h) on H$_2$/CO$_2$ was considerably longer than that of strain P7^T (5·8 h) or that of strain SL1^T (3·5 h). All three strains utilized CO, H$_2$/CO$_2$, ribose, xylose, fructose, glucose, galactose, L-arabinose, mannose, rhamnose, sucrose, cellobiose, melezitose, cellulose, starch, inositol, glycerol, ethanol, propanol, pyruvate, citrate, serine, alanine, histidine, glutamate, aspartate, asparagine, Casamino acids, betaine and choline as growth substrates. Formate,

Table 1. Characteristics of strain P7^T, strain SL1^T and *C. scatologenes* ATCC 25775^T

Temperature and pH range/optimum were determined with fructose as the substrate. Symbols: +, growth; −, no growth.

Characteristic	P7^T	SL1^T	*C. scatologenes* ATCC 25775^T
Growth rate (h^{-1}) with:			
Fructose	0·32	0·29	0·39
CO/CO$_2$	0·16	0·12	0·09
H$_2$/CO$_2$	0·12	0·20	0·04
Temperature range (°C)	24–42	18–42	18–42
Temperature optimum (°C)	37–40	30–37	37–40
pH range	4·4–7·6	4·6–7·8	4·6–8·0
pH optimum	5·0–7·0	5·4–7·5	5·4–7·0
Utilization of:			
Pectin	+	−	+
Fucose	−	+	+
Maltose	−	−	+
Trehalose	+	−	−
Sorbitol	−	−	+
Mannitol	+	+	−
Gluconate	−	+	+
Lactate	−	+	+
Fumarate	−	+	+
Malate	−	+	−
2-Propanol	+	+	−
Butanol	+	+	−
Glutamine	−	−	+
Arginine	−	+	+
Syringate	+	+	−

methanol, D-arabinose, lactose, raffinose, melibiose, amygdalin, succinate, ferulate, vanillate and trimethoxybenzoate did not support the growth of these clostridia.

Differential patterns of substrate utilization for the three strains were observed with pectin, fucose, maltose, trehalose, sorbitol, mannitol, gluconate, lactate, fumarate, malate, 2-propanol, butanol, glutamine, arginine and syringate (Table 1). For example, *C. scatologenes* ATCC 25775^T grew with maltose, sorbitol or glutamine, but these did not support the growth of strain P7^T or strain SL1^T. Strain P7^T did not grow on fucose, gluconate, lactate or arginine, but these substrates were utilized by strain SL1^T and *C. scatologenes* ATCC 25775^T. Strain SL1^T did not grow on pectin and grew on malate, while the opposite was observed for strain P7^T and *C. scatologenes* ATCC 25775^T. These three strains could be distinguished on the basis of growth with these 15 substrates.

The substrate-utilization results for SL1^T are mainly in accord with those reported by Kusel et al. (2000). However, strain SL1^T was reported to utilize formate, vanillate and ferulate in the prior report, and it was also reported that *C. scatologenes* ATCC 25775^T utilized vanillate (Kusel et al., 2000). Other results for *C. scatologenes* ATCC 25775^T differ somewhat from the results of prior work. In the work of Holdeman et al. (1977), *C. scatologenes* ATCC 25775^T did not produce acid from (implying lack of growth with) galactose, maltose, sucrose, cellobiose, melezitose, starch, sorbitol or inositol, but in this study these all served as growth substrates. A later report (Cato et al., 1986) indicated that *C. scatologenes* ATCC 25775^T was negative for utilization of galactose, maltose, sucrose, melezitose, starch or sorbitol, but these compounds did function as growth substrates in this study. All of the substrate-utilization assays were performed independently three times. Each result set for each *Clostridium* species was identical in all three assays. To some degree, the differences in reported substrate utilization reflect legitimate differences in performance of the bacteria, especially if slightly different culture conditions are used.

All three strains were positive in the methyl red test in an MRVP assay (Smibert & Krieg, 1994). All yielded negative results in the Voges–Proskauer reaction, for aesculin hydrolysis, for gelatin hydrolysis, for nitrate reduction and for the production of indole. Strain P7^T was also negative for catalase, oxidase and urease activities.

End-products of metabolism were examined in this study. Strain P7^T converted 600 mmol CO (no. of carbons equivalent to 100 mmol fructose) to CO_2 (264 mmol), ethanol (96 mmol), acetate (12 mmol) and butanol (24 mmol). Strain P7^T converted 100 mmol fructose to CO_2 (280 mmol), ethanol (23 mmol), acetate (81 mmol) and butanol (4 mmol). It was not unusual to detect butyrate as an end-product in other trials. Butyrate was also reported as a minor end-product from strain SL1^T (Kusel et al., 2000). Under similar conditions (i.e. initial culture pH of 6·2

and incubation at 38 °C), strain SL1^T fermented 100 mmol fructose to CO_2 (120 mmol), ethanol (50 mmol), acetate (70 mmol) and butanol (50 mmol), while CO_2 (100 mmol), acetate (200 mmol) and ethanol (50 mmol) were produced by *C. scatologenes* ATCC 25775^T. These values differ from the ratios of end-products from fructose for SL1^T and *C. scatologenes* ATCC 25775^T reported by Kusel et al. (2000). However, end-product ratios in clostridia can change with alterations in pH and other growth conditions (Adler & Crow, 1987).

The phylogenetic relationship between strain P7^T and related *Clostridium* species is shown in Fig. 1. The 16S rRNA gene sequence for strain P7^T was deposited with GenBank under accession number AY170379, and the 16S rRNA gene sequence for strain SL1^T was deposited by Harold Drake's laboratory under accession number Y18813. The 16S rRNA gene sequence of P7^T was very similar to those of strain SL1^T (99·8 %) and *C. scatologenes* ATCC 25775^T (99·7 %). The sequence of strain SL1^T was also very similar to that of *C. scatologenes* ATCC 25775^T (99·7 %), which had been reported previously (99·6 %; Kusel et al., 2000). All three strains are in cluster I (subcluster Ic) of *Clostridium* group I (Collins et al., 1994; Stackebrandt & Hippe, 2001). *Clostridium histolyticum*, *Clostridium limosum* and *Clostridium proteolyticum* are in cluster II of *Clostridium* group I, and the remaining species are in cluster I.

The DNA G+C content for strain P7^T was 31 mol% (nuclease digest), which is similar to the value determined by the DSMZ (32 mol%). The DNA G+C content for strain SL1^T was 32 mol% (nuclease digest), which is similar to the value reported earlier (30 mol%) by Kusel et al. (2000). The
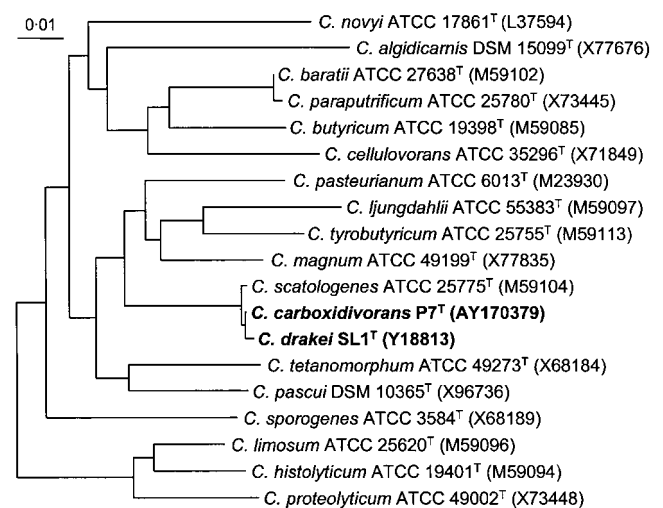

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, for clostridial strain P7^T. The total horizontal distance shown between species indicates the difference between their sequences. Bar, 0·01 substitutions per site.

DNA $G+C$ content for *C. scatologenes* ATCC 25775[T] was 31 mol% (nuclease digest), which is somewhat different from the value reported previously (27 mol%; thermal melting point) by Johnson & Francis (1975).

The results from the 16S rRNA gene sequence analysis and determination of the $G+C$ content showed that these three strains were closely related. Their taxonomic status was investigated further using DNA–DNA reassociation. The DNA–DNA reassociation value of strain P7[T] with respect to strain SL1[T] was 31·8 %, that for strain P7[T] and *C. scatologenes* ATCC 25775[T] was 50·2 % and that for strain SL1[T] and *C. scatologenes* ATCC 25775[T] was 53·0 %. On the basis of the threshold value of 70 % for the definition of species (Wayne *et al.*, 1987), these three clostridia each constitute different species. It had been shown earlier that strains with very similar 16S rRNA sequences could be demonstrated to be distinct species when analysed by DNA–DNA reassociation (Fox *et al.*, 1992), and that strains with a 16S rRNA sequence similarity greater than 97 % require DNA–DNA reassociation analysis to define speciation (Stackebrandt & Goebel, 1994).

As noted above and in Table 1, these three strains could be separated on the basis of substrate-utilization patterns and the slow growth of *C. scatologenes* ATCC 25775[T] on H_2/CO_2, as well as by using DNA–DNA reassociation. The three strains could also be distinguished by using repetitive DNA PCR fingerprinting. This technique should not be used to determine whether a bacterium is a novel species, but can be used to discriminate among the three clostridia. It is presented here because it is a method that is more widely available to laboratories than DNA–DNA reassociation. The results of gel electrophoresis of the PCR products from DNAs, using the BOXA1R primer, are available as Supplementary Fig. S2 in IJSEM Online. All three strains had a band of approximately 1190 bp and a band of approximately 400 bp. Strain SL1[T] and *C. scatologenes* ATCC 25775[T] each had a band of approximately 1350 bp. *C. scatologenes* ATCC 25775[T] also had PCR product bands of 580, 345 and 250 bp. Strain SL1[T] showed unique gel bands of >2000 and 720 bp. Strain P7[T] had a unique band of 830 bp.

The above results demonstrate that strain P7[T] and strain SL1[T] should be considered as representing distinct species of the genus *Clostridium*. We propose the name *Clostridium carboxidivorans* for strain P7[T], on the basis of its ability to utilize CO readily as a substrate. We propose the name *Clostridium drakei* for strain SL1[T], in recognition of the contributions that Harold L. Drake has made to our understanding of the microbiology of acetogens. In both instances, this expands the repertoire of species of acetogens that are members of the genus *Clostridium*, and that can produce C_4 compounds in addition to acetate as end-products of metabolism. This also adds, in addition to *C. ljungdahlii*, to the known acetogenic species of *Clostridium* that can produce ethanol as a product from C_1 substrates in addition to acetate, and expands upon this

solvent-producing ability in that these novel species can produce butanol as well.

Description of *Clostridium carboxidivorans* sp. nov.

Clostridium carboxidivorans (car.bo.xi.di.vo′rans. N.L. neut. n. *carboxidum* carbon monoxide; L. part. adj. *vorans* devouring; N.L. part. adj. *carboxidivorans* carbon monoxide-devouring).

Gram-positive, motile rods ($0·5 \times 3$ μm) occurring singly and in pairs. Cells rarely sporulate, but spores are subterminal to terminal with slight cell swelling. Obligate anaerobe with an optimum growth temperature of 38 °C and an optimum pH of 6·2. Grows autotrophically with H_2/CO_2 or CO and chemoorganotrophically with ribose, xylose, fructose, glucose, galactose, L-arabinose, mannose, rhamnose, sucrose, cellobiose, trehalose, melezitose, pectin, starch, cellulose, inositol, mannitol, glycerol, ethanol, propanol, 2-propanol, butanol, citrate, serine, alanine, histidine, glutamate, aspartate, asparagine, Casamino acids, betaine, choline and syringate. Methanol, D-arabinose, fucose, maltose, lactose, raffinose, melibiose, amygdalin, sorbitol, gluconate, lactate, malate, succinate and arginine do not support growth. Acetic acid, ethanol, butyrate and butanol are the end-products of metabolism. Cultures are methyl red-positive, but negative for the Voges–Proskauer reaction, aesculin hydrolysis, gelatin hydrolysis, nitrate reduction, indole production, catalase, oxidase and urease. The DNA $G+C$ content is 31–32 mol%.

The type strain, strain P7[T] ($=$ ATCC BAA-624[T] $=$ DSM 15243[T]), was isolated from an agricultural settling lagoon in Oklahoma, USA. 16S rRNA gene sequence analysis showed that *C. carboxidivorans* is very closely related to *C. scatologenes* ATCC 25775[T] and *C. drakei*, but DNA reassociation analysis showed that *C. carboxidivorans* is a distinct species, with a reassociation value of 50 % with respect to *C. scatologenes* ATCC 25775[T] and a reassociation value of 32 % with respect to *C. drakei*.

Description of *Clostridium drakei* sp. nov.

Clostridium drakei (dra′ke.i. N.L. gen. n. *drakei* of Drake, in recognition of Harold L. Drake's contributions to our understanding of the physiology and ecology of acetogens).

Original description in Kusel *et al.* (2000). Gram-negative, motile rods ($0·6 \times 3–4$ μm). Forms terminal spores. Obligate anaerobe with an optimum growth temperature of 30–37 °C and an optimum pH of 5·5–7·5. Grows autotrophically with H_2/CO_2 or CO and chemoorganotrophically with ribose, xylose, fructose, glucose, galactose, fucose, L-arabinose, mannose, rhamnose, sucrose, cellobiose, melezitose, starch, cellulose, inositol, mannitol, gluconate, glycerol, ethanol, propanol, 2-propanol, butanol, citrate, malate, fumarate, lactate, serine, alanine, histidine, glutamate, aspartate, asparagine, arginine, Casamino acids, betaine, choline and syringate. Methanol, D-arabinose,

maltose, lactose, trehalose, raffinose, melibiose, amygdalin, sorbitol and succinate do not support growth. Acetic acid, ethanol, butyrate and butanol are the end-products of metabolism. Cultures are methyl red-positive, but negative for the Voges–Proskauer reaction, aesculin hydrolysis, gelatin hydrolysis, nitrate reduction and indole production. The DNA G + C content is 30–32 mol%.

The type strain, strain SL1[T] (= ATCC BAA-623[T] = DSM 12750[T]), was isolated from sediment collected from an acidic coal-mine pond in east-central Germany. 16S rRNA gene sequence analysis showed that *C. drakei* is very closely related to *C. scatologenes* ATCC 25775[T] and *C. carboxidivorans*, but DNA reassociation analysis showed that *C. drakei* is a distinct species, with reassociation values of 53 % with respect to *C. scatologenes* ATCC 25775[T] and 32 % with respect to *C. carboxidivorans* P7[T].

Acknowledgements

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