RESEARCH LETTER

Carbon metabolism of the moderately acid-tolerant acetogen *Clostridium drakei* isolated from peat

Anita S. Gößner[1], Flynn Picardal[2], Ralph S. Tanner[3] & Harold L. Drake[1]

[1]Department of Ecological Microbiology, University of Bayreuth, Bayreuth, Germany; [2]School of Public and Environmental Affairs, Indiana University, Bloomington, IN, USA; and [3]Department of Botany and Microbiology, University of Oklahoma, Norman, OK, USA

Correspondence: Harold L. Drake, Department of Ecological Microbiology, University of Bayreuth, 95440 Bayreuth, Germany. Tel.: +49 0 921 555 640; fax: +49 0 921 555 793; e-mail: hld@uni-bayreuth.de

Received 29 June 2008; accepted 29 July 2008. First published online 15 August 2008.

DOI:10.1111/j.1574-6968.2008.01313.x

Editor: Simon Silver

Keywords
acetogenesis; acetyl-CoA pathway; *Clostridium drakei*; methanogenesis; skatole; wetlands.

Abstract

A moderately acid-tolerant, malodorous bacterium, strain FP, was isolated from peat that had a pore water pH of *c.* 4.2. The 16S rRNA gene sequence of FP was closely related to that of acetogens *Clostridium drakei*, *Clostridium scatologenes*, and *Clostridium carboxidivorans*. The DNA–DNA reassociation values obtained with DNA from FP and that of these three acetogens approximated 80%, 64%, and 59%, respectively, indicating that FP was a new strain of *C. drakei*. FP had broad pH and temperature ranges (3.6–7.4 and 5–40 °C, respectively), and metabolized a wide range of substrates, including cellobiose, glucose, xylose, vanillate, ferulate, lactate, propanol, formate, H_2–CO_2, and CO–CO_2. Acetate was the primary reduced end product, and substrate/product stoichiometries were indicative of acetogenesis at circumneutral pH. Butyrate and H_2 became significant products from glucose at low pH. FP tolerated and could consume moderate amounts of O_2. These results (1) demonstrate that peat can harbor acetogens with a broad substrate range and tolerance to transient exposure to O_2, and (2) confirm that *C. drakei*, the type strain of which was originally isolated from an acidic coal mine pond, occurs in moderately acidic habitats.

Introduction

Acetogenic bacteria utilize the acetyl-CoA 'Wood-Ljungdahl' pathway for the reductive synthesis of acetate from CO_2 (Wood & Ljungdahl, 1991; Drake *et al.*, 2006). Although acetogens have traditionally been considered to be strict anaerobes, they occur in environments subject to fluctuations in O_2, such as aerated soils (Peters & Conrad, 1995; Wagner *et al.*, 1996; Kuhner *et al.*, 1997; Gößner *et al.*, 1999; Küsel *et al.*, 1999), plant roots (Küsel *et al.*, 1999, 2001; Gößner *et al.*, 2006), and gastrointestinal tracts with steep O_2 gradients (Breznak, 1994; Brune *et al.*, 1995). It is, therefore, not surprising that certain acetogens have several mechanisms by which they deal with oxidative stress (Das *et al.*, 2001, 2005; Küsel *et al.*, 2001; Karnholz *et al.*, 2002; Boga *et al.*, 2003; Drake & Gößner, 2008; Drake *et al.*, 2008).

Moderately acidic wetlands (e.g. bogs and fens) constitute an important global reserve of soil carbon and are characterized by low concentrations of inorganic electron acceptors and highly dynamic O_2 gradients (e.g. Williams & Crawford, 1984; Westermann, 1993; Paul *et al.*, 2006). Such habitats emit methane and have methanogenic communities, and have also been postulated to contain acetogens that might be linked to acetate flux (Shannon & White, 1994, 1996; Horn *et al.*, 2003; Bräuer *et al.*, 2004; Metje & Frenzel, 2005; Hamberger *et al.*, 2008). Preliminary studies demonstrated that acetogenic enrichments could be readily obtained with peat obtained from Buck Hollow Bog, a moderately acidic bog in Michigan. The objectives of this study were to isolate an acetogen from this moderately acidic peat and investigate its physiological response to acidity and O_2.

Materials and methods

Site description and inoculum

The peat used as the inoculum was obtained from the Buck Hollow Bog in the Edwin S. George Reserve in Kaiserville, Michigan. The bog is dominated by *Sphagnum* moss species and a nonwoody, vascular plant, *Scheuchzeria palustris*. The biogeochemistry of this site has been documented

© 2008 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

previously (Shannon & White, 1994, 1996). Pore waters exhibit a pH of 4.2 ± 0.2 and a dissolved organic carbon concentration of 4.2 ± 1.7 mM C. Sterile mason jars were completely filled with peat and bog water from a depth of 8–15 cm below the bog surface. Samples were transferred to anoxic tubes inside an O_2-free chamber. Peat was stored at 4 °C for 10 days in crimp-sealed tubes equipped with butyl rubber stoppers before initiating enrichments.

Medium

The anoxic, carbonate-buffered, yeast extract medium (YE medium) contained vitamins, trace metals, and reducing agents, and was prepared as described (Daniel *et al.*, 1990). Medium was dispensed under CO_2 into 27-mL culture tubes (7 mL medium per tube) that were then sealed and autoclaved. Anoxic stock solutions of substrates were filter sterilized and added by syringe injection using O_2-free techniques. Unless otherwise indicated, (1) culture tubes were incubated in an horizontal, static position, (2) cultivation was in anoxic YE medium with a 1 atm (1013 mbar) CO_2 headspace, (3) the initial concentrations of soluble substrates approximated 5–6 mM, (4) H_2 or CO, when added, were provided at 300 mbar overpressure, and (5) the temperature of incubation was 28 °C. The 300 mbar H_2 or CO overpressure is equivalent to an initial concentration of 34 mM, if all of the gas is calculated to be present in the aqueous phase.

Enrichment and isolation

Enrichment tubes were prepared in an O_2-free chamber and contained 10 mL YE medium, 2 g wet peat, 20 mM 2-bromoethanesulfonate to inhibit methanogens (Gunsalus *et al.*, 1978), and both 5 mM vanillate and H_2–CO_2 as cosubstrates. Acetate-producing cultures were transferred to fresh medium every 1–2 weeks. Enrichments were initiated at two pH values (4.3 and 6.8) and two temperatures (5 and 25 °C). Because growth and acetate production was very slow at low pH and low temperature, only the enrichments at 25 °C and circumneutral pH were carried forward due to time constraints. 2-Bromoethanesulfonate was omitted from the medium after four transfers, and the enrichment was streaked onto 5 mM vanillate-supplemented YE medium solidified with 1% Gelrite and incubated under H_2–CO_2 to obtain pure cultures.

Electron microscopy

Samples were prepared by passing an aliquot of culture through a 0.45-μm filter followed by overnight fixation in a solution of 0.1 M phosphate buffer, pH 6.8, containing 3% glutaraldehyde. After ethanol dehydration, the filters were dried, mounted, gold-coated, and viewed at 15 kV using a JEOL JSM-5800 LV (Japanese Electron Optics Laboratory, Tokyo, Japan) scanning electron microscope.

Phylogenetic analysis and DNA–DNA hybridization

A total of 1424 bases of the 16S rRNA gene of FP were sequenced. PCR products were obtained with the bacterial primer pair GM3/GM4 (Muyzer *et al.*, 1995). Sequencing was done by MWG Biotech AG (Ebersberg, Germany) followed by a public database (GenBank, EMBL) search. DNA–DNA hybridization was performed as described elsewhere (Liou *et al.*, 2005).

Additional methods

Growth was measured as $OD_{660\,nm}$ with an optical path width of 1.6 cm. Organic substrates and products were determined using HPLC and gases were determined using GC (Daniel *et al.*, 1990). Results are indicative of replicate experiments.

Accession numbers

FP was deposited at the German Collection of Microorganisms and Cell Cultures [DSMZ (Braunschweig, Germany)] under accession number DSM 14770. The 16S rRNA gene sequence of FP was deposited at the EMBL Nucleotide Sequence Database (Cambridge, UK) under accession number AJ427628.

Results

Isolation and morphology of FP

Sequential passages of colonies in liquid and on solid media yielded a microscopically pure culture that produced long rods (10–50 μm) (Fig. 1). Longer rods may have been chains of cells that had not fully formed septa and thus could not be resolved microscopically as distinct cells. Fresh cultures were motile and stained weakly Gram-negative; spores were not apparent. The malodorous nature of cultures of FP was similar to that of the skatole-producing bacteria *Clostridium drakei*^{T} and *Clostridium scatologenes*^{T} (Whitehead *et al.*, 2008). [Note: *C. drakei*^{T} was described initially as *C. scatologenes* SL-1 because its 16S rRNA gene sequence is 99.6% similar to that of *C. scatologenes*^{T} (Küsel *et al.*, 2000). DNA–DNA reassociation analysis revealed that *C. scatologenes* SL-1 was a distinct species (Liou *et al.*, 2005).]

Phylogenetic analysis and DNA–DNA hybridization

The sequence identity of the 16S rRNA gene of FP with that of its three closest cultured relatives *C. drakei*^{T} (EMBL

© 2008 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

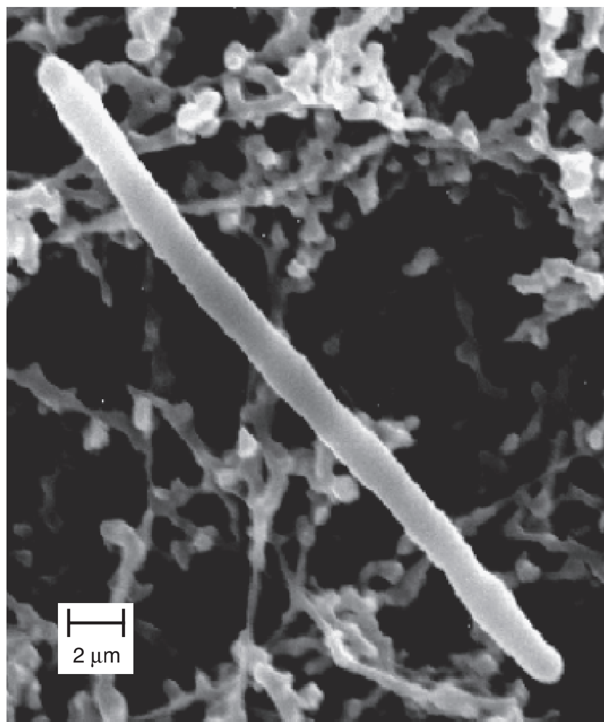

Fig. 1. Electron micrograph of strain FP. The background is the filter matrix.

Y18813), *C. scatologenes*^{T} (EMBL M59104), and *Clostridium carboxidivorans*^{T} (EMBL AY170379) approximated 100%, 99.6%, and 99.6%, respectively. *Clostridium drakei*^{T}, *C. scatologenes*^{T}, and *C. carboxidivorans*^{T} are acetogens (Küsel *et al.*, 2000; Liou *et al.*, 2005), but only *C. drakei*^{T} and *C. scatologenes*^{T} are known to produce skatole (Whitehead *et al.*, 2008). In contrast to these three acetogens, the next closest cultured relative *Clostridium aciditolerans* (EMBL DQ114945; 95.4% sequence similarity to FP) is not an acetogen (Lee *et al.*, 2007). DNA–DNA hybridization of genomic DNA from FP with that of *C. drakei*^{T}, *C. scatologenes*^{T}, or *C. carboxidivorans*^{T} yielded 80%, 64%, and 59% reassociations, respectively. The threshold value for species-level identity is 70% (Wayne *et al.*, 1987), indicating that FP was a new strain of *C. drakei*.

pH and temperature optima

FP grew in glucose-supplemented YE medium over a temperature range of 5–40 °C, with an optimum at 25–30 °C. Growth occurred at pH 3.6–7.4 but was not observed at pH 3.2 or 7.9. Maximal ODs did not vary much from pH 3.6 to 6.8 [e.g. maximum $OD_{660\,nm}$ at pH (initial) 3.6, 4.6, 5.2, 6.0, and 6.8 approximated 0.80, 0.84, 0.87, 0.75, and 0.65, respectively (final pHs were 4.2, 4.6, 4.6, 4.7, and 4.8, respectively)]. The doubling time on glucose over the pH range 4.8–6.8 did not vary significantly and approximated 5 h.

Substrate–product profiles and pH effects

FP metabolized cellobiose, glucose, fructose, xylose, pyruvate, lactate, propanol, ethanol, vanillate (4-hydroxy-3-methoxybenzoate), syringate (3,5-dimethoxy-4-hydroxybenzoate), ferulate [3-(4-hydroxy-3-methoxyphenyl)-2-propenoate], H_2, or CO. FP did not metabolize stachyose, maltose, sucrose, citrate, *N*-acetylglucosamine, oxalate, glyoxylate, methanol, 1,2,3-trimethoxybenzene, or 4-hydroxybenzaldehyde.

Acetate was the main reduced end product formed during growth at circumneutral pH (Table 1). The amount of acetate produced per substrate consumed was indicative of acetogenesis (Drake *et al.*, 2006), further confirming the relatedness of FP to the acetogen *C. drakei*. Traces of H_2 were also formed, especially by CO cultures. CO stimulates the production of H_2 by certain acetogens (Martin *et al.*, 1983; Savage *et al.*, 1987; Daniel *et al.*, 1990). Vanillate was O-demethylated and decarboxylated to catechol (2-hydroxyphenol). Syringate was O-demethylated to a mixture of 5-hydroxyvanillate and gallate (3,4,5-trihydroxybenzoate). Ferulate was converted to hydrocaffeate [3-(3,4-dihydroxyphenylpropionate)], demonstrating that the acrylate side chain was reduced and the methoxyl group was O-demethylated.

Glucose-dependent growth at pH 4.6 yielded butyrate and H_2 (Table 1). The capacity to consume certain substrates was lost at pH 4.6 (e.g. neither vanillate nor CO were effective substrates at pH 4.6). Carbon recoveries from glucose at pH 4.6 were low; it is unknown if this low recovery was due to the formation of nondetected products or the production of an intracellular polymer (e.g. polyhydroxybutyrate).

Response to O_2

Glucose-dependent growth occurred in horizontally incubated static culture tubes containing nonreduced YE medium with up to *c.* 4% O_2 in the headspace (Table 2). Acetate was the main product formed in glucose cultures challenged with O_2. Vanillate-dependent growth was more sensitive to O_2 and marginal above 0.5% O_2 in the headspace (data not shown). Growth was slower and cell yields were lower in the presence of higher amounts of O_2, and glucose cultures consumed up to a maximum of *c.* 2% O_2 in the headspace.

Discussion

Acetogens in the genus *Clostridum* are not monophyletic, in that the closest 16S rRNA gene-based phylogenetic neighbor of a clostridial acetogen may not be an acetogen (Tanner & Woese, 1994; Drake & Küsel, 2005). Indeed, a clostridial

© 2008 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Table 1. Effect of pH on the growth and product profile of FP*

Substrate and amount consumed (mM)	Maximum $OD_{660\,nm}$	Products (mM)			Acetate/substrate ratio†
		Acetate	Butyrate	H_2	
pH 6.8					
Glucose, 5.3	0.74	16.6	0.0	Trace	2.2 (3)
Pyruvate, 5.0	0.21	10.4	0.0	0.2	1.1 (1.25)
Vanillate, 5.7	0.27	8.1	0.0	0.3	0.6 (0.75)
H_2, 25.7	0.12	12.2	0.0	NA	0.3 (0.25)
CO, 14.4	0.12	7.9	0.0	1.4	0.2 (0.25)
None (control)	0.12	4.7	0.0	0.1	NA
pH 4.6					
Glucose, 6.3	0.88	4.2	1.7	3.8	0.2 (3)
Pyruvate, 5.0	0.32	7.9	0.8	0.6	0.9 (1.25)
Vanillate, 0	0	0	0	0	NA
H_2, 15.0	0.13	7.2	0.5	NA	0.3 (0.25)
CO, 0.7	0.15	2.1	0.2	0.3	− 1.6 (0.25)
None (control)	0.11	3.2	0.3	0.5	NA

*Cultivation was in YE medium. Results are the average of duplicate experiments; values between duplicates did not vary by $> 10\%$.
†Acetate values have been corrected for the amount formed in control cultures without substrate. Acetate production in controls primarily results from metabolism of yeast extract components in the medium. Parenthetical values are the theoretical values for homoacetogenesis (Drake *et al.*, 2006).
NA, not applicable.

Table 2. Effect of O_2 on glucose-dependent growth and product profile of FP*

O_2 at T_0 (%)	O_2 at T_{end} (%)	Glucose consumed (mM)	Maximum $OD_{660\,nm}$ (h)†	Products (mM)		Acetate/substrate ratio‡
				Acetate	H_2	
0§	0	5.2	0.69 (50)	16.1	0.2	2.3 (3)
0	0	5.1	0.69 (50)	15.2	0.1	2.2 (3)
0.3	0	5.1	0.69 (55)	16.5	0.7	2.4 (3)
0.8	0.1	5.4	0.68 (120)	17.0	0.9	2.4 (3)
1.9	0.6	5.2	0.59 (168)	16.1	1.1	2.3 (3)
3.9	1.7	5.3	0.42 (172)	14.3	1.6	1.9 (3)

*Unless otherwise indicated, cultivation was in YE medium (pH 6.8) that lacked reducing agents. Growth was not apparent on glucose with 5% O_2 in the headspace. Results are from the second sequential transfer into the indicated medium and are the average of duplicate experiments; values between duplicates did not vary by $> 10\%$.
†Time taken to reach maximum $OD_{660\,nm}$.
‡Acetate value has been corrected for the amount (4.1 mM) formed in control cultures without substrate and without O_2 (acetate production in controls primarily results from metabolism of yeast extract components in the medium). Parenthetical value is the theoretical value for homoacetogenesis (Drake *et al.*, 2006).
§Cultivation was in YE medium that contained reducing agents.

acetogen and nonacetogenic clostridial species can have nearly identical 16S rRNA gene sequences. As illustrated in the present and earlier studies (Liou *et al.*, 2005), genomic DNA–DNA hybridization analysis is often required for resolving the phylogenetic relationships of clostridial acetogens.

The skatole-producing acetogen *C. drakei*^{T} was obtained from acidic pond sediments and displays a broad pH range (Küsel *et al.*, 2000), and the isolation of a strain of this acetogen from acidic peat confirms that *C. drakei* can occur in acidic habitats and also demonstrates that peat can harbor acetogens with the capacity to convert H_2–CO_2 to acetate at moderately low pH. Although cultures of FP were not observed to form spores, old cultures of *C. drakei*^{T} produce terminal and free spores (Küsel *et al.*, 2000; Liou *et al.*, 2005), and it seems likely that FP can sporulate under certain *in situ* conditions.

Moderately acidic peat from bogs and fens are known to harbor acetogens (Horn *et al.*, 2003; Bräuer *et al.*, 2004; Metje & Frenzel, 2005; Hamberger *et al.*, 2008). In contrast to *C. drakei*, most acetogens isolated to date come from circumneutral pH habitats and have pH optima that are above pH 6 (Drake *et al.*, 2006). Acidity is one of the main *in situ* factors that inhibits acetogens (Baronofsky *et al.*,

© 2008 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

1984; Wang & Wang, 1984; Wiegel, 1994). For example, the classic, model acetogen, *Moorella thermoacetica*^{T}, with which the acetyl-CoA pathway was resolved, has a pH optimum of *c.* 6.8 and a pH range of 5.7–7.7 (Drake & Daniel, 2004). The pH optima of an acetogen might be influenced by the substrate it metabolizes, and the *in situ* pH range of an acetogen would thus be affected by *in situ* conditions.

In comparison with FP and *C. drakei*^{T}, *C scatologenes*^{T} has a more alkaline pH range (pH 4.6–8.0) (Liou *et al.*, 2005) and dissimilates H_2 or CO to acetate very poorly (Küsel *et al.*, 2000). FP produced significant amounts of butyrate and H_2 at low pH. Butyrate, formed via a pathway involving the aldol condensation of two acetyl-CoA molecules (Buckel, 2005), can be an end product of certain acetogens, including clostridial species (Sharak-Genthner *et al.*, 1981; Krumholz & Bryant, 1985). Acetic acid and butyric acid have similar p*K*a values (4.76 and 4.82, respectively). Butyrate fermentation yields less ATP per glucose consumed than does homoacetogenesis (i.e. 3 ATP vs. 4 ATP, respectively). Nonetheless, butyrate production would be more advantageous than homoacetogenesis at low pH because less acid is produced per glucose consumed (i.e. butyrate fermentation yields one butyrate per glucose consumed, whereas homoacetogenesis yields three acetate per glucose consumed). FP metabolized methoxyl, acrylate, or carboxyl groups of aromatic compounds, transformations indicative of many acetogens (Schink, 1994; Müller *et al.*, 2004; Drake *et al.*, 2006). However, the capacity to metabolize all three of these substituent groups by a single acetogen is not widespread among acetogens.

FP tolerated and consumed minimal amounts of O_2. Acetogens have various oxidoreductases (e.g. peroxidase, NADH oxidase, catalase, and superoxide dismutase) for dealing with oxidative stress (Das *et al.*, 2001, 2005; Küsel *et al.*, 2001; Karnholz *et al.*, 2002; Boga *et al.*, 2003), and some acetogens may form commensal interactions with organisms capable of consuming O_2 (Gößner *et al.*, 1999, 2006). *Clostridium glycolicum* RD-1, isolated from sea grass roots, tolerates O_2 by redirecting glucose-derived electron flow to ethanol fermentation rather than acetogenesis (Küsel *et al.*, 2001).

Under standard conditions, the reduction of CO_2 to methane is more thermodynamically favorable than the reduction of CO_2 to acetate (Schink, 1994; Drake *et al.*, 2006). However, acetogens appear to be more persistent than methanogens in certain habitats (Brauman *et al.*, 1992; Degraeve *et al.*, 1994; Nozhevnikova *et al.*, 1994; Küsel & Drake, 1995; Peters & Conrad, 1995; Wagner *et al.*, 1996; Kotsyurbenko *et al.*, 2004). Acetogens appear to have a greater tolerance to O_2, low temperature, and acidity than methanogens, and can likewise engage alternative redox processes by which they conserve energy (Drake *et al.*, 2006). Indeed, the acetogen *Moorella perchloratireducens*^{T} respires (per)chlorate to chloride and O_2, a redox process that is extremely oxidative (Balk *et al.*, 2008). The characteristics of O_2 tolerance, broad pH and temperature ranges, and wide substrate range constitute multiple strategies by which *C. drakei* might be competitive in moderately acidic wetlands.

Acknowledgements

Support for this study was provided by the Deutsche Forschungsgemeinschaft (DFG Dr310/3-2) and the University of Bayreuth. F.P. was funded during the initial portions of this research at the University of Bayreuth via a Short Term Faculty Exchange fellowship from Indiana University. The authors thank Barry Stein for assistance with electron microscopy, Jeff White for assistance with collection of peat samples, and Peter Schumann for coordinating the DNA–DNA hybridization assays at the DSMZ.

References

Balk M, van Gelder T, Weelink SA & Stams AJM (2008) (Per)chlorate reduction by the thermophilic bacterium *Moorella perchloratireducens* sp. nov., isolated from underground gas storage. *Appl Environ Microbiol* **74**: 403–409.

Baronofsky JJ, Schreurs WJA & Kashket ER (1984) Uncoupling by acetic acid limits growth of and acetogenesis by *Clostridium thermoaceticum*. *Appl Environ Microbiol* **48**: 1134–1139.

Boga HI, Ludwig W & Brune A (2003) *Sporomusa aerivorans* sp. nov., an oxygen-reducing homoacetogenic bacterium from the gut of a soil-feeding termite. *Int J Syst Evol Microbiol* **53**: 1397–1404.

Bräuer SL, Yavitt JB & Zinder SH (2004) Methanogenesis in McLean Bog, an acidic peat bog in upstate New York: stimulation by H_2/CO_2 in the presence of rifampicin, or by low concentrations of acetate. *Geomicrobiol J* **21**: 433–443.

Brauman A, Kane MD, Labat M & Breznak JA (1992) Genesis of acetate and methane by gut bacteria of nutritionally diverse termites. *Science* **257**: 1384–1387.

Breznak JA (1994) Acetogenesis from carbon dioxide in termite guts. *Acetogenesis* (Drake HL, ed), pp. 303–330. Chapman and Hall Inc., New York.

Brune A, Emerson D & Breznak JA (1995) The termite gut microflora as an oxygen sink: microelectrode determination of oxygen and pH gradients in guts of lower and higher termites. *Appl Environ Microbiol* **61**: 2681–2687.

Buckel W (2005) Special clostridial enzymes and fermentation pathways. *Handbook on Clostridia* (Dürre P, ed), pp. 177–220. CRC Press, Boca Raton, FL.

Daniel SL, Hsu T, Dean SI & Drake HL (1990) Characterization of the H_2- and CO-dependent chemolithotrophic potentials of the acetogens *Clostridium thermoaceticum* and *Acetogenium kivui*. *J Bacteriol* **172**: 4464–4471.

© 2008 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Das A, Coulter ED, Kurtz Jr DM & Ljungdahl LG (2001) Five-gene cluster in *Clostridium thermoaceticum* consisting of two divergent operons encoding rubredoxin oxidoreductase – rubredoxin and rubrerythrin-type flavodoxin – high-molecular-weight rubredoxin. *J Bacteriol* **183**: 1560–1567.

Das A, Silaghi-Dumitrescu R, Ljungdahl LG & Kurtz Jr DM (2005) Cytochrome *bd* oxidase, oxidative stress, and dioxygen tolerance of the strictly anaerobic bacterium *Moorella thermoacetica*. *J Bacteriol* **187**: 2020–2029.

Degraeve KG, Grivet JP, Durand M, Beaumatin P, Cordelet C, Hannequart G & Demeyer D (1994) Competition between reductive acetogenesis and methanogenesis in the pig large-intestinal flora. *J Appl Bacteriol* **76**: 55–61.

Drake HL & Daniel SL (2004) Physiology of the thermophilic acetogen *Moorella thermoacetica*. *Res Microbiol* **155**: 869–883.

Drake HL & Gößner AS (2008) Acetogens: anaerobic gamblers at the oxic–anoxic interface. *Microbes and the Environment: Perspectives and Challenges* (Liu SJ & Drake HL, eds), pp. 59–66. Science Press, Beijing.

Drake HL & Küsel K (2005) Acetogenic clostridia. *Handbook on Clostridia* (Dürre P, ed), pp. 719–746. CRC Press, Boca Raton, FL.

Drake HL, Küsel K & Matthies C (2006) Acetogenic prokaryotes. *The Prokaryotes* (Balows A, Trüper HG, Dworkin M, Harder W & Schleifer KH, eds), pp. 354–420. Springer, New York.

Drake HL, Gößner AS & Daniel SL (2008) Old acetogens, new light. *Incredible Anaerobes: From Physiology to Genomics to Fuels* (Wiegel J, Maier RJ & Adams MWW, eds), pp. 100–128. New York Academy of Sciences, Boston, MA.

Gößner A, Devereux R, Ohnemüller N, Acker G, Stackebrandt E & Drake HL (1999) *Thermicanus aegyptius* gen. nov., sp. nov., isolated from oxic soil, a facultative microaerophile that grows commensally with the thermophilic acetogen *Moorella thermoacetica*. *Appl Environ Microbiol* **65**: 5124–5133.

Gößner AS, Küsel K, Acker G, Lovell CR & Drake HL (2006) Trophic interaction of the aerotolerant anaerobe *Clostridium intestinale* and the acetogen *Sporomusa rhizae* sp. nov. isolated from roots of the black needlerush *Juncus roemerianus*. *Microbiology* **152**: 1209–1219.

Gunsalus RP, Romesser JA & Wolfe RS (1978) Preparation of coenzyme-M analogs and their activity in methyl coenzyme-M reductase system of methanobacterium-thermoautotrophicum. *Biochemistry* **17**: 2374–2377.

Hamberger A, Horn MA, Dumont MG, Murrell JC & Drake HL (2008) Anaerobic consumers of monosaccharides in a moderately acidic fen. *Appl Environ Microbiol* **74**: 3112–3120.

Horn MA, Matthies C, Küsel K, Schramm A & Drake HL (2003) Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat. *Appl Environ Microbiol* **69**: 74–83.

Karnholz A, Küsel K, Gößner A, Schramm A & Drake HL (2002) Tolerance and metabolic response of acetogenic bacteria toward oxygen. *Appl Environ Microbiol* **68**: 1005–1009.

Kotsyurbenko OR, Chin KJ, Glagolev MV, Stubner S, Simankova MV, Nozhevnikova AN & Conrad R (2004) Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog. *Environ Microbiol* **6**: 1159–1173.

Krumholz LR & Bryant MP (1985) *Clostridium pfennigii* sp. nov. uses methoxyl groups of monobenzoids and produces butyrate. *Int J Syst Bacterial* **35**: 454–456.

Kuhner CH, Frank C, Grießhammer A, Schmittroth M, Acker G, Gößner A & Drake HL (1997) *Sporomusa silvacetica* sp. nov., an acetogenic bacterium isolated from aggregated forest soil. *Int J Syst Bacteriol* **47**: 352–358.

Küsel K & Drake HL (1995) Effects of environmental parameters on the formation and turnover of acetate by forest soils. *Appl Environ Microbiol* **61**: 3667–3675.

Küsel K, Pinkart HC, Drake HL & Devereux R (1999) Acetogenic and sulfate-reducing bacteria inhabiting the rhizoplane and deep cortex cells of the seagrass *Halodule wrightii*. *Appl Environ Microbiol* **65**: 5117–5123.

Küsel K, Dorsch T, Acker G, Stackebrandt E & Drake HL (2000) *Clostridium scatologenes* strain SL1 isolated as an acetogenic bacterium from acidic sediments. *Int J Syst Evol Microbiol* **20**: 537–546.

Küsel K, Karnholz A, Trinkwalter T, Devereux R, Acker G & Drake HL (2001) Physiological ecology of *Clostridium glycolicum* RD-1, an aerotolerant acetogen isolated from sea grass roots. *Appl Environ Microbiol* **67**: 4734–4741.

Lee Y-J, Romanek CS & Wiegel J (2007) *Clostridium aciditolerans* sp. nov., an acid-tolerant spore-forming anaerobic bacterium from constructed wetland sediment. *Int J Syst Evol Microbiol* **57**: 311–315.

Liou JS-C, Balkwill DL, Drake GR & Tanner RS (2005) *Clostridium carboxidovorans* 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. *Int J Syst Evol Microbiol* **55**: 2085–2091.

Martin DR, Lundie LL, Kellum R & Drake HL (1983) Carbon monoxide-dependent evolution of hydrogen by the homoacetate-fermenting bacterium *Clostridium thermoaceticum*. *Curr Microbiol* **8**: 337–340.

Metje M & Frenzel P (2005) Effect of temperature on anaerobic ethanol oxidation and methanogenesis in acidic peat from a northern wetland. *Appl Environ Microbiol* **71**: 8191–8200.

Müller V, Inkamp F, Rauwolf A, Küsel K & Drake HL (2004) Molecular and cellular biology of acetogenic bacteria. *Strict and Facultative Anaerobes: Medical and Environmental Aspects* (Nakano MM & Zuber P, eds), pp. 251–281. Horizon Bioscience, Norfolk.

Muyzer G, Teske A, Wirsen CO & Jannasch HW (1995) Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Arch Microbiol* **164**: 165–172.

Nozhevnikova AN, Kotsyurbenko OR & Simankova MV (1994) Acetogenesis at low temperature. *Acetogenesis* (Drake HL, ed), pp. 416–431. Chapman and Hall Inc., New York.

© 2008 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Paul S, Küsel K & Alewell C (2006) Reduction processes in forest wetlands: tracking down heterogeneity of source/sink functions with a combination of methods. *Soil Biol Biochem* **38**: 1028–1039.

Peters V & Conrad R (1995) Methanogenic and other strictly anaerobic bacteria in desert soil and other oxic soils. *Appl Environ Microbiol* **61**: 1673–1676.

Savage DM, Wu Z, Daniel SL, Lundie Jr LL & Drake HL (1987) Carbon monoxide-dependent chemolithotrophic growth of *Clostridium thermoautotrophicum*. *Appl Environ Microbiol* **53**: 1902–1906.

Schink B (1994) Diversity, ecology, and isolation of acetogenic bacteria. *Acetogenesis* (Drake HL, ed), pp. 197–235. Chapman and Hall Inc., New York.

Shannon RD & White JR (1994) A three-year study of controls on methane emissions from two Michigan peatlands. *Biogeochemistry* **27**: 35–60.

Shannon RD & White JR (1996) The effects of spatial and temporal variations in acetate and sulfate on methane cycling in two Michigan peatlands. *Limnol Oceanogr* **41**: 435–443.

Sharak-Genthner BR, Davies CL & Bryant MP (1981) Features of rumen and sewage sludge strains of *Eubacterium limosum*, a methanol- and H_2-CO_2-utlizing species. *Appl Environ Microbiol* **42**: 12–19.

Tanner RS & Woese CR (1994) A phylogenetic assessment of the acetogens. *Acetogenesis* (Drake HL, ed), pp. 254–272. Chapman and Hall Inc., New York.

Wagner C, Grießhammer A & Drake HL (1996) Acetogenic capacities and the anaerobic turnover of carbon in a Kansas prairie soil. *Appl Environ Microbiol* **62**: 494–500.

Wang G & Wang DIC (1984) Elucidation of growth inhibition and acetic acid production by *Clostridium thermoaceticum*. *Appl Environ Microbiol* **47**: 294–298.

Wayne LG, Brenner DJ, Colwell RR *et al.* (1987) International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**: 463–464.

Westermann P (1993) Wetland and swamp microbiology. *Aquatic Microbiology, an Ecological Approach* (Ford TE, ed.), pp. 215–238. Blackwell Scientific Publications, Oxford.

Whitehead TR, Price NP, Drake HL & Cotta MA (2008) Catabolic pathway for the production of skatole and indole acetic acid by the acetogen *Clostridium drakei*, *Clostridium scatologenes*, and swine manure. *Appl Environ Microbiol* **74**: 1950–1953.

Wiegel J (1994) Acetate and the potential of homoacetogenic bacteria for industrial applications. *Acetogenesis* (Drake HL, ed), pp. 484–504. Chapman and Hall, Inc., New York.

Williams RT & Crawford RL (1984) Methane production in Minnesota peatlands. *Appl Environ Microbiol* **47**: 1266–1271.

Wood HG & Ljungdahl LG (1991) Autotrophic character of acetogenic bacteria. *Variations in Autotrophic Life* (Shively JM & Barton LL, eds), pp. 201–250. Academic Press, San Diego, CA.

© 2008 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved