Clostridium clariflavum sp. nov. and *Clostridium caenicola* sp. nov., moderately thermophilic, cellulose-/cellobiose-digesting bacteria isolated from methanogenic sludge

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Two novel anaerobic, moderately thermophilic and cellulose-/cellobiose-digesting bacteria, EBR45^T and EBR596^T, were isolated from anaerobic sludge of a cellulose-degrading methanogenic bioreactor. Phylogenetic analysis based on 16S rRNA gene sequences indicated that these strains belonged to cluster III within the low-G+C-content Gram-positive bacteria. The close relatives of EBR45^T were *Clostridium straminisolvens* DSM 16021^T (sequence identity, 94.6 %) and *Clostridium thermocellum* DSM 1237^T (93.4 %). The closest relative of EBR596^T was *Clostridium stercorarium* DSM 8532^T (95.9 %). Both isolates were rod-shaped sporulators, growing optimally at 60 °C. EBR45^T was Gram-staining-reaction-variable and non-motile, formed bright-yellow colonies on solid media, and grew on a relatively narrow range of carbohydrates including cellulose and cellobiose. EBR596^T was Gram-staining-reaction-negative and motile, formed glossy white colonies and grew on cellobiose and various carbohydrates except cellulose. Major fatty acid compositions were 16 : 0 iso, 16 : 0 and 16 : 0 dimethylacetal (strain EBR45^T) and 15 : 0 iso, 16 : 0 iso, 15 : 0 anteiso and 17 : 0 anteiso (strain EBR596^T). The DNA G+C contents were 36.9 mol% (EBR45^T) and 51.1 mol% (EBR596^T). Based on the phenotypic and phylogenetic data and genomic distinctiveness, strains EBR45^T and EBR596^T represent two novel species, for which the names *Clostridium clariflavum* sp. nov. (type strain EBR45^T =DSM 19732^T =NBRC 101661^T) and *Clostridium caenicola* sp. nov. (type strain EBR596^T =DSM 19027^T =NBRC 102590^T) are proposed.

The Gram-positive bacterial genus *Clostridium* was first proposed by Prazmowski in 1880. To date, the names of over 190 species of this genus have been validly published (http://www.bacterio.cict.fr/c/clostridium.html). Most of the members are chemo-organotrophs that can utilize carbohydrates and/or proteinaceous compounds as energy sources. In the classic taxonomy, the organisms had only to meet four criteria to be classified as a member of *Clostridium*, i.e. endospore formation, obligately anaerobic growth, a Gram-positive cell wall and no ability to perform dissimilatory reduction of sulfate. The genus was thus highly divergent and did not form a monophyletic group. To clarify this phylogenetic incoherence, Collins *et al.* (1994) proposed reclassification of the genus *Clostridium* and related taxa into newly established phylogenetic groups based on the 16S rRNA gene sequences. Following this, Wiegel *et al.* (2006) proposed a new approach: that members of *Clostridium* cluster I, including the type strain, comprise the genus *Clostridium sensu stricto* and that those remaining outside this cluster are reclassified within different phylogenetic groups. According to this criterion, the members of cluster III, which includes industrially important cellulose digesters, should be distinguished from *Clostridium sensu stricto*, but appropriate reclassification has not yet been carried out due to insufficient information with regard to the phylogenetic and physiological properties of its constituents.

We study the application of an anaerobic thermophilic methanogenic bioreactor to the utilization of municipal solid wastes. Recently, we succeeded in isolating an

Abbreviation: DMA, dimethylacetal.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains EBR45^T and EBR596^T are AB186359 and AB221372.

effective cellulose-degrading strain of the genus *Clostridium*, strain EBR45^T, which played a major role in the efficient operation of the bioreactor that digested artificial municipal waste containing homogenized photocopy paper (Shiratori *et al.*, 2006). The result of 16S rRNA gene sequence-based phylogenetic analysis indicated that strain EBR45^T and another isolate from the same sludge, strain EBR596^T, were representatives of novel species within *Clostridium* cluster III. In this paper, we describe the taxonomic characterization of these isolates.

Unless noted otherwise, cultivation of strains EBR45^T and EBR596^T was performed using M solution, which was prepared by mixing 980 ml basal solution with 10 ml each of vitamin solution and mineral solution. The basal solution had the following composition (l^{-1}; all chemicals from Kokusan unless indicated otherwise): 0.4 g KH_2PO_4, 0.4 g $K_2HPO_4 . 3H_2O$, 1.0 g NH_4Cl, 0.1 g $MgCl_2 . 6H_2O$, 2.0 g yeast extract (Difco), 6.0 g $NaHCO_3$, 0.5 g cysteine hydrochloride monohydrate, 0.25 g $Na_2S . 9H_2O$ (Yoneyama) and 0.001 g resazurin. The vitamin solution had the following composition (l^{-1}): 2 mg biotin, 2 mg folic acid, 10 mg pyridoxine hydrochloride, 5 mg thiamine hydrochloride, 5 mg riboflavin, 5 mg nicotinic acid, 5 mg DL-calcium pantothenate, 0.1 mg vitamin B_{12}, 5 mg *p*-aminobenzoic acid and 5 mg lipoic acid. The mineral solution had the following composition (l^{-1}): 4.5 g nitrilotriacetic acid, 0.4 g $FeCl_2 . 4H_2O$, 0.12 g $CoCl_2 . 6H_2O$, 0.01 g $AlK(SO_4)_2$, 1.0 g NaCl, 0.02 g $CaCl_2$, 0.01 g $Na_2MoO_4 . 2H_2O$, 0.1 g $MnCl_2 . 4H_2O$, 0.1 g $ZnCl_2$, 0.01 g H_3BO_3, 0.01 g $CuSO_4 . 5H_2O$ and 0.02 g $NiCl_2$. M solution was supplied with various cellulosic substrates to prepare each culture medium. Mc medium contained 0.5 % (w/v) cellobiose in M solution. Mpc medium contained 1.0 % (w/v) cellulose powder (type D; Advantec) in M solution. The bacteria were cultivated routinely in a screw-capped test tube under an oxygen-free nitrogen atmosphere without shaking at 55 °C (for strain EBR45^T) or 60 °C (for strain EBR596^T) and maintained as a glycerol suspension (10 %, w/v) at −80 °C. The reference strains *Clostridium thermocellum* DSM 1237^T, *C. stercorarium* subsp. *stercorarium* DSM 8532^T and *C. straminisolvens* JCM 21531^T were obtained from the DSMZ or JCM and cultured in Mc medium for analysis of cellular fatty acid composition.

Cell morphology was observed under a Zeiss Axioskop 2 optical microscope (Carl Zeiss Microimaging) and a JEM-1200EX electron microscope (JEOL). For transmission electron microscopy, cells were fixed with 2.0 % (v/v) glutaraldehyde and 2.0 % (v/v) osmium tetroxide. The samples were embedded in Epoxy resin (Epon 812) and ultrathin sections of samples were prepared with an LKB-8800 ultramicrotome. The samples were stained with 2.0 % uranyl acetate and lead citrate. After vapour deposition treatment with carbon, the samples were observed.

The following physiological and biochemical characteristics were studied using the methods described by Holdeman *et al.* (1977): Gram-staining, motility, aesculin hydrolysis, nitrate reduction, sulfate reduction, indole production, casein digestion, gelatin liquefaction and activities of lecithinase, lipase and catalase. The potential for degradation of cellulose was tested using M solution supplemented with 1.0 % (w/v) each of filter paper (filter paper no. 1; Whatman), powdered cellulose (type D; Advantec), microcrystalline cellulose (Serva), photocopy paper (NBS Ricoh), newspaper and CM-cellulose (Wako). The temperature range for growth (30, 37, 40, 50, 55, 60, 65 and 70 °C) was determined in Mc medium. The pH range (pH 5.0–9.0 at intervals of 0.5 or 1.0 pH unit) for growth was determined in Mc medium adjusted by addition of 1 M HCl or NaOH. Tolerance of salinity was tested in Mc medium supplemented with NaCl at 0–1 % NaCl (at intervals of 0.1 %) and 2, 3 and 5 % NaCl (w/v). The potential for colony formation and oxygen tolerance at various oxygen concentrations (0, 1, 2, 4 and 6 %) were studied using Mc and Mpc solid medium (each broth medium solidified with 1.5 % agar in a Petri dish) in an Anoxomat Mark II system (Mart Microbiology). Substrate utilization was studied in PY medium (Holdeman *et al.*, 1977) and M solution supplemented with various carbohydrates (1.0 %, w/v). Growth was judged by the increase of optical density or decrease of the culture broth pH after 5–7 days cultivation. Production of organic acids and gases (H_2 and CO_2) was analysed by HPLC with an electric conductivity monitor (Shimadzu) and gas chromatography (model GC323; GL Science), respectively.

As partially described in a previous report (Shiratori *et al.*, 2006), cells of strains EBR45^T and EBR596^T were thermophilic, spore-forming, flagellated rods (Fig. 1a, b). Phenotypic characteristics of the two isolates are summarized in Table 1 and in the species descriptions. Colonies of strain EBR45^T on Mc and Mpc solid medium were round, convex, about 1–2 mm in diameter, bright yellow in colour and had a slightly undulating margin. Colonies of strain EBR596^T on Mc solid medium were similar to strain EBR45^T in form and size but had a glossy white colour. Although the Gram-staining reaction of both isolates was negative or variable, the cell-wall ultrastructure resembled that of Gram-positive bacteria (Fig. 1c, d). Both strains retained clear S-layer structures at the outermost surface but they exhibited highly diverged cell-wall structures (longitudinal sections; insets in Fig. 1c, d). The salinity ranges (NaCl concentration) for growth of strains EBR45^T and EBR596^T were 0–0.7 % (w/v) (optimum 0.4 %, no growth $\geqslant$2.0 %) and 0–1.5 % (optimum 1.0 %), respectively. Both strains were anaerobic, as demonstrated by their inability to grow under aerobic conditions (no growth at O_2 concentrations of $\geqslant$4 % for EBR45^T and $\geqslant$2 % for EBR596^T). Strain EBR45^T digested various cellulose substrates (purified cellulose, papers and CM-cellulose) and accumulated an orange–yellow pigment on the surface of cellulose materials, while strain EBR596^T was non-cellulolytic. Strain EBR45^T did not utilize amygdalin, arabinose, glucose, maltose, mannitol, salicin or starch (each supplied

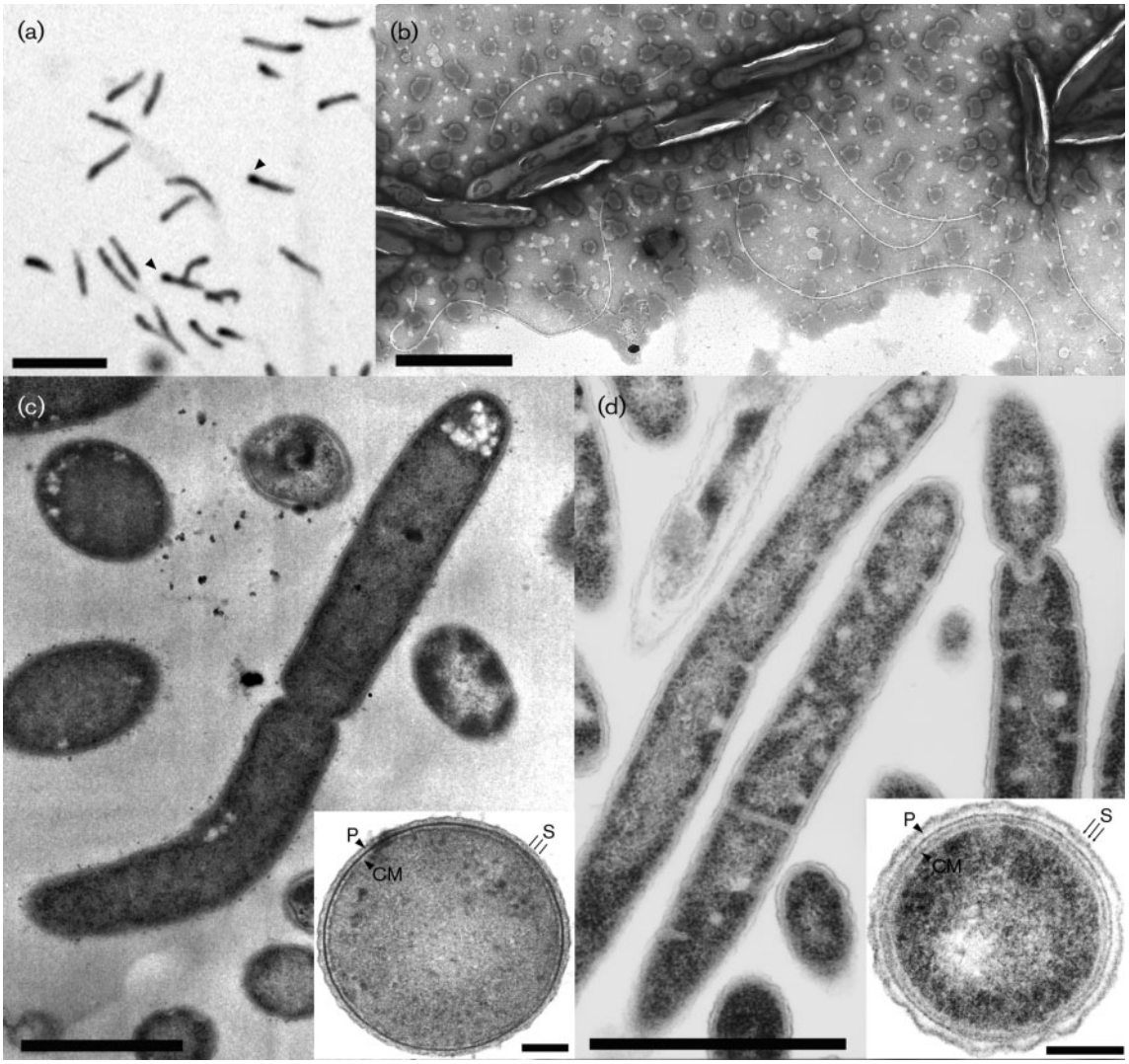

Fig. 1. Micrographs of strains EBR45^T and EBR596^T. (a) Optical micrograph of cells of strain EBR596^T grown in a cellobiose-utilizing liquid culture. Arrows indicate spores within vegetative cells. Bar, 5 μm. (b) Electron micrograph of negatively stained cells of strain EBR596^T. Bar, 1 μm. Optical micrographs of strain EBR45^T have been published previously (Shiratori *et al.*, 2006). (c, d) Electron micrographs of cross sections and longitudinal sections (inset) of strains EBR45^T (c) and EBR596^T (d). CM, Cytoplasmic membrane; P, peptidoglycan layer; S, S-layer. Bars, 1 μm (c, d) and 0.1 μm (insets).

at 1.0 %) as carbon and energy sources, while strain EBR596^T utilized all of them. These features imply that these strains played different roles in the biomass degradation in the original methanogenic bioreactor.

Neither strain utilized any of the following carbohydrates: adonitol, dulcitol, erythritol, fructose, galactose, glycerol, glycogen, inositol, inulin, lactose, mannose, melibiose, melezitose, raffinose, rhamnose, ribose, sorbitol, sorbose, sucrose, trehalose, xylitol and xylose. The main fermentation products of the two strains in a cellobiose-utilizing culture were H_2, CO_2, lactate, acetate and ethanol. Strain EBR45^T also produced a detectable amount of formate. Both strains tested negative for the following biochemical properties: nitrate and sulfate reduction, indole production, casein digestion, gelatin liquefaction and lecithinase, lipase and catalase activities.

DNA was extracted by using phenol/chloroform extraction followed by polyethylene glycol precipitation, based on the method described by Marmur (1961) with some modification. The DNA G+C contents of strains EBR45^T and EBR596^T determined by an HPLC method (Mesbah & Whitman, 1989) were 36.9 and 51.1 mol%, respectively (mean of three measurements). The 16S rRNA gene sequences were amplified by PCR with a bacterial domain-specific primer set, 27F/1492R (Wang *et al.*, 2007). PCR was performed on a T1 Thermocycler (Biometra) with Ex *Taq* polymerase (Takara Bio). The PCR protocol included an initial denaturation period of

Table 1. Differential phenotypic characteristics of strains EBR45^T and EBR596^T and thermophilic members of cluster III in the low-G+C-content Gram-positive bacteria

Taxa: 1, *Clostridium clariflavum* sp. nov. EBR45^T; 2, *Clostridium caenicola* sp. nov. EBR596^T; 3, *C. thermocellum* (data from McBee, 1954; Ng *et al.*, 1977; Wiegel, 1980); 4, *C. straminisolvens* CSK1^T (unless indicated, data from Kato *et al.*, 2004); 5, *C. stercorarium* subsp. *stercorarium* (Madden, 1983); 6, *C. stercorarium* subsp. *leptospartum* (Toda *et al.*, 1988); 7, *C. stercorarium* subsp. *thermolacticum* (Le Ruyet *et al.*, 1985). All taxa are positive for cellobiose utilization and aesculin hydrolysis. +, Positive; −, negative; W, weak; ND, data not available.

Characteristic	1	2	3	4	5	6	7
Isolation source	Sludge	Sludge	Widespread	Rice straw	Compost	Compost	Compost
Colony colour	Bright yellow	White	Yellow	Tan–yellow	Cream	No pigment	No pigment
Temperature for growth (°C)							
Range	40–65	50–70	50–68	45–65	ND	45–71	50–70
Optimum	55–60	60	60–64	50–55	65	60	60–65
Cell size (μm)							
Length	2.0–5.0	1.8–4.8	2.5–5.0	3.0–8.0	2.0–4.0	4.5–15	2.7–7.7
Width	0.4–0.5	0.4–0.6	0.5–0.7	0.5–1.0	0.3–0.4	0.25–0.45	0.7–0.8
Gram reaction	Variable	−	−	−*	−	−	−
Flagella	+	+	+	ND	+	−	+
Motility	−	+	+/−	−	+	−	+
Utilization of:							
Arabinose	−	+	−	ND	−	−	−
Cellulose	+	−	+	+	+	+	+/−
Fructose	−	−	−	−	+	−	+
Glucose	−	+	−	−	+	+	+
Lactose	−	−	−	−	+	+	+
Mannitol	−	W	−	−	−	−	−
Starch	−	+	−	−	ND	ND	+
Sucrose	−	−	−	−	+	−	+
Fermentation products†	F, A, L, E	A, L, E	A, L, E	A, E, (L)	A, L, E	A, E, (IP)	A, L, E
DNA G+C content (mol%)	36.9	51.1	38–39	41.3	39	43	41

*Data from this study.

†F, Formate; A, acetate; L, lactate; E, ethanol; IP, isopentanol. Products found as traces are given in parentheses.

94 °C for 4 min, 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, an extension period of 72 °C for 3 min, and incubation at 4 °C until further processing. The nearly complete 16S rRNA gene sequences of strains EBR45^T (1560 bp) and EBR596^T (1435 bp) were determined directly by using a BigDye terminator v3.1 cycle sequencing kit on an ABI 3130 Genetic Analyzer (Applied Biosystems). Analysis of the sequences was performed with the software package MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignment of sequences by CLUSTAL W (Thompson *et al.*, 1994) and SEAVIEW (Galtier *et al.*, 1996). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. A bootstrap analysis (1000 replications) was carried out to evaluate the topology of the resulting tree (Felsenstein, 1985). The evolutionary distance matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969).

The neighbour-joining tree of 16S rRNA gene sequences, which was also supported by the maximum-parsimony method, showed that the two isolates were members of the thermophilic group of cluster III (Collins *et al.*, 1994; Wiegel *et al.*, 2006) within the low-G+C-content Gram-positive bacteria (Fig. 2). Sequence similarity calculations using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) indicated that strain EBR45^T showed highest similarities to *C. straminisolvens* DSM 16021^T (sequence identity 94.6 %) and *C. thermocellum* DSM 1237^T (93.4 %). The closest relatives of strain EBR596^T were *C. stercorarium* subsp. *stercorarium* DSM 8532^T, *C. stercorarium* subsp. *leptospartum* DSM 9219^T and *C. stercorarium* subsp. *thermolacticum* DSM 2910^T (sequence identities 95.0–95.9 %). These identity scores are low enough, according to Stackebrandt & Goebel (1994), to justify the description of strains EBR45^T and EBR596^T as representatives of novel species of cluster III. These results support the view that strains EBR45^T and EBR596^T can be discriminated from their closest relatives at the species level (Wayne *et al.*, 1987).

Fatty acid methyl esters were extracted and analysed according to the standard protocol of the Sherlock Microbial Identification System (version 5.0; MIDI). The cellular fatty acid profiles of strains EBR45^T and EBR596^T and related type strains belonging to the cluster III are

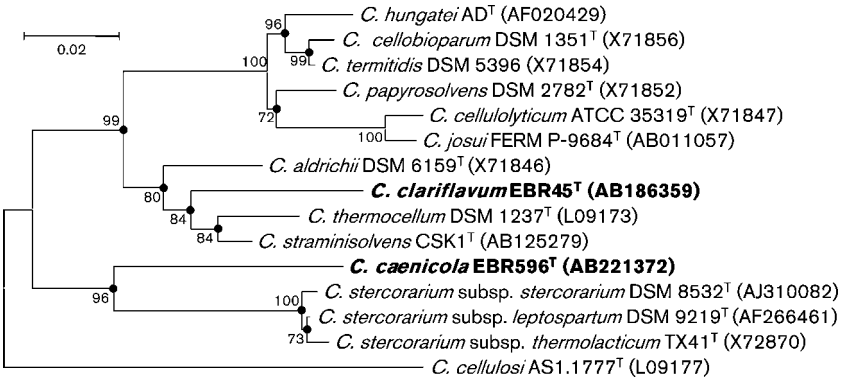

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strains EBR45^T and EBR596^T among cluster III within the low-G+C-content Gram-positive bacteria. Numbers at nodes represent bootstrap values (expressed as percentages of 1000 resampled datasets) obtained in the neighbour-joining analysis. Solid circles indicate branching supported by the maximum-parsimony method. The sequence of *Clostridium cellulosi* AS1.1777^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

shown in Table 2. The major fatty acids of strain EBR45^T were 16:0, 16:0 iso and 16:0 dimethylacetal (DMA), which accounted for 60.6 % of the total fatty acids. The major fatty acids of strain EBR596^T were 15:0 iso, 16:0 iso, 15:0 anteiso and 17:0 anteiso, which accounted for 60.5 % of the total fatty acids. This result was consistent with the fatty acid pattern of Gram-positive, spore-forming, low-G+C-content and thermophilic bacteria, which usually contain long-chain (C_{16}–C_{18}) saturated forms as the predominant fatty acids (Chan *et al.*, 1971; Kaneda, 1991). The fatty acid profile of strain EBR45^T differed from that of *C. straminisolvens* JCM 21531^T and *C. thermocellum* DSM 1237^T in the presence of 15:0 (0.62 %), 18:1ω9*c* (0.57 %) and summed feature 13 (15:0 anteiso DMA and/or 14:0 2-OH; 0.20 %). Strain EBR596^T could be differentiated from *C. stercorarium* subsp. *stercorarium* DSM 8532^T in the presence of branched forms 13:0 iso (1.36 %), 16:0 3-OH (0.40 %), 17:0 anteiso 3-OH (1.31 %) and 19:0 Δ9,10 and Δ11,12 DMA (1.54 % in total).

The members of *Clostridium* cluster III (Collins *et al.*, 1994), which currently consists of 12 cellulolytic species/subspecies, are phenotypically heterogeneous (e.g. this cluster includes both mesophiles and thermophiles). In addition, they exhibit a broad range of DNA G+C content (28–43 mol%). Despite this heterogeneity, it is known that cluster III is clearly distinguished from not only *Clostridium* cluster I (*sensu stricto*) but also clusters II and IV in terms of 16S rRNA gene phylogeny (Collins *et al.*, 1994; Wiegel *et al.*, 2006). The result of this phylogenetic analysis clearly shows that strains EBR45^T and EBR596^T fall into the thermophilic group of cluster III (Fig. 2). This indicates that these two novel isolates are closely related to the members of the cluster III, although the significant phylogenetic distances from the closest relatives (Fig. 2) imply that the two isolates are affiliated with taxa that are distinct from others in the cluster. In addition, strains EBR45^T and EBR596^T could be distinguished from their relatives as follows: strain EBR45^T differed from its phylogenetic relatives, *C. thermocellum* and *C. straminisolvens*, in its temperature range for growth and DNA G+C content; strain EBR45^T produced formate as a fermentation product, whereas the two related species did not; strain EBR596^T differed from its close relatives, the subspecies of *C. stercorarium*, in its substrate-utilization patterns and DNA G+C content. Strain EBR596^T exhibited the highest DNA G+C content within cluster III. Fatty acid content profiling also clearly differentiated both the novel isolates from their relatives.

Based on the above evidence, strains EBR45^T and EBR596^T are proposed to be representatives of two novel species of *Clostridium* cluster III in the low-G+C-content Gram-positive bacteria, for which the names *Clostridium clariflavum* sp. nov. and *Clostridium caenicola* sp. nov. are proposed.

Description of *Clostridium clariflavum* sp. nov.

Clostridium clariflavum (cla.ri.fla′vum. L. adj. *clarus* clear, bright, shining, brilliant; L. neut. adj. *flavum* yellow; N.L. neut. adj. *clariflavum* bright yellow, the colour of the colonies or pigment).

Moderately anaerobic, thermophilic and chemo-organotrophic. Cells are straight or slightly curved rods (0.4–0.5 × 2.0–5.0 μm). Gram-type positive but Gram-staining reaction is variable. Non-motile, although retarded peritrichous flagella are present. Spores are oval and subterminal. On solid agar containing cellulose or cellobiose as a carbon source, it forms bright yellow, round colonies (1–2 mm in diameter). Temperature range for growth is 45–65 °C (optimum 55–60 °C). pH range for growth is pH 6.0–8.0 (optimum pH 7.5). The salinity (NaCl) range for growth is 0–0.7 % (w/v) (optimum 0.4 % w/v). Colony formation is observed under anaerobic conditions (O_2<4 %). Cellulose and cellobiose are utilized as sole carbon and energy sources; fermentation products are hydrogen, carbon dioxide, acetate, lactate, ethanol and a small amount of formate. The following compounds are not utilized: glucose, adonitol, amygdalin, arabinose, dulcitol, erythritol, fructose, galactose, glycerol, glycogen, inositol, inulin, lactose, maltose, mannitol, mannose, melibiose, melezitose, raffinose, rhamnose, ribose, salicin,

Table 2. Cellular fatty acid composition (%) of strain EBR45^T, strain EBR596^T and type strains of thermophilic species of cluster III within the low-G+C-content Gram-positive bacteria

Strains: 1, *C. clariflavum* sp. nov. EBR45^T; 2, *C. caenicola* sp. nov. EBR596^T; 3, *C. thermocellum* DSM 1237^T; 4, *C. straminisolvens* JCM 21531^T; 5, *C. stercorarium* subsp. *stercorarium* DSM 8532^T. All data were obtained in this study. Major fatty acids are shown in bold.

Fatty acid	1	2	3	4	5
13:0 iso	–	1.36	–	–	–
13:0 anteiso	–	0.26	–	–	–
13:0 iso 3-OH	1.37	0.08	–	0.33	–
14:0	3.35	1.50	–	0.66	2.13
14:0 iso	0.94	1.99	–	0.36	–
14:0 DMA	3.22	0.09	0.51	0.74	1.03
15:0	0.62	–	–	–	–
15:0 iso	1.67	**18.85**	–	0.77	1.73
15:0 anteiso	–	**9.51**	–	0.23	0.41
15:0 iso DMA	1.74	0.58	0.41	0.71	0.79
16:0	**20.43**	5.92	**16.59**	**22.87**	**52.21**
16:0 3-OH	–	0.40	–	–	–
16:0 iso	**23.70**	**22.97**	**11.80**	**28.31**	1.97
16:0 DMA	**16.47**	0.29	**20.39**	**13.13**	**19.75**
16:1	–	–	–	–	–
16:1ω7*c*	–	1.18	–	–	**5.25**
16:0 aldehyde	4.83	0.06	5.27	4.30	–
17:0 iso	5.42	8.18	4.79	6.43	4.15
17:0 anteiso	1.22	**9.18**	0.98	2.65	3.04
17:0 anteiso 3-OH	–	1.31	–	–	–
17:0 anteiso DMA	–	0.21	0.51	0.58	0.87
18:0	0.73	0.24	5.39	1.38	2.81
18:0 iso	0.85	0.19	14.59	3.77	–
18:0 DMA	0.63	–	1.17	0.48	–
18:0 aldehyde	–	–	–	0.47	–
18:1	–	–	–	–	–
18:1ω9*c*	0.57	0.67	–	–	0.21
19:0 Δ9,10 DMA	–	1.32	–	–	–
19:0 Δ11,12 DMA	–	0.22	–	–	–
20:0	–	–	–	–	1.73
Unknown 16.107	7.87	0.34	**15.71**	**10.41**	0.28
Unknown 17.103	1.84	0.15	1.87	0.80	0.50
Summed feature 1*	1.70	0.30	–	0.21	0.35
Summed feature 3*	–	4.69	–	0.22	0.22
Summed feature 13*	0.20	0.51	–	–	0.22

*Summed feature 1 contained one or more of 13:1, 14:0 aldehyde and 11:1 2-OH; summed feature 3 contained 15:0 iso aldehyde and/or an unknown fatty acid; summed feature 13 contained 15:0 anteiso DMA and/or 14:0 2-OH.

sorbitol, sorbose, sucrose, trehalose, xylitol and xylose. Hydrolysis of aesculin is positive, while hydrolysis of starch, casein or gelatin is negative. Negative for nitrate and sulfate reduction, indole production, lecithinase, lipase and catalase. The major cellular fatty acids are 16:0, 16:0 iso and 16:0 DMA. The genomic G+C content of the type strain is 36.9 mol%.

The type strain is EBR45^T (=DSM 19732^T =NBRC 101661^T).

Description of *Clostridium caenicola* sp. nov.

Clostridium caenicola (cae.ni.co'la. L. n. *caenum* mud, sludge; L. suff. *-cola* dweller; N.L. n. *caenicola* sludge dweller).

Obligately anaerobic, thermophilic and chemo-organotrophic. Cells are motile, lophotrichously flagellated (a few flagella at a polar attachment point) and straight or slightly curved rods (0.4–0.6 × 1.8–4.8 μm). Gram-staining reaction is negative but shows Gram-positive cell-wall structure. Spores are oval and subterminal. Colonies are glossy white in colour on solid agar containing cellobiose as a carbon source. Temperature range for growth is 50–70 °C (optimum 60 °C). pH range for growth is pH 6.0–8.0 (optimum pH 6.5). The salinity (NaCl) range for growth is 0–1.5 % (w/v) (optimum 1.0 % w/v). Colony formation is observed under anaerobic conditions (O_2 <2 %). Cellobiose, amygdalin, arabinose, glucose, maltose, mannitol, salicin and starch are utilized as carbon and energy sources. The following compounds are not utilized: cellulose, adonitol, dulcitol, erythritol, fructose, galactose, glycerol, glycogen, inositol, inulin, lactose, mannose, melibiose, melezitose, raffinose, rhamnose, ribose, sorbitol, sorbose, sucrose, trehalose, xylitol and xylose. Fermentation products are hydrogen, carbon dioxide, acetate, lactate and ethanol. Hydrolysis of aesculin is positive, while hydrolysis of casein and gelatin is negative. Negative for nitrate and sulfate reduction, indole production, lecithinase, lipase and catalase. The major cellular fatty acids are 15:0 iso, 16:0 iso, 15:0 anteiso and 17:0 anteiso. The genomic G+C content of the type strain is 51.1 mol%.

The type strain is EBR596^T (=DSM 19027^T =NBRC 102590^T).

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