International Journal of Systematic and Evolutionary Microbiology (2002), **52**, 801–807 DOI: 10.1099/ijs.0.01998-0

Reclassification of *Clostridium hydroxybenzoicum* as *Sedimentibacter hydroxybenzoicus* gen. nov., comb. nov., and description of *Sedimentibacter saalensis* sp. nov.

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Strain ZF2^T, isolated from freshwater sediment, is a motile, rod-shaped, Gram-positive, endospore-forming, amino acid- and pyruvate-utilizing, anaerobic bacterium. It requires yeast extract for growth. Carbohydrates are not utilized. The optimal temperature and pH for growth are 37 °C and 6·8–7·3, respectively. The G+C content of the DNA is 34·0 mol%. A 16S rDNA sequence analysis of strain ZF2^T revealed that the highest similarity (94·4%) was shared with *Clostridium hydroxybenzoicum* JW/Z-1^T. Strain ZF2^T, however, was not able to carboxylate phenol or to decarboxylate 4-hydroxybenzoate, which are characteristic properties of strain JW/Z-1^T. The degree of 16S rDNA relatedness, together with the physiological and chemotaxonomic properties, suggest that strain ZF2^T represents a novel species that is clearly distinct from *Clostridium hydroxybenzoicum* JW/Z-1^T. In a phylogenetic dendrogram, both strains form a separate cluster that is peripherally associated with the *Peptostreptococcus* group (cluster XIII) of the clostridia and the lineage of *Helcococcus kunzii*. Strains ZF2^T and JW/Z-1^T show a somewhat deeper branching from the cluster XII clostridia *Clostridium purinolyticum* and *Clostridium acidiurici*. The latter strains possessed the closest 16S rDNA similarity (between 88·4 and 90·7%), but were clearly separated by phenotypic markers. Therefore, a new genus, *Sedimentibacter* gen. nov., is described, comprising *Sedimentibacter hydroxybenzoicus* gen. nov., comb. nov., as the type species of the genus, with JW/Z-1^T (= ATCC 51151^T = DSM 7310^T) as the type strain, and the novel species *Sedimentibacter saalensis* sp. nov., with strain ZF2^T (= DSM 13558^T = ATCC BAA-283^T) as the type strain.

Keywords: anaerobic low-G+C Gram-positive bacteria, Firmicutes, *Clostridium hydroxybenzoicum*, 16S rDNA, *Sedimentibacter*

INTRODUCTION

Clostridium hydroxybenzoicum JW/Z-1^T was isolated from a freshwater pond sediment during the investigation of anaerobic mineralization of 2,4-dichlorophenol, which was dechlorinated to phenol and further degraded, via carboxylation, to hydroxybenzoate (Zhang & Wiegel, 1990b, 1992; Zhang *et al*., 1994). The reversible carboxylation/decarboxylation reaction was assigned to organisms similar, or identical, to strain JW/Z-1^T. The biochemistry of this reaction has been studied in detail (Zhang & Wiegel, 1994; He & Wiegel, 1995, 1996). From its morphological, chemotaxonomic and physiological properties, this strain was assigned to the clostridia. However, analyses of

Published online ahead of print on 14 December 2001 as DOI 10.1099/ijs.0.01998-0.

Abbreviations: 3,4-OHB, 3,4-dihydroxybenzoate; 4-OHB, 4-hydroxybenzoate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA sequence of *Sedimentibacter saalensis* strain ZF2^T is AJ404680.

the 16S rDNA of species of *Clostridium* and other related genera have revealed that the genus *Clostridium* is polyphyletic (Collins *et al*., 1994), and several species have been reclassified within newly created genera. In the case of strain JW/Z-1^T, the 16S rRNA gene sequence forms a separate line of descent that is only peripherally associated with cluster XIII and diverges from cluster XII of the clostridia (Zhang *et al*., 1994; Collins *et al*., 1994).

A novel isolate, ZF2^T, was obtained from sediment of the Saale River in Germany by isolation from an anaerobic, 2,4,6-trichlorophenol-dehalogenating enrichment culture (Breitenstein *et al*., 2001) by using an agar-shake dilution series. This strain lacked both dehalogenating and carboxylation/decarboxylation activities, but 16S rDNA sequence analysis revealed a high level of relatedness to strain JW/Z-1^T. On the basis of phylogenetic and phenotypic properties, we propose the reclassification of *C. hydroxybenzoicum* to *Sedimentibacter hydroxybenzoicus* gen. nov., comb. nov., as the type species of *Sedimentibacter* gen. nov., strain JW/Z-1^T being the type strain. The newly isolated strain ZF2^T is described as a novel species in this genus, under the designation *Sedimentibacter saalensis* sp. nov.

METHODS

Organisms, media and culture conditions. Strain ZF2^T was isolated from a 10^{-5} dilution step in an agar-shake dilution series of an anaerobic, dehalogenating, mixed culture enriched from sediment of the River Saale (Germany) by using 25 mM pyruvate and 100 μM 2,4,6-trichlorophenol as substrates (Breitenstein *et al*., 2001). A pure culture was obtained through three consecutive serial dilutions in agar shakes containing 25 mM pyruvate and 0·1 % yeast extract. *C. hydroxybenzoicum* strain JW/Z-1^T (= DSM 7310^T) was obtained from the DSMZ. If not mentioned otherwise in the text, both strains were normally grown in minimal medium DC consisting of medium 1 (Holliger *et al*., 1992) containing (l^{-1}) 10 mM phosphate, trace elements according to Holliger *et al*. (1993), 16 nM Na_2SeO_3, 12 nM Na_2WoO_4, 63 nM 1,4-naphthoquinone and 5 g yeast extract, supplemented with 10 mM L-arginine and 10 mM glycine or 25 mM pyruvate. The cultivation of the strains was carried out in volumes of 50–80 ml in 125 ml serum bottles or in 10 ml aliquots in Hungate tubes under a N_2/CO_2 (4:1) atmosphere at 30 °C in the dark. Endospore formation was studied on Brewer's anaerobic agar. To test heat resistance, cells were rinsed off the agar with minimal medium, incubated in Hungate tubes for 30 min submerged in an 80 °C water bath and then tested for growth with glycine and arginine. All experiments were performed in triplicate, unless otherwise indicated.

Growth parameters. The temperature range for growth was determined, using medium DC supplemented with 25 mM pyruvate, to be between 4 and 40 °C. The pH range for growth was studied in medium DC containing glycine and L-arginine and was adjusted to pH values in the range 5·5–9·0 with 50 mM MES, phosphate or pyrophosphate buffer and suitable volumes of 1 M HCl or 1 M KOH prior to autoclaving under a N_2 atmosphere. Growth was monitored by measuring the increase in optical density at 600 nm.

Physiological characterization. Acid formation from carbohydrates, aesculin hydrolysis, indole production and gelatinase and urease activities were tested for by using API 20A strips (bioMérieux). Cells were pre-grown in minimal medium with 0·3 % (w/v) yeast extract and glycine and L-arginine, harvested by centrifugation and then washed twice with 10 mM anaerobic phosphate buffer before inoculation of the API strips; the strips were incubated anaerobically at 30 °C and monitored after 24 and 48 h. Sulfide production, indole formation and motility were tested for by using sulfide indole motility (SIM) medium according to Holdeman *et al*. (1977). Nitrate reduction was analysed using medium containing 0·5 % yeast extract and supplemented with 25 mM pyruvate and 0·1 % KNO_3 and commercially supplied test strips for the semi-quantitative detection of nitrate and nitrite.

Fermentation products. Volatile and non-volatile fatty acids were analysed by GC with Shimadzu-GC 14A equipment incorporating an FFAP capillary column (25 m × 0·25 mm internal diameter, 0·25 μm film thickness) and a flame-ionization detector. Helium served as the carrier gas. The injector and detector were heated to 240 and 270 °C, respectively. The volatile (i) and non-volatile fatty acids (ii) were analysed using the following temperature programmes: (i) initial column temperature 100 °C for 3 min, increased at a rate of 3 °C min^{-1} to 136 °C and at a rate of 40 °C min^{-1} to 220 °C; (ii) initial temperature 100 °C for 6 min, increased at a rate of 3 °C min^{-1} to 115 °C and at a rate of 40 °C min^{-1} to 220 °C. Culture supernatants were pre-treated according to Nanninga & Gottschal (1985) before direct measurement. Hydrogen was measured in the headspaces of Hungate tubes containing medium DC with 0·5 % yeast extract and 25 mM pyruvate. Incubation was for 8 days at 30 °C using a Reduction Gas Analyzer RGA2 (Traceanalytical) with N_2 as the carrier gas, a 60/80 Molsieve 5A column and the following settings: oven and detector temperatures, 105 and 265 °C, respectively; gas flow, 20 ml min^{-1}; pre-pressure, 4 bar; injection volume, 1 ml.

Chemotaxonomic analyses. Lipid fatty acids and isoprenoid quinones were analysed as described by Lechner *et al*. (1995). Cell walls were prepared and the peptidoglycan structure determined by using the methods described by Schleifer & Kandler (1972). Amino acids and peptides were separated and characterized by TLC on cellulose sheets.

Determination of DNA base composition. DNA was isolated and purified from 500 mg freeze-dried cell material as described by Väisänen & Salkinoja-Salonen (1989) with the modification that DNA extraction was carried out repeatedly with chloroform/isoamyl alcohol (24:1, v/v) in the presence of 1 M $NaClO_4$. The G+C content (mol %) was determined by HPLC of purified DNA digested to the nucleoside level according to the method of Tamaoka & Komagata (1984).

Microscopic examinations. Cell morphology and motility were investigated by phase-contrast microscopy and transmission electron microscopy using cells from the exponential growth phase (12 h) and the stationary phase (20 h, 48 h and 2 weeks old) grown in medium DC with either pyruvate or glycine and arginine as the carbon and energy source. Cells were negatively stained with 2 % (w/v) tungstophosphoric acid for electron microscopy. Gram-staining was performed according to standard methods (Gerhardt, 1981) with *Escherichia coli* K-12 (DSM 498) and *Bacillus subtilis* (DSM 347) as the respective controls.

Carboxylation and decarboxylation assay. The carboxylation of phenol and catechol was analysed using cell suspensions prepared as described by Zhang & Wiegel (1994) from cells

pre-grown in mineral medium containing 10 mM L-arginine and 10 mM glycine plus 6 mM 4-hydroxybenzoate (4-OHB) or 4 mM 3,4-dihydroxybenzoate (3,4-OHB), respectively, as potential inducers of the reversible decarboxylases (Zhang & Wiegel, 1990a). The decarboxylation of 4-OHB and 3,4-OHB was tested for using supernatants of cultures grown in mineral medium supplemented with 10 mM L-arginine and 10 mM glycine plus 1·5 mM 4-OHB or 1·5 mM 3,4-OHB, respectively. Measurement of the carboxylation of phenol and the decarboxylation of 4-OHB was performed by HPLC on a LiChrospher 100-RP 18 column (250 × 4 mm, 10 μm, 100 Å) at 35 °C using a mobile phase of methanol/water (1:1), acidified to pH 3 with acetic acid, at a constant flow rate of 1 ml min^{-1}. The wavelength used for detection was 260 nm. The carboxylation/decarboxylation of catechol/3,4-OHB was analysed using a mobile phase of methanol/water (5:95) at a constant flow rate of 0·9 ml min^{-1} and a detection wavelength of 275 nm. Cells of strain JW/Z-1^T were used as positive controls for the carboxylation and decarboxylation reactions.

Determination and analysis of 16S rRNA gene sequence. The 16S rRNA gene was PCR-amplified using the universal eubacterial primers fD1 and rP2 (Weisburg *et al.*, 1991) from 1 μl of a suspension of one colony in 50 μl water and subjected to five freeze/thaw cycles. Purification, cloning and bidirectional sequencing of the amplified PCR product were performed as described previously (Breitenstein *et al.*, 2001). The sequence was used for similarity searches against the EMBL and GenBank databases, using the FASTA 3 program (Pearson, 1990). Multiple sequence alignments were performed using the CLUSTAL W program, version 1.7 (Thompson *et al.*, 1994). Positions containing unidentified nucleotides were excluded from the alignment, which was subsequently based on 1305 nt (28–1400 bp, *E. coli* numbering). The phylogenetic analyses were performed with the PHYLIP 3.5c program package (Felsenstein, 1993). Evolutionary distances were calculated with the DNADIST program according to the algorithm of Jukes & Cantor (1969). The unrooted phylogenetic tree was constructed using the neighbour-joining method and the Jukes–Cantor correction (Jukes & Cantor, 1969). Bootstrapping was performed with the programs SEQBOOT, DNADIST and CONSENSE.

RESULTS AND DISCUSSION

Cell morphology

Cells of strain ZF2^T were rod-shaped (0·5–0·7 μm wide; 3·7–6·0 μm long), occurring singly or in chains of two cells up to 12 or more cells. In addition, long filaments of up to 130 μm appeared (Fig. 1a), sometimes having only a few, or no, visible septations. However, staining with 4′,6-diamidino-2-phenylindole (DAPI; 0·05 μg ml^{-1}) indicated the presence of approximately one nucleoid every 4–6 μm. Cells were motile by three to four peritrichously inserted flagella (Fig. 1b). Pili up to 1 μm in length and 5 nm thick were present only on some of the cells (not shown). Motility could be demonstrated in SIM medium and under the light microscope when a freshly withdrawn sample was studied immediately. No flagella were described for strain JW/Z-1^T (Zhang *et al.*, 1994). However, under the conditions described here for ZF2^T, flagella were also found on some cells in preparations of strain JW/Z-1^T. Gram-staining of ZF2^T resulted in a faint blue colour that was regarded as positive in comparison with the reference strains. Heat-resistant (80 °C, 30 min), oval endospores were formed on Brewer's anaerobic agar.

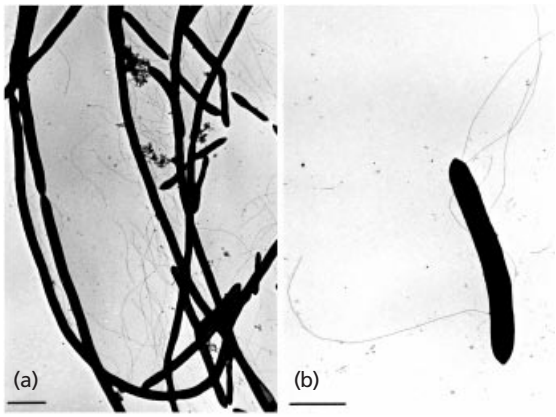

Fig. 1. Transmission electron micrographs of strain ZF2^T, showing long filaments (a) and a single flagellated cell (b) present in a 20-h culture. Bars, 2·5 (a) and 1 (b) μm.

Biochemical and physiological characteristics

Growth requirements, temperature and pH range. Strain ZF2^T is an obligately anaerobic, chemo-organotrophic bacterium. The optimal growth temperature was 37 °C, and growth was observed between 14 and 40 °C; no growth was observed at 4 or 45 °C. The pH range for growth was 5·8–8·0, the optimum being between pH 6·8 and 7·3. No growth occurred at pH 5·5 or 8·5. Strain ZF2^T required at least 0·01 % yeast extract for growth. The maximum growth yield was obtained when 0·3–0·5 % yeast extract was added to medium DC containing 25 mM pyruvate.

Substrate utilization and acid production. The amino acids L-arginine, glycine and L-lysine had different effects on the growth of strain ZF2^T when added singly or in combination to medium containing 0·5 % (w/v) yeast extract (Table 1). Glycine and L-lysine supported growth as single substrates and were fermented to almost equimolar amounts of acetate and acetate plus butyrate, respectively. The amino acid fermentation of strain ZF2^T resembled that of *C. hydroxybenzoicum* strain JW/Z-1^T (Zhang *et al.*, 1994) except for the L-lysine fermentation. Strain ZF2^T produced both acetate and butyrate from L-lysine, whereas strain JW/Z-1^T largely produced butyrate (Zhang *et al.*, 1994). However, the increased acetate production from lysine plus glycine at the expense of butyrate indicated that glycine functioned as the electron acceptor for both strains. The addition of arginine to glycine led to an increase in growth and acetate formation, suggesting a Stickland-type reaction with glycine reduction and arginine oxidation to acetate (Seto, 1980; Andreesen *et al.*, 1989). However, the addition of arginine alone did

Table 1. Growth and volatile fatty acid production by strain ZF2^T in minimal medium with and without the addition of certain amino acids

All amino acids were added at 10 mM. A value for growth of 100 % was equivalent to an OD_{600} of 0·28. The medium contained 0·5 % yeast extract. The concentrations of fatty acids determined were corrected for the amounts of fatty acids present in the yeast extract at time zero.

Addition(s)	Growth (%)	Concentration of volatile fatty acid produced (mM)				
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate
None	100	3·3	0·45	0	1·2	0·11
Arg	71	3·2	0·33	0	0	0
Gly	179	14·1	0·94	0·14	0	0·70
Lys	257	11·0	0·60	0	11·3	0·34
Arg + Gly	261	20·0	0·62	0·10	1·9	0·56
Gly + Lys	261	27·0	1·30	0·42	7·8	0·80

Table 2. Growth and production of volatile and non-volatile fatty acids in minimal medium containing 0·5 % yeast extract

The initial OD_{600} was 0·1. Values are means of three replicate cultures in early stationary phase (after 19·5 h for strain ZF2^T and 27·5 h for strain JW/Z-1^T). Values were corrected for the amounts of fatty acids present in the yeast extract at time zero.

Strain	Substrate	Growth (OD_{600})	Concentration of fatty acid produced (mM)					
			Acetate	Butyrate	Lactate	Propionate	Isobutyrate	Isovalerate
ZF2^T	Pyruvate (25 mM)	0·88	11·7	5·0	0·5	0·5	0·1	0·07
	None	0·45	1·8	1·9	0	0·4	0	0·05
JW/Z-1^T	Pyruvate (25 mM)	0·54	12·0	0·9	0·2	0·5	0·6	1·00
	None	0·20	2·0	1·4	0·2	0·3	0·3	0·50

not support growth. This was also described for strain JW/Z-1^T (Zhang *et al*., 1994) and is in contrast to the situation for many clostridia, which can use the energy-providing arginine deiminase pathway (Andreesen *et al*., 1989). Strain JW/Z-1^T converted arginine to ornithine (Zhang *et al*., 1994), perhaps because of an arginase reaction (Cunin *et al*., 1986). In the presence of glycine, ornithine was converted to alanine (Zhang *et al*., 1994), suggesting that ornithine might function as the electron donor, as it does in many amino-acid-utilizing clostridia (Andreesen *et al*., 1989).

The growth of strains ZF2^T and JW/Z-1^T on 25 mM pyruvate was compared (Table 2). In the case of ZF2^T, the optical density increased twofold relative to that for growth on minimal medium containing 0·5 % yeast extract but no pyruvate. Strain JW/Z-1^T was described as not growing with pyruvate as the substrate (Zhang *et al*., 1994). However, under the conditions applied here, pyruvate fermentation was also observed for strain JW/Z-1^T, although with a lower growth yield compared with strain ZF2^T. The main fermentation products of strain ZF2^T were acetate and butyrate, suggesting that pyruvate was oxidized to acetate, which, in part, might have served as an electron acceptor in the formation of butyrate. Strain JW/Z-1^T formed almost the same amount of acetate but smaller amounts of butyrate and larger amounts of isobutyrate and isovalerate (Table 2). The latter branched-chain fatty acids were probably formed from the respective amino acids present in the yeast extract (Andreesen *et al*., 1989). Electrons produced during oxidative metabolism were probably transferred to acetate to form butyrate, as has been described for *Clostridium aceticireducens* (Girbal *et al*., 1997). This is supported by the fact that neither strain produced hydrogen under these conditions.

Strain ZF2^T did not produce acids from glucose, mannitol, lactose, sucrose, maltose, salicin, xylose, arabinose, glycerol, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose or trehalose in the API 20A test system. Uric acid, hypoxanthine, xanthine, guanine and adenine (10 mM each) did not stimulate

Table 3. Characteristics of strain ZF2^T compared with those of strain JW/Z-1^T

+, Positive; −, negative. Cells of both strains were slightly curved, motile rods. The following characteristics tested positive for both strains: fermentation of pyruvate, glycine and lysine and of arginine plus glycine; formation of H_2S in SIM medium. Both contained the peptidoglycan type A1α, L-Lys-direct and the cellular fatty acids 14:0, 16:0, 18:0, 16:1 *cis*-9 and 18:1 *cis*-11 and the dimethyl acetals 16:0 and 18:0. The following characteristics tested negative for both strains: catalase, gelatin hydrolysis, nitrate reduction, fermentation of carbohydrates and purines and production of H_2.

Characteristic	Strain ZF2^T	Strain JW/Z-1^T*
Cell size (μm)	0·5–0·7 × 3·7–6·0	0·35–0·67 × 2·5–5·1
Spore shape	Oval	Round
Gram-stain reaction	+	−
G+C content (mol%)	34·0	35·5
Optimum temp. (°C)	37	33–34
Optimum pH	6·8–7·3	7·2–8·2
Indole production	−	+
Decarboxylation of:		
4-OHB	−	+
3,4-OHB	−	+

* Data were taken partly from Zhang *et al.* (1994).

growth of strain ZF2^T when added to the minimal medium in which selenite was present. This is in accordance with the data reported for JW/Z-1^T (Zhang *et al.*, 1994).

Biochemical properties. Strain ZF2^T did not hydrolyse aesculin or gelatin. No indole was produced from tryptophan and catalase and urease were absent. Nitrate was not reduced. Strain ZF2^T produced H_2S from thiosulfate when grown in SIM medium (Holdeman *et al.*, 1977).

Carboxylation and decarboxylation ability. *C. hydroxybenzoicum* JW/Z-1^T has been described as reversibly decarboxylating 3,4-OHB to catechol and 4-OHB to phenol (Zhang & Wiegel, 1990a, 1994). Carboxylation/decarboxylation was studied with the two strains in parallel. Cell suspensions of JW/Z-1^T formed carboxylated products from phenol or catechol in the 0·1 mM range, as described by Zhang & Wiegel (1994), and converted 2 mM 4-OHB and 1 mM 3,4-OHB completely to phenol and catechol within 50 and 85 h, respectively. However, strain ZF2^T neither carboxylated phenol or catechol nor decarboxylated 3,4-OHB or 4-OHB (under identical conditions).

Chemotaxonomic data. Purified cell walls of strain ZF2^T contained muramic acid, glucosamine and the amino acids lysine, glutamate and alanine in the molar ratio 1:1:2. The fingerprints of chromatograms of the partial hydrolysate were consistent with the peptidoglycan type A1α, L-Lys-direct. The lipid fatty acid composition of cells grown with 40 mM pyruvate was as follows: fatty acids, 12:0 (1·4%), 14:0 (13·2%), 16:1 *cis*-7 (4·4%), 16:1 *cis*-9 (10·9%), 16:0 (11·8%), 18:1 *cis*-9 (7·7%) and 18:0 (6·8%); aldehydes, 16:0 (1·0%) and 18:0 (1·0%); dimethyl acetals, 16:1 *cis*-9 (2·1%), 16:0 (5·0%), 18:1 *cis*-9 (5·5%), 18:1 *cis*-11 (3·9%) and 18:0 (6·5%). All other components were below 1%. The lipid fatty acid composition of *C. hydroxybenzoicum* JW/Z-1^T was investigated under identical conditions and was shown to possess the same spectrum of fatty acids, albeit in slightly different proportions (data not shown), e.g. fatty acids 14:0 (27·7%) and 16:0 (13·1%), and dimethyl acetals 16:0 (11·2%) were significantly increased at the expense of the unsaturated fatty acids 16:1 and 18:1, which were present in smaller proportions. Cluster analysis of the cellular fatty acids of both strains using MIDI software (Microbial ID) revealed 22·3 Euclidian distances, suggesting that the two strains belong to two different species within a genus. Quinones were not detected in strain ZF2^T. The G+C content of the DNA of strain ZF2^T (34·0 mol%) was slightly lower than that obtained for strain JW/Z-1^T (35·5 mol%) (Zhang *et al.*, 1994); both values were determined by HPLC measurement and are well within the range required for members of the same genus.

The characteristics determined for strain ZF2^T are summarized in Table 3 and compared with those of strain JW/Z-1^T.

Phylogeny of strain ZF2^T

The almost complete 16S rRNA gene sequence (1434 nt, 28–1492 bp, *E. coli* numbering; Brosius *et al.*, 1978) of strain ZF2^T was determined. Comparative sequence searches of the EMBL and GenBank databases revealed that the 16S rDNA sequence was related to those of the *Clostridium* subphylum of the Gram-positive bacteria (Fig. 2). Within this subphylum, the highest sequence identity, 94·4%, was obtained with the 16S rRNA sequence of *C. hydroxybenzoicum* JW/Z-1^T (Zhang *et al.*, 1994), whereas values for similarity to other members of the clostridial subphylum were much lower (below 91%). The sequences of strains ZF2^T and JW/Z-1^T form a common cluster, clearly separated from the *Peptostreptococcus* branch of clostridial cluster XIII and from the cluster XII organisms *Clostridium purinolyticum* and *Clostridium acidiurici* (Collins *et al.*, 1994). The latter two organisms (possessing 16S rDNA similarities to strains ZF2^T and JW/Z-1^T of 88·4–90·7%) are specialized for the degradation of purines (Andreesen *et al.*, 1989), which were not attacked by strains JW/Z-1^T (Zhang *et al.*, 1994) and ZF2^T.

Thus, strain ZF2^T is described as a novel species, *Sedimentibacter saalensis* sp. nov., within the novel genus *Sedimentibacter*, *Sedimentibacter hydroxybenzoicus* gen. nov., comb. nov., being the type species.

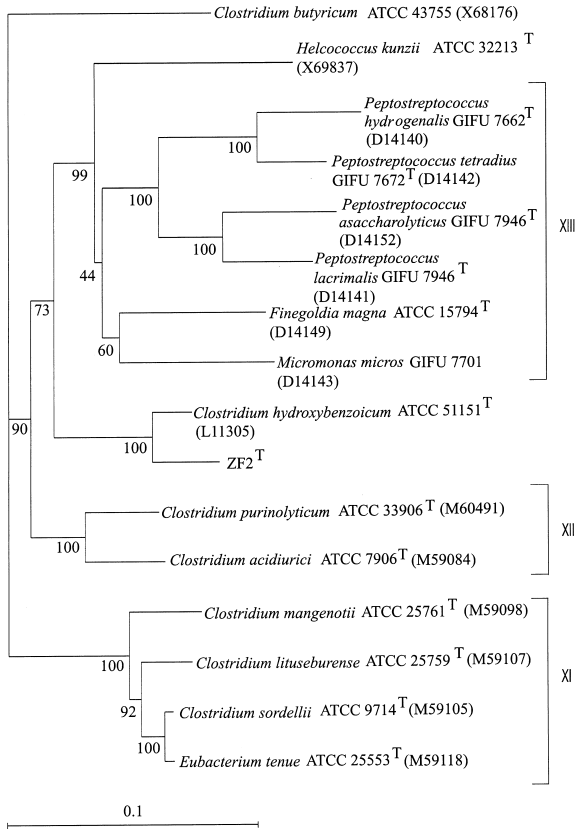

Fig. 2. Phylogenetic tree indicating the position of strain ZF2^T as a close relative of *Clostridium hydroxybenzoicum* JW/Z-1^T within the radiation of selected representatives of the low-G+C, Gram-positive bacteria. *Clostridium butyricum* (cluster I) was used as an outgroup. Percentage bootstrap values are based on 500 replicates and are indicated at the branching points. Accession numbers are given in parentheses. Roman numerals indicate the corresponding clostridial clusters according to Collins *et al.* (1994). Bar, 0·1 nucleotide substitutions per site.

Description of *Sedimentibacter* gen. nov.

Sedimentibacter (Se.di.men.ti.bac′ter. N.L. masc. n. *sedimentum* sediment; N.L. n. *bacter* masc. equivalent of Gr. neut. n. *bakterion* rod or staff; N.L. masc. n. *Sedimentibacter* rod from sediment, referring to its origin).

Cells are slightly curved rods with peritrichously arranged flagella. Spores might be formed. The Gram type positive cell wall is of the A1α, L-Lys-direct peptidoglycan type. Growth is strictly anaerobic and requires yeast extract and is supported by the fermentation of pyruvate or of amino acids in a Stickland-type reaction. H_2 is not produced. Carbohydrates are not fermented. The purines uric acid, adenine, hypoxanthine, guanine and xanthine are not utilized. Catalase and urease are absent. *Sedimentibacter* represents a novel line of descent in the branch of low-G+C, Gram-positive bacteria, according to 16S rDNA sequence analysis. Members of the genus *Sedimentibacter* can be distinguished from each other by their 16S rDNA sequences. The type species is *Sedimentibacter hydroxybenzoicus*.

Description of *Sedimentibacter hydroxybenzoicus* comb. nov.

Basonym: *Clostridium hydroxybenzoicum* (Zhang *et al.* 1994).

Properties of strain JW/Z-1^T are as described previously (Zhang & Wiegel, 1994), with the exception of the new information that flagellated cells can be observed occasionally and that pyruvate is fermented in yeast-extract-containing medium. The type strain is JW/Z-1^T (= ATCC 51151^T = DSM 7310^T).

Description of *Sedimentibacter saalensis* sp. nov.

Sedimentibacter saalensis (saa.len′sis. N.L. adj. *saalensis* referring to the German River Saale, from which the organism was isolated).

Cells are strictly anaerobic, slightly curved rods that are 0·5–0·7 μm in diameter and 3·7–6·0 μm long, occurring singly, in pairs or in chains of up to 12 cells. In addition, long filaments with no visible septation occur. Motile by peritrichously arranged flagella. Gram-staining of different growth phases gives a positive result. The cell wall type is A1α, L-Lys-direct. Oval spores (0·7 μm diameter) are formed on complex medium. The temperature range for growth is 14–40 °C, the optimum temperature being 37 °C. The pH range for growth is 5·8–8·0; there is a broad optimum range of pH 6·8–7·3. Requires yeast extract for growth. The addition of 25 mM pyruvate, 10 mM glycine or 10 mM L-lysine, or of combinations of glycine plus arginine or glycine plus lysine, to yeast-extract-containing medium supports growth. The major fermentation products during growth on pyruvate are acetate and butyrate, whereas propionate, lactate and traces of isobutyrate and isovalerate are minor products (in a medium containing 0·5% yeast extract); H_2 is not formed. No acid production from glucose, mannitol, lactose, sucrose, maltose, salicin, xylose, arabinose, glycerol, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose or trehalose. Uric acid, adenine, hypoxanthine, guanine and xanthine are not utilized. Tests for catalase and urease activity, indole production, reduction of nitrate to nitrite, gelatin liquefaction and hydrolysis of aesculin are negative. Forms sulfide. The major fatty acids are 14:0, 16:1 *cis*-9 and 16:0. The genomic DNA G+C content is 34·0 mol%. The type strain, ZF2^T (= DSM 13558^T = ATCC BAA-283^T), was isolated from freshwater sediment of the River Saale (Germany).

ACKNOWLEDGEMENTS

This work was supported by the Graduiertenförderung of Sachsen-Anhalt, Germany, and the Fonds der Chemischen Industrie. The authors are grateful to Gerd Hause (Biozentrum Halle) for electron microscopy and Hendrik

Ballerstedt (TNO Environment, Energy and Process Innovation, Apeldoorn, The Netherlands) for hydrogen measurement.

REFERENCES

Andreesen, J. R., Bahl, H. & Gottschalk, G. (1989). Introduction to the physiology and biochemistry of the genus *Clostridium*. In *Clostridia. Biotechnology Handbooks 3*, pp. 27–62. Edited by N. P. Minton & D. L. Clarke. New York: Plenum.

Breitenstein, A., Saano, A., Salkinoja-Salonen, M., Andreesen, J. R. & Lechner, U. (2001). Analysis of a 2,4,6-trichlorophenol-dehalogenating enrichment culture and isolation of the dehalogenating member *Desulfitobacterium frappieri* strain TCP-A. *Arch Microbiol* **175**, 133–142.

Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.

Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H. & Farrow, J. A. E. (1994). The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* **44**, 812–826.

Cunin, R., Glansdorff, N., Pierard, A. & Stalon, V. (1986). Biosynthesis and metabolism of arginine in bacteria. *Microbiol Rev* **50**, 314–352.

Felsenstein, J. (1993). PHYLIP: phylogenetic inference package, version 3.5. Seattle: University of Washington.

Gerhardt, P. (1981). *Manual of Methods for General Bacteriology*. Washington, DC: American Society for Microbiology.

Girbal, L., Örlygsson, J., Reinders, B. J. & Gottschal, J. C. (1997). Why does *Clostridium acetireducens* not use interspecies hydrogen transfer for growth on leucine? *Curr Microbiol* **35**, 155–160.

He, Z. & Wiegel, J. (1995). Purification and characterization of an oxygen-sensitive reversible 4-hydroxybenzoate decarboxylase from *Clostridium hydroxybenzoicum*. *Eur J Biochem* **229**, 77–82.

He, Z. & Wiegel, J. (1996). Purification and characterization of an oxygen-sensitive, reversible 3,4-dihydroxybenzoate decarboxylase from *Clostridium hydroxybenzoicum*. *J Bacteriol* **178**, 3539–3543.

Holdeman, L. V., Cato, E. P. & Moore, W. E. C. (1977). *Anaerobe Laboratory Manual*, 4th edn. Blacksburg, VA: Virginia Polytechnic Institute and State University Anaerobe Laboratory.

Holliger, C., Schraa, G., Stams, A. J. M. & Zehnder, A. J. B. (1992). Enrichment and properties of an anaerobic mixed culture reductively dechlorinating 1,2,3-trichlorobenzene to 1,3-dichlorobenzene. *Appl Environ Microbiol* **58**, 1636–1644.

Holliger, C., Schraa, G., Stams, A. J. M. & Zehnder, A. J. B. (1993). A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. *Appl Environ Microbiol* **59**, 2991–2997.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Lechner, U., Baumbach, R., Becker, D., Kitunen, V., Auling, G. & Salkinoja-Salonen, M. (1995). Degradation of 4-chloro-2-methylphenol by an activated sludge isolate and its taxonomic description. *Biodegradation* **6**, 83–92.

Nanninga, H. J. & Gottschal, J. C. (1985). Amino acid fermentation and hydrogen transfer in mixed cultures. *FEMS Microbiol Ecol* **31**, 261–269.

Pearson, W. R. (1990). Rapid and sensitive sequence comparison with FASTP and FASTA. *Methods Enzymol* **183**, 63–98.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

Seto, B. (1980). The Stickland reaction. In *Diversity in Bacterial Respiratory Systems*, pp. 49–64. Edited by C. J. Knowles. Boca Raton, FL: CRC Press.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Väisänen, O. & Salkinoja-Salonen, M. (1989). Use of phage typing and fatty acid analysis for the identification of bacilli isolated from food packaging paper and board machines. *Syst Appl Microbiol* **12**, 103–111.

Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.

Zhang, X. & Wiegel, J. (1990a). Isolation and partial characterization of a *Clostridium* sp. transforming *p*-hydroxybenzoate and 3,4-dihydroxybenzoate and producing phenols as the final transformation products. *Microb Ecol* **20**, 103–122.

Zhang, X. & Wiegel, J. (1990b). Sequential anaerobic degradation of 2,4-dichlorophenol in freshwater sediments. *Appl Environ Microbiol* **56**, 1119–1127.

Zhang, X. & Wiegel, J. (1992). The anaerobic degradation of 3-chloro-4-hydroxybenzoate in freshwater sediment proceeds via either chlorophenol or hydroxybenzoate to phenol and subsequently to benzoate. *Appl Environ Microbiol* **58**, 3580–3585.

Zhang, X. & Wiegel, J. (1994). Reversible conversion of 4-hydroxybenzoate and phenol by *Clostridium hydroxybenzoicum*. *Appl Environ Microbiol* **60**, 4182–4185.

Zhang, X., Mandelco, L. & Wiegel, J. (1994). *Clostridium hydroxybenzoicum* sp. nov., an amino acid-utilizing, hydroxybenzoate-decarboxylating bacterium isolated from methanogenic freshwater pond sediment. *Int J Syst Bacteriol* **44**, 214–222.