Propionicimonas paludicola gen. nov., sp. nov., a novel facultatively anaerobic, Gram-positive, propionate-producing bacterium isolated from plant residue in irrigated rice-field soil

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Two propionate-producing strains (Wd^T and Wf) that were isolated anaerobically from plant residue of irrigated rice-field soil in Japan were characterized phenotypically and phylogenetically. The growth rate of strain Wd^T was very slow in basal medium, but both growth and propionate production were stimulated significantly by the addition of cyanocobalamin. Strain Wf grew well in basal medium and produced substantial amounts of fermentation products, including propionate. Other phenotypic and phylogenetic characteristics of the two isolates were almost identical. Both were facultatively anaerobic, but much better growth was observed under anaerobic conditions. Cells were Gram-positive, non-motile, non-spore-forming and pleomorphic rods with irregular V- or crescent-shaped cell arrangements. Fermentation products from glucose in the presence of excess cyanocobalamin were acetate, lactate, a small amount of succinate and CO₂, in addition to propionate. Both oxidase and catalase activities were negative. The strains possessed *meso*-diaminopimelic acid in their peptidoglycan and their major cellular fatty acids were C_{13:0}, anteiso-C_{15:0} and C_{15:0}. The isolates had high genomic DNA G + C contents (68.7 and 67.4 mol%, respectively). Menaquinones MK-9(H₄) and MK-10(H₄) were the predominant respiratory quinones. Phylogenetic analysis based on 16S rDNA sequences placed both strains in the *Actinobacteria*, with *Micropruina glycogenica* as their closest relative (sequence similarity values of 95.8 and 95.7 %, respectively). *Micrococcus phosphovor* and *Friedmanniella antarctica* were also related closely to the isolates. As their morphological, physiological and chemotaxonomic characteristics were distinctly different from those of any related species, *Propionicimonas paludicola* gen. nov., sp. nov. is proposed to accommodate these strains. The type strain of the novel species is Wd^T (=JCM 11933^T = DSM 15597^T).

INTRODUCTION

Rice serves as the principal food for nearly half of the world's population; about 90 % of the total area of rice fields in the world is found in Asia. Rice fields are differentiated into four ecosystems: irrigated, rain-fed,

deepwater and upland fields. The former three soils (about 90 % of the global area) (Wassmann *et al.*, 2000b) become anoxic after flooding and the methanogenic microbial community develops with active degradation of organic matter (Takai, 1970; Seiler *et al.*, 1984; Glissmann & Conrad, 2000). Thus, the rice-field ecosystem is considered to be one of the major sources of global atmospheric methane (Khalil, 2000). Many studies have investigated anaerobic microbial communities in anoxic rice-field soil (Janssen *et al.*, 1997; Großkopf *et al.*, 1998; Henckel *et al.*, 1999; Hengstmann *et al.*, 1999) and novel anaerobic micro-organisms have been isolated (Rajagopal *et al.*, 1988; Asakawa *et al.*, 1995; Chin *et al.*, 1998, 1999; Rosencrantz *et al.*, 1999; Wind *et al.*, 1999; Satoh *et al.*, 2002).

Japan is located in the rice-producing district of Asia and rice cultivation using irrigated fields has been practiced for

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Abbreviations: DAP, diaminopimelic acid; RS plot, rice-straw plot; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains Wd^T and Wf are AB078858 and AB078859, respectively.

Micrographs of strains Wd^T and Wf are available as supplementary material in IJSEM Online.

a long time. Rice straw and remaining plant residue, such as rice stubble and roots, are ploughed into the soil at the beginning of the growing season to act as organic fertilizers. Such additions often result in an increase in methane emission rates from the fields (Yagi & Minami, 1990; Watanabe *et al.*, 1995; Wassman *et al.*, 2000a). In a previous study, we reported the enumeration and isolation of anaerobic fermentative bacteria from rice-plant residue ploughed into the soil of a Japanese rice field (Akasaka *et al.*, 2003). Of the 47 bacterial isolates that were analysed by means of 16S rDNA sequence, five strains in the *Actinobacteria* commonly produced propionate from glucose. This group was phylogenetically distinct and was tentatively named the 'propionate-producing *Actinobacteria* group'. We have investigated the phenotypic characteristics of representative strains of this group and from the results obtained, we propose a novel genus and species, *Propionicimonas paludicola* gen. nov., sp. nov., for these isolates.

METHODS

Bacterial strains. Strains W1, Wd^T (=JCM 11933^T=DSM 15597^T), Wf (=JCM 11934=DSM 15598), K2 and K5 in the 'propionate-producing *Actinobacteria* group' were isolated previously from plant-residue samples collected from the rice straw (RS) plot in the Shonai Branch of the Yamagata Agricultural Experimental Station (Fujishima-machi, Yamagata, Japan) during the rice-growing season (Akasaka *et al.*, 2003). Rice straw had been applied annually to this plot for more than 20 years. Cultivation practices for rice plants and characteristics of the soil were described previously (Ueki *et al.*, 1999, 2000; Kaku *et al.*, 2000; Hattori *et al.*, 2001, 2002). Strains were isolated by selecting individual colonies after counting anaerobic bacteria on plant residue collected from the soil. Methods used have been described previously (Hungate, 1966; Holdeman *et al.*, 1977; Akasaka *et al.*, 2003). Strains W1, Wd^T and Wf were isolated from rice-straw samples, whereas strains K2 and K5 were derived from rice stubble and roots. Strains W1, K2 and K5 were isolated in May, during the flooding period of the field, and the other strains were isolated in August, during the intermittent irrigation period (Akasaka *et al.*, 2003).

Culture conditions. Strains were cultivated anaerobically at 30 °C by using peptone/yeast extract (PY) medium as basal medium with oxygen-free, 95% N₂/5% CO₂ mixed gas as the headspace, as described previously (Akasaka *et al.*, 2003). PY medium supplemented with (l⁻¹) 0.25 g each of glucose, cellobiose, maltose and soluble starch, as well as 50 ml plant residue extract (RE) and 15 g agar (Difco), was designated PY4SR agar and used for maintenance of the strains in agar slants. Plant residue collected from the RS plot during the flooding period was autoclaved (120 °C for 30 min) with a fivefold amount (wet-weight basis) of deionized H₂O and the supernatant obtained after centrifugation was used as RE for the provision of growth factors. PY liquid medium supplemented with 10 g glucose l⁻¹ (PYG medium) was usually used for cultivation of the cells. For PY liquid medium, cyanocobalamin (cobalamin) (vitamin B₁₂; Kanto Chemical) was added at 50 µg l⁻¹ as a growth factor as described below, unless stated otherwise. Media were adjusted to pH 7.3 with 1 M NaOH. For estimation of optimal growth conditions, strains were cultivated at different temperatures (10–50 °C), pH values (4.6–7.5 in PYG medium) and NaCl concentrations (0–5.0% in PYG medium). Growth in liquid medium was monitored by changes in OD₆₆₀.

Morphological characteristics. Morphology of cells was observed by Gram-staining, phase-contrast microscopy and transmission electron microscopy (TEM). For TEM, a centrifuged cell pellet was fixed with 5% (v/v) glutaraldehyde and 1% (v/v) osmium tetroxide. Ultrathin sections of the sample, embedded in epoxy resin (Kushida, 1980), were prepared with a Reichert ultramicrotome. Samples were stained with uranyl acetate and lead citrate and examined with a Hitachi H-7000 transmission electron microscope.

Physiological and biochemical characterization. Growth of strains under aerobic conditions was examined by plate culture on nutrient agar (Nissui Pharmacy) and PY4SR agar. Spore formation was assessed after Gram-staining, as well as under phase-contrast microscopy and by subsequent growth in PYG medium of cells exposed to 80 °C for 10 min. Oxidase, catalase and nitrate-reducing activities were determined according to methods described previously (Satoh *et al.*, 2002; Akasaka *et al.*, 2003). Utilization of carbon sources was tested in PY liquid medium with each substrate added at 10 g l⁻¹ (for mono-, di-, tri- and polysaccharides and sugar alcohols) or 30 mM (for alcohols and organic acids). Fermentation products were analysed as described previously (Ueki *et al.*, 1986; Akasaka *et al.*, 2003). Glucose concentrations in the media were measured with a D-glucose kit (Boehringer Mannheim) according to the manufacturer's protocol. All phenotypic characterizations, including the following chemotaxonomic analyses, were carried out in duplicate.

Quinone, fatty acid and cell-wall analyses. Quinones were extracted from freeze-dried cells with chloroform/methanol (2:1, v/v) and n-hexane. Extracts were purified by using Sep-Pak Plus (Waters) and analysed by reverse-phase HPLC for identification (Tamaoka *et al.*, 1983). Whole-cell fatty acids were converted to methyl esters by treatment with anhydrous methanolic HCl (Ueki & Suto, 1979; Komagata & Suzuki, 1987). Methyl esters were extracted with n-hexane and analysed by using a GC-mass spectrometer (M7200A GC/3DQMS; Hitachi) equipped with a DB-5ms capillary column coated with 5% phenylmethylpolysiloxane at a thickness of 250 nm (Hanada *et al.*, 2002). The presence of diaminopimelic acid (DAP) isomers in the cell-wall peptidoglycan was determined by TLC (no. 5716; Merck) after hydrolysis with 6 M HCl at 100 °C for 18 h (Komagata & Suzuki, 1987).

DNA base composition. Genomic DNA was extracted according to a method described previously (Kamagata & Mikami, 1991). Extracted DNA was digested with P1 nuclease by using a Yamasa GC kit (Yamasa Shoyu) and its G+C content was measured by HPLC (model LC-6A system; Shimadzu) equipped with a CLC-ODS column (6 × 150 mm; Shimadzu).

16S rDNA sequence and phylogenetic analysis. 16S rDNA of strains was extracted according to a method described previously (Akasaka *et al.*, 2003) and amplified by PCR. PCR-amplified 16S rDNA was sequenced by using a Thermo Sequenase Primer Cycle Sequencing kit (Amersham Biosciences) and a model 4000L DNA sequencer (LI-COR). Multiple alignments of isolates' sequences with reference sequences in GenBank were performed with the BLAST program (Altschul *et al.*, 1997). A phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987) by using the CLUSTAL W program (Thompson *et al.*, 1994). All gaps and unidentified base positions in the alignments were excluded before assemblages.

RESULTS AND DISCUSSION

Cobalamin requirement of the isolates

The five isolates have 16S rDNA similarity values of 98.0–99.7% to each other and all are facultatively anaerobic,

Gram-positive, irregular rods with a similar morphology (Akasaka *et al.*, 2003). However, the five isolates could be divided into two groups according to their growth in PYG medium. Four isolates (W1, Wd^T, K2 and K5) showed extremely slow growth rates without addition of any growth factors, whereas strain Wf grew well. For slow-growing isolates, 16S rDNA similarities among three strains (W1, Wd^T and K5) were 99.4–99.7 %, whereas those with strain K2 were lower (98.0–98.3 %).

By screening possible growth factors, addition of cobalamin to PYG medium markedly stimulated the growth of the four slow-growing strains. Table 1 shows the effects of cobalamin addition on growth (final OD₆₆₀) and fermentation end products. In PYG medium without added cobalamin, all isolates except strain Wf formed small amounts of products, with lactate as the predominant one. In the presence of added cobalamin, production of propionate was stimulated together with acetate, whilst lactate changed to a minor product. Although strain Wf produced large amounts of fatty acids in PYG medium with no growth factor addition, cobalamin also stimulated its growth and production of propionate, again with a concomitant suppression of lactate production. All isolates produced CO₂ as well as fatty acids, but hydrogen production was not observed.

To further investigate the effects of cobalamin on slow-growing isolates, strain Wd^T was cultivated in the presence of different amounts of cobalamin in PYG medium and time-courses for growth and end products were examined (data not shown). Without added cobalamin, strain Wd^T grew very slowly, showing an arithmetic increase in OD. With addition of 1 µg cobalamin l⁻¹, its growth improved markedly and lactate was produced as the predominant fatty acid from the onset of fermentation. With 20 µg

cobalamin l⁻¹, propionate accumulated rapidly to a much higher concentration than that of any other product, whilst lactate production was suppressed significantly compared to that in the presence of 1 µg cobalamin l⁻¹. The other three slow-growing strains (W1, K2 and K5) showed similar changes in the time-course of growth, depending on cobalamin concentrations.

Strains Wd^T and Wf were selected and characterized in more detail. In later experiments, both isolates were cultured in media that contained an excess amount of cobalamin (50 µg l⁻¹).

Colony and cell morphologies

Strains Wd^T and Wf grew anaerobically on PY4SR agar and produced white colonies of 2–3 mm diameter after 2–3 days incubation. Cells were Gram-positive, pleomorphic, often slightly curved rods, 0.4–0.5 µm in diameter and 1.4–2.2 µm in length. Irregular V- or crescent-shaped cell arrangements were observed frequently (see Supplementary Figs a and c, available as supplementary material in IJSEM Online). Cells were non-motile under phase-contrast microscopy. TEM showed that cells had a cell-wall structure typical of Gram-positive bacteria (Supplementary Figs b and d). Electron-translucent regions in the cells revealed the presence of intracellular storage compounds. Spore formation was not observed and cells treated at 80 °C for 10 min did not grow.

Physiological and biochemical characteristics

Strains Wd^T and Wf had very similar physiological and biochemical characteristics. Both grew weakly in air on both PY4SR and nutrient agar, but much better growth was observed under anaerobic conditions. Both utilized arabinose, xylose, fructose, galactose, glucose, mannose,

Table 1. Effects of addition of cyanocobalamin on growth and fermentation products of isolates in the propionate-producing group

All values were determined after 5 days incubation. –, Without cyanocobalamin added; +, with cyanocobalamin added; ND, not detected.

Strain	Cyanocobalamin*	OD ₆₆₀	pH	Fatty acids produced (mmol l ⁻¹)			
				Acetate	Propionate	Lactate	Succinate
W1	–	0.28	6.22	0.54	0.80	2.29	0.11
	+	2.21	4.60	8.12	17.5	0.49	ND
Wd ^T	–	0.71	5.70	2.46	0.59	4.26	2.45
	+	2.04	4.42	8.06	17.7	3.21	0.89
K2	–	0.53	5.67	0.92	0.74	4.27	0.32
	+	2.11	4.52	9.47	19.0	ND	ND
K5	–	0.29	6.26	1.29	0.64	3.95	0.36
	+	1.63	4.48	6.49	20.5	1.69	0.38
Wf	–	1.87	4.35	8.12	12.3	15.2	0.53
	+	2.40	4.34	13.8	24.7	6.01	0.27

*Amounts of cyanocobalamin added: strains Wd^T, K5 and Wf, 10 µg l⁻¹; strains W1 and K2, 50 µg l⁻¹.

cellobiose, maltose, sucrose, trehalose, glycerol and mannitol as carbon sources, whereas utilization of ribose and lactose was poor. Both strains grew on pyruvate and lactate; very weak growth was observed with malate, fumarate and succinate. Neither strain utilized fucose, rhamnose, sorbose, melibiose, melezitose, raffinose, cellulose, glycogen, soluble starch, xylan, adonitol, dulcitol, erythritol, inositol, sorbitol, ethanol, methanol or propanol as growth substrates. Other physiological characteristics of the two isolates are shown in Table 2.

Chemotaxonomic characteristics

Strains Wd^T and Wf also had similar chemotaxonomic characteristics. The major cellular fatty acids in both were C_{13:0} (11.8% for strain Wd^T and 10.5% for strain Wf), anteiso-C_{15:0} (31.0 and 39.6%, respectively) and C_{15:0} (40.6 and 34.1%, respectively). Branched C_{14:0} (5.1 and 5.5%, respectively), C_{14:0} (1.5 and 2.8%, respectively), C_{16:0} (1.7 and 1.4%, respectively) and C_{17:0} (4.1 and 1.5%, respectively) were also detected as minor components. Major respiratory quinones of both strains were MK-9(H₄) and MK-10(H₄), with peak area ratios of 59:41 for strain Wd^T and 77:23 for strain Wf. Other chemotaxonomic features are given in Table 2.

Phylogenetic analysis based on 16S rDNA sequences

A phylogenetic tree based on 16S rDNA sequences was constructed (Fig. 1). Sequence similarity between the 16S rDNA of strains Wd^T and Wf was 99.1%; both strains formed a cluster close to the genera *Micropruina* (Shintani *et al.*, 2000), *Microlunatus* (Nakamura *et al.*, 1995) and *Friedmanniella* (Schumann *et al.*, 1997) in the *Actinobacteria*. The closest relative of both strains was *Micropruina glycogenica* (Shintani *et al.*, 2000), with sequence similarities of 95.8 and 95.7%, respectively. The closest relative to strain Wd^T found in GenBank was environmental clone SJA-181 (Fig. 1), which was derived from an anaerobic microbial consortium in a trichlorobenzene-transforming bioreactor (von Wintzingerode *et al.*, 1999), with 16S rDNA sequence similarity of 98.3%.

The five strains used in this study were isolated from different plant residues at different sampling times and were thus considered to be part of a ubiquitous anaerobic bacterial group that occurs on plant residue in rice-field soil. Their population density was estimated to be at least of the order of 10⁸–10⁹ c.f.u. (g dry wt plant residue)⁻¹ (Akasaka *et al.*, 2003).

Propionate is an important intermediate in methanogenic decomposition of organic matter in anoxic rice-field soils (Dolfing, 1988; Glissmann & Conrad, 2000; Chin *et al.*, 2001; Chin & Janssen, 2002). The striking effects of cobalamin on growth and fermentation of the isolates suggest that survival of the group on plant residue at a rather high population density depends on the availability

of cobalamin from the environment. A similar requirement for cobalamin has been reported in a ruminal propionate-producing bacterium, *Prevotella ruminicola* (Strobel, 1992).

Many propionate-producing bacteria produce propionate through conversion of succinyl-CoA to methylmalonyl-CoA, which is catalysed by a vitamin B₁₂-dependent methylmalonyl-CoA isomerase (Roth *et al.*, 1996). Stimulation of propionate production by added cobalamin suggests that this pathway is present in the isolates described here. Many other anaerobes, including methanogens and acetogens, synthesize cobalamin endogenously for their own use and several reactions, including reductive dehalogenation of chlorinated compounds, depend on cobalamin or vitamin B₁₂ (Krone *et al.*, 1989; Stupperich *et al.*, 1990; White & Zhou, 1993; Roth *et al.*, 1996). Thus, cobalamin synthesized by various microbial groups may be released into the environment, where it may support the growth of cobalamin-requiring bacteria.

Phenotypic and phylogenetic characteristics of strains Wd^T and Wf were almost the same, except for a requirement for cobalamin for their optimal growth. *Micropruina glycogenica* was the most closely related known species to both isolates, based on 16S rDNA sequence as described above, followed by *Microlunatus phosphovorus* (Nakamura *et al.*, 1995) (similarity of 94.7–95.8%) and *Friedmanniella antarctica* (Schumann *et al.*, 1997) (similarity of 93.1%), all in the family *Propionibacteriaceae* within the *Actinobacteria* (Garrity & Holt, 2001) (Fig. 1). These data agree with the base compositions of their genomic DNA, which indicate that they both belong to the high-G+C Gram-positive bacteria. However, many differences exist between the characteristics of these isolates and those of other closely related species (Table 2).

Their cellular morphologies show that the isolates are irregular rods, whereas other related species have spherical cells. The isolates are facultatively anaerobic and catalase-negative and grow well under anoxic conditions, whereas the other three species are strictly aerobic and catalase-positive. Both *Micropruina glycogenica* and *Microlunatus phosphovorus* have oxidase and nitrate-reducing activities, but the novel isolates do not. Furthermore, their cellular fatty acid composition profiles are different from those of their close relatives: major fatty acids of the isolates are C_{13:0}, anteiso-C_{15:0} and C_{15:0}, whereas those of the other three are iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0} or C_{16:0}. Fatty acids C_{13:0} and C_{15:0} are absent from, or only minor components in, other related species. Furthermore, most predominant fatty acids in their relatives, except for anteiso-C_{15:0}, are absent from, or only minor components in, the isolates described here. In addition to MK-9(H₄), both isolates contain MK-10(H₄) as a predominant menaquinone, whereas the other three species do not (Nakamura *et al.*, 1995; Schumann *et al.*, 1997; Maszenan *et al.*, 1999; Shintani *et al.*, 2000). Among their close relatives, only *Micropruina glycogenica* has meso-DAP in the peptidoglycan of the cell wall (Shintani *et al.*, 2000).

Table 2. Phenotypic characteristics of strains Wd^T and Wf and related species in the *Actinobacteria*

Taxa: 1, Wd^T; 2, Wf; 3, *Micropruina glycogenica* [data from Shintani *et al.* (2000)]; 4, *Microlunatus phosphovor* [data from Nakamura *et al.* (1995)]; 5, *Friedmanniella antarctica* [data from Schumann *et al.* (1997)]. All organisms are Gram-positive, non-motile and non-spore-forming. +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5
Habitat	Plant residue in paddy soil	Plant residue in paddy soil	Activated sludge reactor	Activated sludge reactor	Antarctic sandstone
Cell shape	Irregular rods	Irregular rods	Cocci	Cocci	Cocci
Cell size (µm)	0.4–0.5 × 1.8–2.0	0.4–0.5 × 1.4–2.2	0.5–2.2	0.8–2.0	0.5–2.2
Colour of colony	White	White	White	Cream	Orange
Growth temperature (°C):					
Optimum	35	35	30	25–30	22
Range	10–40	10–40	20–30	5–35	9–25
Growth pH:					
Optimum	6.5	6.5	7.0	7.0	6.0–7.2
Range	4.5–7.5	4.5–7.5	6.0–8.0	5.0–9.0	5.1–8.7
NaCl concentration range for growth (%)	0–2	0–2	0–3	0–6	0–2
O ₂ requirement	Facultative anaerobe	Facultative anaerobe	Aerobe	Aerobe	Aerobe
Cobalamin requirement	+	–	ND	ND	ND
Oxidase	–	–	+	+	–
Catalase	–	–	+	+	+
Nitrate reduction	–	–	+	+	–
Acid production from glucose	+	+	+	+	–
DNA G + C content (mol%)	68.7	67.4	70.5	67.9	73.0
Major quinone	MK-9(H ₄), MK-10(H ₄)	MK-9(H ₄), MK-10(H ₄)	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄)
Major cellular fatty acids	C _{13:0} , anteiso-C _{15:0} , C _{15:0}	C _{13:0} , anteiso-C _{15:0} , C _{15:0}	iso-C _{14:0} , anteiso-C _{15:0} , iso-C _{16:0} , C _{16:0}	iso-C _{15:0} , anteiso-C _{15:0} , iso-C _{16:0}	iso-C _{15:0} , anteiso-C _{15:0}
Peptidoglycan	meso-DAP	meso-DAP	meso-DAP	LL-DAP	LL-DAP

Fig. 1. Neighbour-joining tree showing the phylogenetic relationship of strains Wd^T, Wf and related species in the *Actinobacteria*, based on 16S rDNA sequences. Bootstrap values (%) are shown for nodes with >50% support in bootstrap analysis of 1000 replicates. The sequence of *Escherichia coli*, which belongs to the γ -*Proteobacteria* (Garrity & Holt, 2001), was used as the outgroup. Bar, 1% estimated difference in nucleotide sequence position.

The isolates produce propionate in the presence of excess cobalamin and their fermentation patterns resemble those of *Propionibacterium* spp. (Cummins & Johnson, 1986) and *Propioniferax* spp. (Yokota *et al.*, 1994; Pitcher & Collins, 1991) in the family *Propionibacteriaceae* (Garrity & Holt, 2001). Despite this, they are only distantly related to *Propionibacterium propionicum* and *Propioniferax innocua*, based on 16S rDNA sequence data (similarities of 91.5–92.0 and 90.0%, respectively) (Fig. 1). Furthermore, both *Propionibacterium propionicum* and *Propioniferax innocua* contain LL-DAP in their cell-wall peptidoglycan and MK-9(H₄) as the sole menaquinone (Maszenan *et al.*, 1999). The predominant cellular fatty acids in *Propioniferax innocua* are also iso-C_{15:0} and anteiso-C_{15:0} (Schumann *et al.*, 1997).

On the basis of both the phylogenetic and phenotypic characteristics described above, we conclude that Wd^T and Wf should be assigned to the same species within a novel genus, *Propionicismonas* gen. nov., with *Propionicismonas paludicola* sp. nov. as the type species.

Description of *Propionicismonas* gen. nov.

Propionicismonas (Pro.pi.on.i.ci.mo'nas. N.L. n. *acidum* propionic acid; Gr. fem. n. *monas* a unit, monad; N.L. fem. n. *Propionicismonas* propionic acid-producing monad).

Cells are Gram-positive, irregular rods. Facultatively anaerobic chemo-organotroph. Non-motile and non-spore-forming. Mesophilic. Oxidase-, catalase- and nitrate reduction-negative. Propionate is produced anaerobically from glucose. Cell-wall peptidoglycan contains *meso*-DAP; major cellular fatty acids are C_{13:0}, anteiso-C_{15:0} and C_{15:0}. MK-9(H₄) and MK-10(H₄) are the major quinones. Genomic DNA G+C content is 68.7 mol%. On the basis of 16S rDNA sequences, the bacterium belongs to the class *Actinobacteria*. The genera *Micropruina*, *Microlunatus* and *Friedmanniella* are related most closely to this genus. The type species is *Propionicismonas paludicola*.

Description of *Propionicismonas paludicola* sp. nov.

Propionicismonas paludicola (pa.lu.di'co.la. L. n. *palus*, -udis swamp, marsh; L. suff. -cola derived from *incola* inhabitant, dweller; N.L. masc. n. *paludicola* an inhabitant of swamps).

Gram-positive, irregular, often slightly curved rods, 0.4–0.5 µm in diameter and 1.8–2.0 µm in length and frequently arranged in irregular V- or crescent-shapes. Optimal growth occurs at 35 °C and pH 6.5. Grows in the presence of up to 2.0% (w/v) NaCl. Cells produce acetate, propionate, lactate, succinate and CO₂ anaerobically from glucose. Growth and production of propionate are both stimulated by addition of cyanocobalamin to the medium. Cells grow on arabinose, xylose, fructose, galactose, glucose, mannose, cellobiose, maltose, sucrose, trehalose, glycerol, mannitol, pyruvate and lactate, but growth is scarce on ribose, lactose, malate, fumarate and succinate. Fucose, rhamnose, sorbose, melibiose, melezitose, raffinose, cellulose, glycogen, soluble starch, xylan, adonitol, dulcitol, erythritol, inositol, sorbitol, ethanol, methanol and propanol are not utilized. Chemo-taxonomic characteristics of the species are the same as those described for the genus.

The type strain is Wd^T (=JCM 11933^T=DSM 15597^T). Isolated from rice-plant residue ploughed into the soil of a Japanese rice field.

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