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Ruminococcus hydrogenotrophicus sp. nov., a new H_2/CO_2-utilizing acetogenic bacterium isolated from human feces

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Abstract A new H_2/CO_2-utilizing acetogenic bacterium was isolated from the feces of a non-methane-excreting human subject. The two strains S5a33 and S5a36 were strictly anaerobic, gram-positive, non-sporulating coccobacilli. The isolates grew autotrophically by metabolizing H_2/CO_2 to form acetate as sole metabolite and were also able to grow heterotrophically on a variety of organic compounds. The major end product of glucose and fructose fermentation was acetate; the strains also formed ethanol, lactate and, to a lesser extent, isobutyrate and isovalerate. The G+C content of DNA of strain S5a33 was 45.2 mol%. 16S rRNA gene sequencing demonstrated that the two acetogenic isolates were phylogenetically identical and represent a new subline within *Clostridium* cluster XIVa. Based on phenotypic and phylogenetic considerations, a new species, *Ruminococcus hydrogenotrophicus*, is proposed. The type strain of *R. hydrogenotrophicus* is S5a33 (DSM 10507). Furthermore, H_2/CO_2 acetogenesis appeared to be a common property of most of the species phylogenetically closely related to strain S5a33 (*Clostridium coccoides*, *Ruminococcus hansenii*, and *Ruminococcus productus*).

Key words *Ruminococcus hydrogenotrophicus* · Human feces · Anaerobic bacteria · Acetogenesis · H_2/CO_2-utilizing acetogens · Phylogeny

Introduction

Reductive acetogenesis is a microbial metabolic pathway leading to the synthesis of acetate from CO_2 or other one-carbon compounds. This process has been demonstrated in various anoxic ecosystems such as soils, sediments, muds, sewage (Dolfing 1988) and the gastrointestinal tract of rodents (Prins and Lankhorst 1977), pigs (De Graeve et al. 1994), ruminants (Mackie and Bryant 1994), termites (Breznak 1994), and humans (Wolin and Miller 1994; Bernalier et al. 1996a). Whereas methanogenesis appears to be the predominant pathway of H_2-recycling in various animal guts, H_2/CO_2-acetogenesis may be an important mechanism of H_2-uptake in the hindgut of wood-feeding termites and in the colon of some human individuals (Breznak 1994; Wolin and Miller 1994; Bernalier et al. 1996a). H_2/CO_2-acetogenesis is of great interest for human nutrition and health by decreasing the total gas volume in the colon and by producing a non-gaseous metabolite that is an energy source for eukaryotic cells. While 33% of the total acetate produced in the termite gut may be formed by H_2/CO_2-acetogenesis (Breznak and Kane 1990), Wolin and Miller (1983) have estimated that this reductive pathway could be responsible for 27–30% of the total acetate production in the colon of human individuals harboring low numbers of methanogenic Archaea (30–50% of the European population). Furthermore, it has been demonstrated that the fecal microflora of non-methane-producing subjects principally metabolized $H_2 + CO_2$ to acetate, whereas the methane-producing ones mainly used H_2 and CO_2 to form methane (Lajoie et al. 1988; Bernalier et al. 1996a). A negative correlation between the populations of methanogens and H_2/CO_2-utilizing acetogens was also observed in human colon (Doré et al. 1995b).

Whereas several H_2-consuming acetogenic species have been isolated from various ecosystems such as sewage, sludge, sediments (Dolfing 1988), and the digestive tract of termites (Breznak 1994) or ruminants (Sharak-Genthner and Bryant 1982; Greening and Leedle 1989; Rieu-Lesme et al. 1995), the H_2/CO_2-utilizing acetogenic population of the human colon has not been studied extensively. A few acetogenic strains have been isolated recently (Wolin and Miller 1993) but were reportedly poor H_2-consumers. To learn more about the diversity and the

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role in H_2-reutilization of the H_2/CO_2-utilizing acetogenic population of the human colon, Bernalier et al. (1996b) have isolated approximately 20 acetogenic strains from fecal samples of non-methane-excreting subjects. In the present paper, we report the description of a new species of these H_2/CO_2-utilizing acetogens from the human colon, *Ruminococcus hydrogenotrophicus*.

Materials and methods

Source of organisms and isolation of bacteria

Acetogenic strains were isolated from feces of a non-methane-excreting human subject. Strains S5a33 and S5a36, which showed similar morphology, were selected for further investigations. Strain S5a33 (DSM 10507) was deposited in the Deutsche Sammlung von Mikroorganismen (Braunschweig, Germany).

Enrichments of acetogenic bacteria were carried out from a fecal sample of a non-methane-excreting subject harboring a very low number of methanogenic Archaea ($<10^2$ per gram wet weight feces). Serial tenfold dilutions of the fecal specimen were performed in an anaerobic mineral solution (Doré et al. 1995a) and further inoculated into a semi-synthetic liquid medium (AC-21 modified medium) containing H_2/CO_2 (60 : 40, v/v; 202 kPa) as energy source (Doré et al. 1995 a,b; Bernalier et al. 1996 a,b). Enrichments were obtained from the highest dilution tubes showing bacterial growth, gas consumption, and acetate production as described previously (Bernalier et al. 1996b). After three transfers of the enriched cultures, isolates were obtained by using roll tubes (Hungate 1969) of homologous medium solidified with 2% agar and with H_2/CO_2 as energy source. Colonies were transferred to tubes containing liquid medium. Cultures were considered to be pure after three to five successive isolations from roll tubes, at which time a single morphology type could be observed by phase contrast microscopy.

Culture conditions and media

The liquid and solid media were prepared using strictly anoxic techniques with 100% CO_2 gas (Hungate 1969). The semi-synthetic medium used for enrichment, isolation, and further routine cultivation of the strains was the AC-21 medium (Breznak et al. 1988) modified in our laboratory and containing (per liter): 0.2 g KH_2PO_4, 0.25 g NH_4Cl, 0.5 g KCl, 0.15 g $CaCl_2 \cdot 2H_2O$, 0.6 g $MgCl_2 \cdot 6H_2O$, 0.1 g Na_2SO_4, 0.5 g yeast extract, 2 g tryptone, 1 ml trace elements solution, 0.1 ml tungstate-selenium solution, 5 ml vitamin solution, 1 ml 1% (w/v) resazurin, 30 ml 1 M $NaHCO_3$ and 20 ml 1.25%/1.25% (w/v) cysteine/sulfur. The trace elements solution was from Widdel et al. (1983). The tungstate-selenium solution was 0.1 mM Na_2WO_4 and 0.1 mM Na_2SeO_3 in 20 mM NaOH. The vitamin solution added was that described by Greening and Leedle (1989). After inoculation, the culture headspace gas was replaced with H_2/CO_2 (60 : 40, v/v) at an initial pressure of 202 kPa when these gases were used as substrate.

Microscopy and cell wall analysis

Cell morphology was studied by phase-contrast microscopy and by electron microscopy after negative staining of the whole cell with uranyl-acetate 2%. Gram-test was performed using the conventional stain and the KOH-test according to Buck (1982). Cell wall structure was observed on thin sections by transmission electron microscopy. Cells were fixed in 2% glutaraldehyde for 15 h at 4° C and then treated with 2% OsO_4 for 15 h at 4° C. Cells were embedded in eppon-812, and thin sections were stained by uranyl-acetate and lead citrate. Electron micrographs were obtained with a 400 Philips electron microscope.

Growth and nutrition studies

The catalase activity was measured by mixing 1 ml of bacterial suspension with a few drops of H_2O_2 (30%). The production of O_2 gas (bubbles) indicated a positive test. The presence of a cytochrome oxidase was examined by placing a bacterial colony on a filter paper disk saturated with dimethyl-*p*-phenylenediamine. A positive test showed the appearance of a purple-red color of the disk due to the oxidation of the compound. Bacterial growth was measured by determining the optical density of cultures at 600 nm (OD_{600}) with a Spectronic 20D spectrophotometer (Bioblock Scientific, Illkirch, France).

The growth dependency of the acetogenic strain on yeast extract and tryptone was studied by varying their concentrations in AC-21 modified medium (0–0.5 g/l and 0–2 g/l, respectively) containing H_2/CO_2 or glucose as substrate. The influence on bacterial growth of rumen fluid addition (10%) to the AC-21 medium was also observed. The OD_{600} of triplicate cultures was followed during 5 days at 37° C.

To estimate the temperature dependence of growth, glucose-grown cultures of strain S5a33 were incubated at temperatures varying from 20 to 45° C. The OD_{600} of cultures was followed during incubation for 24 h. The effect of pH variations on bacterial growth was studied by changing the $CO_2/NaHCO_3$ ratio of the AC-21 modified medium (Costilow 1981) and measuring the growth of cultures (OD_{600}) during 24 or 48 h of incubation at 37° C. For each temperature and pH tested, three tubes were inoculated.

The H_2/CO_2 metabolism of growing cells of strain S5a33 was studied on the AC-21 modified medium using H_2/CO_2 (60 : 40, v/v) at 202 kPa pressure as energy source. Control cultures were incubated under N_2/CO_2 (60 : 40, v/v) at 150 kPa. Bacterial growth (OD_{600}) was followed during 96 h of incubation at 37° C. Three culture tubes (one control and two assays) were harvested after 0, 6, 24, 30, 48, 54, 72, 78, and 96 h of incubation and were stored at 4° C. H_2 consumption was determined by direct manometric measurement of the partial pressure using a Capshuhelic-type manometer (Dwyer Instruments, Michigan City, Mich., USA) and gas phase analysis by gas chromatography (De Graeve et al. 1994). Acetate production was measured enzymatically (Boehringer Mannheim, Meylan, France).

The utilization of various organic compounds by the strain was determined using the AC-21 modified medium containing 5 or 10 mM of substrate and a gas phase composed of 100% CO_2. The test was considered positive when the bacterium could maintain its growth after three transfers on the same substrate. In addition, the OD_{600} of the culture had to be twice that observed on the basal substrate-free medium after 24 h of incubation at 37° C.

Fermentation studies

The stoichiometry of H_2/CO_2 metabolism of strain S5a33 was calculated from the total amount of H_2 consumed and acetate produced after 96 h of incubation at 37° C on the AC-21 modified medium containing H_2/CO_2 (60 : 40, v/v; 202 kPa) as sole energy substrate.

The H_2-dependent incorporation of $^{13}CO_2$ into ^{13}C-acetate by cell suspensions was measured by NMR. Bacteria were grown in 1-l flasks containing 400 ml of AC-21 modified medium with H_2/CO_2 as sole energy substrate. They were harvested by centrifugation at 12,000 × *g* for 20 min and resuspended in a supplemented phosphate buffer (Bernalier et al. 1996a) containing 50 mM $NaH^{13}CO_3$. The cells were incubated under H_2/N_2 (80 : 20, v/v) or 100% N_2 (control) at 101 kPa for 20 h at 37° C. At the end of incubation, the suspensions were centrifuged at 12,000 × *g* for 20 min, and supernatants were sampled. Total acetate productions were determined enzymatically (Boehringer Mannheim), whereas ^{13}C-labeled organic acids were analyzed by NMR as described by Bernalier et al. (1996a).

The glucose and fructose fermentations were studied by incubating the strain during 20 h at 37° C on the AC-21 modified medium containing 1 g/l of one of these substrates. At the end of in-

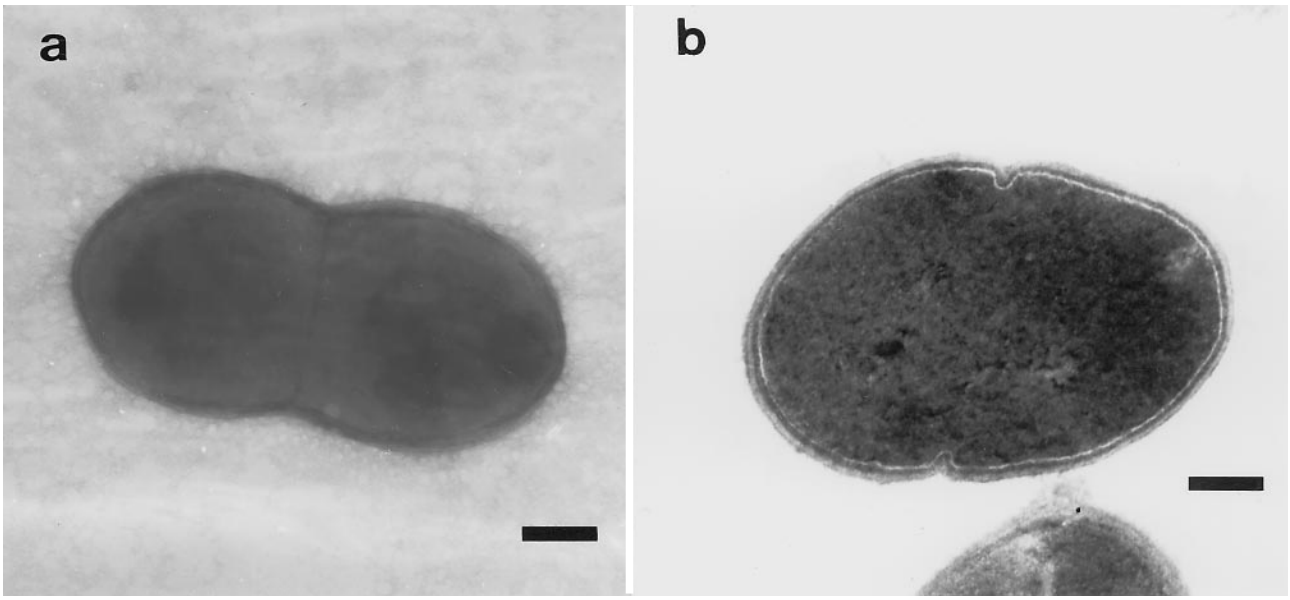

Fig. 1a,b Morphology of strain S5a33. **a** Transmission electron micrograph after negative staining (bar 0.18 μm). **b** Transmission electron micrograph of a thin section (bar 0.11 μm)

cubation, short-chain fatty acids in the supernatant fluids were determined by capillary gas chromatography after conversion to tertiary butyldimethylsilyl derivates (Richardson et al. 1989).

16S rDNA sequence and DNA G+C content determinations

DNA was extracted from rice-grain-sized cell pellets of acetogenic strains (S5a33 and S5a36) by the method of Lawson et al. (1989). Using primers ARI (5′-GAGAGTTTGATCCTGGCTCAGGA-3′) and pH (5′-AAGGAGGTGATCCAGCCGCA-3′), the almost complete 16S rRNA genes were amplified by PCR. The PCR products were purified using a Prep-A-Gene kit (Biorad, Hercules, Calif., USA) according to the manufacturer's instructions and sequenced using a Dye-Dideoxy Terminator Cycle Sequence kit (Applied Biosystems, Foster City, Calif., USA) and a model 373A automatic DNA sequencer (Applied Biosystems). Data base searches with the Fasta program from the GCG package (Devereux et al. 1984) were used to determine the nearest neighbors of the acetogenic isolates. Sequences of these strains were obtained from the EMBL and RDP data bases, and a multiple alignment was constructed using the GCG program Pileup (Devereux et al. 1984). The alignment was verified and corrected manually. Because of alignment uncertainties in the V1 region, approximately the first 100 bases were omitted from further analyses. The programs Pretty (Devereux et al. 1984) and DNAdist (Felsenstein 1989) were used to calculate a distance matrix, and a phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei 1987) using the program Neighbor (Felsenstein 1989). To assess the stability of the groupings, bootstrap analysis using 500 replications was performed with the programs Seqboot, DNAdist, Neighbor, and Consense of the Phylip package. The base composition of the DNA was determined by HPLC by the Taxolab of Pasteur Institute (Paris, France).

Determination of the acetogenic activity of different bacterial species phylogenetically related to strain S5a33

The capacity of different bacterial species phylogenetically closely related to strain S5a33 to use H_2/CO_2 and to form acetate was studied. Strains of *Ruminococcus* (*Peptostreptococcus*) *productus* (DSM 3507), *R. productus* (DSM 2950^T), *Clostridium coccoides* (DSM 935^T), *Ruminococcus* (*Streptococcus*) *hansenii* (DSM 20583^T), and *Ruminococcus obeum* (ATCC 29174^T) were grown on the AC-21 modified medium with H_2/CO_2 (60 : 40, v/v) at 202 kPa as substrate. Control cultures incubated under N_2/CO_2 (60 : 40, v/v) were also carried out. Bacterial growth (OD_{600}) was estimated after 6 days of incubation at 37° C, and H_2 consumption and acetate production were determined as described above.

Results

Isolation of bacteria

H_2/CO_2-utilizing acetogens were enriched, on AC-21 modified medium from the 10^{-5} dilution tubes showing the highest H_2/CO_2 consumption and acetate production associated with bacterial growth. 2-Bromoethanesulfonic acid was omitted in the subsequent transfers of the enriched cultures. Whereas the gas pressure in headspace decreased after 4 days of incubation, no methane was detected in these enrichments. From the third transfer, ten strains were isolated from roll tubes. Among these H_2-consuming isolates, two strains (S5a33 and S5a36) exhibited similar morphology and gram-staining reaction. Consequently, one of these, strain S5a33, was selected for further physiological studies.

Colonial and cellular morphology

Colonies developed on roll tubes in AC-21 modified medium with H_2/CO_2 as energy source were white to slightly brown, translucent, circular with entire margins, and about 1.0–2.0 mm in diameter after 7 days of incubation. Subsurface colonies of 48-hour-old cultures grown with glucose as carbon source were more irregular with undulated edges. Cells of strain S5a33 were coccobacillus-shaped with an average size of 0.7 × 0.6 μm (Fig. 1a). They were usually present singly or in pairs. Cells stained

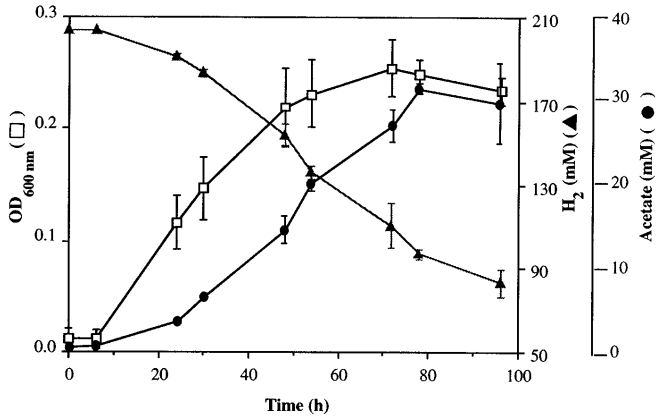

Fig. 2 Growth, H_2 utilization, and acetate production by strain S5a33. Cells were incubated for 96 h at 37° C on the AC-21 modified medium with H_2/CO_2 (60 : 40, v/v) at 202 kPa pressure as energy source. Growth was followed by measurement of the OD_{600} of the culture. Figures represents the mean values ± standard deviation of three determinations

gram-positive, and thin sections examined by transmission electron microscopy showed a gram-positive structure of the cell wall (Fig. 1b). Negatively stained cells revealed the absence of flagella (Fig. 1a). Viable cells could not be recovered from cultures held at 80° C for 10 min, and no spores were detected either on H_2/CO_2-grown or on glucose-grown cultures.

Nutrition and growth

Strain S5a33 was strictly anaerobic and required an O_2-free medium at a redox potential sufficient to decolorize resazurin (E'_0 = –50 mV). The cells did not possess catalase or cytochrome oxidase. The presence in the medium of yeast extract (0.5 g/l) or, to a lesser extent, tryptone (2 g/l) was not required, but stimulated growth. The addition of rumen fluid (10%) had no effect. With glucose as substrate, the optimal growth temperature was 35–37° C, with growth occurring from 30 to 45° C. Strain S5a33 grew from an initial pH of 6.0 up to 7.0, with an optimum at pH 6.6.

The bacterium grew autotrophically with H_2/CO_2 as sole energy source. When the bacteria were grown at 37° C on AC-21 modified medium with H_2/CO_2 (60 : 40, v/v) at 202 kPa in the gas phase as substrate, they exhibited a doubling time of 26.4 h and achieved a maximal optical density (OD_{600}) of 0.25 after 72 h (Fig. 2). H_2 consumption and acetate production occurred during the exponential and stationary growth phases (Fig. 2). At the end of incubation (96 h), the strain had consumed 121 mM of H_2 and produced 30 mM of acetate as sole metabolite. Growth and acetate production were considerably less when the strain was grown in the same medium with N_2/CO_2 in the gas phase (maximal OD_{600} = 0.1 and total acetate production = 3 mM).

Strain S5a33 could also grow heterotrophically and utilized a variety of substrates including cellobiose, fructose, galactose, glucose, lactose, maltose (poor growth), mannose (poor growth), raffinose, salicin, trehalose, pyruvate, and formate. The bacterium was able to hydrolyze esculin. No growth was observed on amygdalin, arabinose, inulin, lactose, mannitol, melibiose, methanol, rhamnose, sorbitol, soluble starch, saccharose, betaine, and benzoate. The doubling times of cultures grown at 37° C with glucose or fructose as energy source were close to 2 or 3 h, respectively (Table 1). Under these conditions, the maximal optical density (OD_{600}) measured was 0.9–1.0.

Fermentation products and stoichiometry

The stoichiometry of H_2/CO_2 metabolism by strain S5a33 was consistent with the theoretical equation of reductive acetogenesis: $4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$ (Table 1). When the bacterium was incubated under H_2 and $^{13}CO_2$, ^{13}C-acetate was the sole metabolite detected by NMR. ^{13}C-acetate was equally labeled in the methyl and carboxyl groups. Double-labeled acetate represented 72% of the total labeled acetate, confirming the total synthesis of acetate from $H_2 + CO_2$ by strain S5a33.

During heterotrophic growth, acetate was also the major fermentation product (Table 1). However, strain S5a33 usually formed other metabolites during organic substrate utilization. For example, the fermentation of glucose or fructose led to the production of acetate, lactate, ethanol, and, to a lesser extent, isobutyrate and isovalerate (Table 1). In all cases, the carbon recovery was close to 100%.

Table 1 Fermentation profiles of strain S5a33 after growth on various substrates. Cultures were incubated for 20 h with glucose (1 g/l) or fructose (1 g/l) as carbon source, or incubated for 96 h under a gas phase composed of H_2/CO_2 (60 : 40, v/v; 202 kPa) as energy source. Numbers correspond to mean values of three determinations. The percentage of C recovery does not take into account the cell carbon

Substrates	Doubling time h	End products (mmol/100 mmol substrate used)					C recovery %
		Actetate	Isobutyrate	Isovalerate	Lactate	Ethanol	
H_2/CO_2	26.4	49.5	0.0	0.0	0.0	0.0	99.0
Glucose	1.8	216.0	7.8	11.9	19.2	15.1	104.0
Fructose	2.7	148.0	7.4	6.0	46.0	17.3	89.0

16S rRNA sequence analysis and G+C content of DNA

The primary 16S rRNA gene sequence for strains S5a36 consisted of 1458 nucleotides. Only the first 827 nucleotides were determined for strain S5a33, and the sequence was 100% identical to that of strain S5a36. Searches in the EMBL data base demonstrated that these acetogenic strains showed the highest sequence similarity with members of *Clostridium* cluster XIVa, as described by Collins et al. (1994). Therefore, sequences for known members of this cluster were included in further phylogenetic analyses. Sequences of the species *Peptostreptococcus productus* strains ATCC 27340^T and ATCC 43917 (strain Marburg), and *Ruminococcus gnavus* strain ATCC 29149^T were also determined in order to complete or verify the data available from the EMBL and RDP data libraries. The sequence similarities within cluster XIVa ranged from 85 to 99%. Strains S5a33 and S5a36 showed no specifically high sequence similarity with any of the members of cluster XIVa (85 to 94%). A bootstrapped phylogenetic tree of *Clostridium* cluster XIVa is shown in Fig. 3. The G+C content of the DNA of strain S5a33 was 45.2 ± 0.2 mol%.

Acetogenic activity of some different bacterial species from *Clostridium* cluster XIVa

Strains of *Ruminococcus productus* (DSM 3507) and *Clostridium coccoides* (DSM 935^T) were able to consume large amounts of H_2 and produce acetate as sole metabolite. The quantities of H_2/CO_2 used by these species after 6 days of incubation were similar to those observed for strain S5a33. In contrast, strains of *R. productus* (DSM 2950^T) and *Ruminococcus hansenii* (DSM 20583^T) were shown to be poor H_2/CO_2 utilizers. However, the small amounts of H_2/CO_2 used by these bacteria were totally metabolized to acetate. In all cases, the amounts of acetate produced by the strains were consistent with the stoichiometric equation of reductive acetogenesis. *Ruminococcus obeum* (ATCC 29174^T) was the only species tested that was unable to grow and metabolize H_2/CO_2.

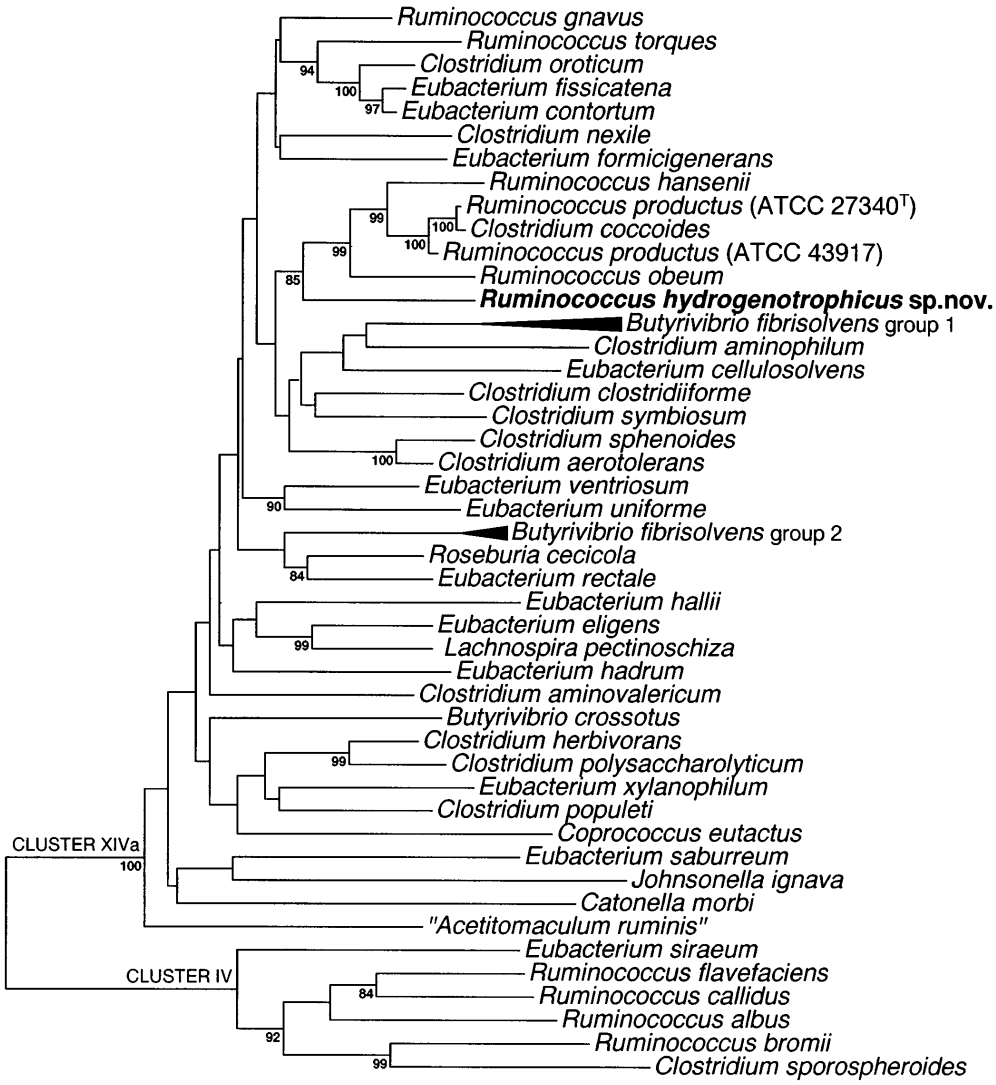

Fig. 3 Phylogenetic tree constructed using the neighbor-joining method and showing the position of *Ruminococcus hydrogenotrophicus* sp. nov. (strain S5a33) within *Clostridium* cluster XIVa (Collins et al. 1994). Some representatives of *Clostridium* cluster IV, which includes *Ruminococcus flavefaciens*, were also included for comparative purposes. Significant bootstrap values (> 90%), expressed as percentages of 500 replications, are indicated at the branching points. *Butyrivibrio fibrisolvens* group 1 and group 2 refer to two phylogenetic groups found within this species. The type strain is included in group 1 (Willems et al. 1996)

Discussion

Two obligately anaerobic, acetogenic strains designated S5a33 and S5a36 were recovered from the feces of a non-methane-excreting human. Both isolates were gram-positive, non-sporulating coccobacilli with similar phenotypic characteristics. They were typical H_2/CO_2-utilizing acetogenic bacteria. Autotrophic growth occurred with H_2/CO_2 and formate. Acetate was the sole metabolite formed from these substrates, as expected from the stoichiometric equation of reductive acetogenesis (Dolfing 1988; Breznak and Kane 1990). Furthermore, the existence of the reductive pathway of acetate formation was demonstrated by the incorporation of $^{13}CO_2$ into acetate by strain S5a33 when it was cultivated in the presence of H_2 in the gas phase. Heterotrophic growth of these isolates was also shown on a variety of organic compounds. The fermentation of glucose and fructose by the acetogenic strain S5a33 was of the mixed-acid type. However, homoacetogenesis, i.e., production of more than 2 mol of acetate per mol of sugar consumed, could only be observed with glucose as carbon source. Heterotrophic growth of the strain was faster and better than autotrophic growth due to a higher energy recovery from organic substrate utilization (Diekert 1990). The nutritional versatility of these acetogenic strains may provide them with an ecological advantage over the methanogenic Archaea present in the colonic ecosystem, which can only use a restricted range of substrates (Miller and Wolin 1986). The ability of strain S5a33 to grow by mixotrophy (i.e., by the simultaneous use of H_2/CO_2 and organic compounds for energy), as demonstrated for *Sporomusa termitida* (Breznak and Blum 1991), is now under investigation. Futhermore, the low doubling time of this strain observed during acetogenesis from H_2/CO_2 compares with the transit time in the human colon, which has been reported to vary from 25 to 67 h (Macfarlane and Cummings 1991).

16S rRNA gene sequencing demonstrated that the two strains S5a33 and S5a36 are genealogically identical and are phylogenetically members of a suprageneric grouping designated *Clostridium* cluster XIVa (Collins et al. 1994). This cluster comprises a range of phenotypically diverse organisms (including cocci and rods, spore-formers and non-spore-formers) bearing a variety of generic designations (e.g., *Butyrivibrio*, *Clostridium*, *Coprococcus*, *Eubacterium*, *Lachnospira*, *Roseburia*, *Ruminococcus*). Considerable internal phylogenetic structure is present within this suprageneric grouping (Fig. 3), and it is recognized that members of this cluster are in need of extensive taxonomic revision (Collins et al. 1994; Rainey and Janssen 1995). The new acetogenic isolates form a separate subline within cluster XIVa and show a loose association with a small subgroup consisting of *Ruminococcus obeum*, *Ruminococcus hansenii* (formerly *Streptococcus hansenii*), *Ruminococcus productus* (formerly *Peptostreptococcus productus*), and *Clostridium coccoides* (Fig. 3). Sequence divergence values of approximately 7–8% with other species within this subgroup indicate that the acetogenic isolates constitute at least a separate species. The generic affiliation of the acetogenic isolates, however, is problematic. The coccal morphology of strain S5a33 and S5a36, together with the absence of endospores, precludes the assignment of these acetogens to the genus *Clostridium*. In addition, *Clostridium butyricum*, the type species of the genus, and other true clostridia form a quite separate phylogenetic group [designated cluster I (Collins et al. 1994)]. It is pertinent to note that *Ruminococcus flavefaciens*, the type species of the genus *Ruminococcus*, is a member of a separate phylogenetic cluster (Fig. 3) designated *Clostridium* cluster IV (Rainey and Janssen 1995; Willems and Collins 1995). Hence, although the acetogenic isolates are phenotypically closest to *R. hansenii*, *R. obeum*, and *R. productus*, they, like the aforementioned species, can not be considered true phylogenetic members of the genus *Ruminococcus* sensu stricto. Although *R.*

Table 2 Comparison between some properties of strain S5a33 and those from bacterial species phylogenetically closed (+/– poor growth on the substrate, *s* depends on the strain)

Property	*Ruminococcus productus*[a]	*Clostridium coccoides*[b]	*Ruminococcus hansenii*[c]	*Ruminococcus obeum*[d]	Strain S5a33
Endospores formed	–	+	–	–	–
Utilization of:					
H_2-CO_2	s	+	+/–	–	+
Amygdalin	+	+	–	+/–	–
Arabinose	+/–	+	–	+	–
Cellobiose	+	+	–	–	+
Fructose	+	+	–	+	+
Mannitol	+/–	+	–	+/–	–
Mannose	+	+	–	+	+/–
Melibiose	+	+	+	+	–
Rhamnose	–	+	–	+	–
Salicin	+/–	+	–	+/–	–
Sorbitol	+	+	–	+/–	–
Sucrose	+	+	–	+	–
DNA mol% G + C	44–45	43–45	37–38	42	45

[a] Moore and Holdeman (1974); Holdeman et al. (1976)
[b] Kaneuchi et al. (1976)
[c] Holdeman and Moore (1974)
[d] Moore et al. (1976)
[e] Data from the present study

flavefaciens is phylogenetically only remotely related to cluster XIVa, we nevertheless consider the genus *Ruminococcus* to be phenotypically the most appropriate home for this novel acetogenic species. We recognize however, that as with *Ruminococcus gnavus*, *R. hansenii*, *R. obeum*, *R. productus*, and *Ruminococcus torques*, this is a placement of convenience that in the future will require emendation as the taxonomy of cluster XIVa and related clostridial groups, in particular *Ruminococcus*, is resolved. Therefore, based on the results of the present study, we propose that the acetogenic strains S5a33 and S5a36 be designated a new species, *Ruminococcus hydrogenotrophicus* sp. nov., with strain S5a33 as the type strain of the species.

It is pertinent to note that, with the exception of *R. obeum*, all the phylogenetic relatives of *R. hydrogenotrophicus* (viz. *C. coccoides*, *R. hansenii*, and *R. productus*) are able to use H_2/CO_2 and produce acetate as sole metabolite. Therefore, reductive acetogenesis appears to be a common property among most of the members of this small phylogenetic subgroup. Table 2 shows some properties of *R. hydrogenotrophicus* and these close phylogenetic neighbors.

Description of *Ruminococcus hydrogenotrophicus* sp. nov.

Ruminococcus hydrogenotrophicus [hy.dro.ge.no.tro'phi.cus.] Gr. n. *hydoor*, water; Gr. n. *genus*, race, offspring; M.L. *hydrogenium*, hydrogen, that which produces water; Gr.n. *trophos*, one who feeds; M.L. masc. adj. *hydrogenotrophicus*, one who feeds on hydrogen, referring to the ability of the microorganism to grow with H_2/CO_2 as energy source.

Non-motile coccobacillus, 0.7×0.6 μm in size, occurring singly or in pairs. Gram-positive by staining and cell-wall ultrastructure. No heat-resistant endospores formed.

Strictly anaerobic. Oxidase- and catalase-negative. Metabolizes H_2/CO_2 to acetate. Also ferments cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, raffinose, salicin, trehalose, pyruvate, formate, and esculin. Glucose and fructose fermentations yield acetate as major end product, but ethanol and lactate, as well as trace amounts of isovalerate and isobutyrate, are also formed.

pH optimum = 6.6 (range 6.0–7.0); temperature optimum = 35–37°C (range 30–45° C). Yeast extract, tryptone, and rumen fluid are not required for growth.

G+C content of DNA is 45.2 ± 0.2% as determined by HPLC.

Source: human feces from a non-methane-excreting subject.

Type strain: *Ruminococcus hydrogenotrophicus*, strain S5a33. The strain has been deposited in the Deutsche Sammlung von Microorganismen (Braunschweig, Germany) under number DSM 10507. The 16S rRNA gene sequence of *R. hydrogenotrophicus* has been deposited in the EMBL data base under accession number X 95624.

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