Clostridium homopropionicum sp. nov., a new strict anaerobe growing with 2-, 3-, or 4-hydroxybutyrate

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Abstract. From anoxic sewage sludge a new strictly anaerobic, spore-forming bacterium was isolated with 2-hydroxybutyrate as sole substrate. 2-, 3-, and 4-hydroxybutyrate, 4-chlorobutyrate, crotonate, vinylacetate, and pyruvate were fermented to acetate and butyrate. Fructose was converted to acetate, butyrate, butanol, and H_2 . Lactate and acrylate were fermented to acetate and propionate. Cells pregrown with lactate fermented 2-hydroxybutyrate to butyrate, propionate and acetate. No inorganic electron acceptors were reduced. The DNA base ratio was 32.0 ± 1.0 mol % and was similar to that of *Clostridium propionicum*, which was determined to be 35.3 ± 0.5 mol %. Strain LuHBu1 is described as type strain of a new species, *Clostridium homopropionicum* sp. nov. Another isolate obtained from marine sediment degraded 2- and 3-hydroxybutyrate to acetate and butyrate and was in some respects similar to the known species *Ilyobacter polytropus*.

Key words: *Clostridium homopropionicum* sp. nov. — *Clostridium propionicum* — α -Dehydration — γ -Dehydration — Butyrate fermentation — Propionate fermentation — Bioenergetics

Propionate is an important intermediate in anaerobic degradation of organic matter (Zehnder et al. 1982). Its degradation to acetate proceeds via the methylmalonyl-CoA pathway, both in sulfate-reducing bacteria (Stams et al. 1984) and in methanogenic sediment and sludges (Schink 1985) or enrichment cultures (Koch et al. 1983; Houwen et al. 1987). Recently Tholozan et al. (1988) described a temporary accumulation of butyrate in a propionate-degrading methanogenic enrichment culture. NMR-studies with $[1-^{13}C]$ -, $[2-^{13}C]$ - and $[3-^{13}C]$ -propionate revealed that propionate was converted to butyrate by reductive carboxylation, without randomization. Such

a pathway was not compatible with the concepts of anaerobic propionate metabolism existing so far. In an attempt to isolate bacteria able to catalyze interconversions of propionate and butyrate, we chose 2-hydroxybutyrate and 2-aminobutyrate as enrichment substrates.

Materials and methods

Sources of organisms

Enrichment cultures were inoculated with several different sediment and sludge samples of freshwater and marine origin. Strain LuHBu1 was isolated from anoxic digested sludge of the municipal sewage plant in Tübingen, Lustnau, FRG. Strain FMHBu1 was isolated from anoxic sediment of a channel near the fish market in Venice, Italy. *Escherichia coli* K12 (DSM 498), *Propionibacterium freudenreichii* subsp. *shermanii* (DSM 20270) and *Clostridium propionicum* (DSM 1682) were obtained from the Deutsche Sammlung für Mikroorganismen, Braunschweig, FRG. *Methanospirillum hungatei* SK was kindly provided by Dr. F. Widdel, Marburg, FRG.

Media and growth conditions

Carbonate-buffered, sulfide-reduced mineral medium was prepared as previously described (Widdel and Pfennig 1981). It contained 7-vitamin solution (Widdel and Pfennig 1981), selenite-tungstate solution (Tschech and Pfennig 1984) and the trace element solution SL10 (Widdel et al. 1983). The final pH of the medium was adjusted to 7.2–7.4. Freshwater medium contained 0.1% NaCl and 0.04% $MgCl_2 \times 6 H_2O$ whereas saltwater medium contained 2% NaCl and 0.3% $MgCl_2 \times 6 H_2O$. The growth temperature was 28°–30°C. Growth was determined by measuring turbidity in a Bausch and Lomb Spectronic 70 spectrophotometer in 22 ml screw-cap tubes. Substrates were added from sterile, neutralized stock solutions.

Isolation and characterization

For isolation of pure cultures, the agar shake culture method (Pfennig 1978) was applied. Purity was checked microscopically and by growth tests in a medium containing 5 mM fumarate, 3 mM glucose, 0.1% yeast extract, and 10 mM 2-hydroxybutyrate. The Gram type

was determined according to Bartholomew (1962) and to Gregersen (1978) with *Propionibacterium freudenreichii* and *Escherichia coli* as controls. Flagella staining was carried out according to Blenden and Goldberg (1965). For detection of cytochromes, crude cell extracts of fructose-grown cells were prepared by fourtime passage through a French Pressure Cell Press (AMINCO, Silver Spring, USA) at 6.9 MPa. Cell debris was removed by centrifugation for 20 min at 20,000 × g. Redox difference spectra (dithionite-reduced minus air-oxidized) were measured in crude cell-free extracts with a Uvikon 860 spectrophotometer (Kontron, Zürich, Switzerland). The guanine-plus-cytosine content of the DNA was determined by the thermal denaturation method according to De Ley (1970) after extraction as described by Marmur (1961). Capacity for nitrogen fixation was checked with cells pregrown for four generations in ammonia-free mineral medium with 2-hydroxybutyrate as substrate. Acetylene was injected into the gas phase of the culture vessel to a concentration of 10% (v/v), and acetylene and ethylene were determined by gas chromatography after incubation at 30°C for two days.

Induction experiments

Dense cell suspensions (OD 2.5) were prepared with cells pregrown with 2-, 3-, or 4-hydroxybutyrate or lactate in 100 ml infusion bottles (Müller & Krempel, Bülach, Switzerland), each with 10 mM substrate. Cells were harvested in the late log phase by centrifugation under anaerobic conditions for 30 min at 4°C and 4,500 rpm in a Sorvall RC-5 centrifuge. The pellet was washed once with freshwater medium, and the suspension was distributed anoxically at 10 ml each to 20 ml tubes under N₂/CO₂ (90%/10%) gas mixture. The tubes received 2-, 3-, 4-hydroxybutyrate, or lactate at 20 mM concentration. Parallel assays contained the respective substrate plus chloramphenicol (30 mg/l). Product formation was determined by gas chromatography.

Enzyme assays

Assays were performed with crude cell-free extracts of fructose- or lactate-grown cells of strain LuHBu1 using a Hitachi 100-40 spectrophotometer (Hitachi, Tokyo, Japan). Phosphate acetyl transferase and acetate kinase were measured by standard methods (Bergmeyer 1974). Lactate dehydrogenase was measured with reduced Nicotinamide adenine dinucleotide as electron donor (Bergmeyer 1974) or dichlorophenol indophenol as electron acceptor (Molinari and Lara 1960).

Chemical analyses

Fatty acids and butanol were assayed by gas chromatography as described (Platen and Schink 1987). For analysis of propionate and butyrate, operation conditions were as follows: 1 min at 120°C, 25°C/min to 170°C, 1 min at 170°C. Hydrogen was determined by gas chromatography as previously described (Matthies et al. 1989). Acetylene and ethylene were determined with a Carlo Erba GC 6000 gas chromatograph (Milano, Italy) with flame ionization detector. A glass column (2 m × 2 mm) was packed with a molecular sieve (5 Å, 18–50 mesh, Merck, Darmstadt, FRG), column temperature was 140°C, detector and injector temperature 200°C. Sulfide formation from sulfate or sulfur was analyzed qualitatively by a rapid detection test (Cord-Ruwisch 1985), nitrite formation from nitrate with sulfanilic acid and α -naphthylamine (Procházková 1959). Determination of poly- β -hydroxybutyrate as storage material was carried out according to Law and Slepecky (1961). Polyphosphates were detected as described by Drews (1983). Protein was quantified by a modified microbiuret method (Zamenhoff 1957).

Chemicals

All chemicals were of reagent grade quality and obtained from Fluka, Neu-Ulm; Merck, Darmstadt; Sigma, Deisenhofen, FRG; and Aldrich, Steinheim, FRG. Gases were obtained from Messer-Griesheim, Darmstadt, FRG.

Results

Enrichment and isolation

50 ml enrichment cultures in either freshwater or saltwater medium with 10 mM 2-hydroxybutyrate or 2-aminobutyrate as substrate were inoculated with 5 ml anoxic mud from various habitats. After three to four weeks of incubation, subcultures with 2-hydroxybutyrate were inoculated with 5 ml culture fluid. Enrichments with 2-aminobutyrate exhibited slow gas production, but subcultures did not grow. Enrichment of a 2-hydroxybutyrate-degrading bacterium from a propionate-degrading enrichment culture (provided by J. L. Tholozan, Lille, France) failed as well. Butyrate and acetate were the main products formed from 2-hydroxybutyrate; propionate was detected at low concentrations (0.5 mM). After five to six transfers, the cultures contained mainly one single type of bacterium. A long, motile, sporeforming rod prevailed in the freshwater enrichment culture inoculated with sewage sludge, whereas a short, non-motile rod was enriched from marine sediment.

The prevailing bacteria were isolated in two subsequent dilution series with 10 mM 2-hydroxybutyrate and 0.05% yeast extract, and transferred into liquid mineral medium. Purity was checked by microscopic control after growth in selective or complex test media. Strain FMHBu1 required 0.05% yeast extract for growth in pure culture.

Morphology and cytological characterization

Cells of the marine strain FMHBu1 were short rods with rounded ends, 1.0 × 2.5–4.5 µm in size (Fig. 1a). The gram Type after staining and the KOH-test was negative. Treatment with Lugol's potassium iodide-iodine solution (Drews 1983) stained areas at the cell end brown indicating the presence of glycogen-like polysaccharides. Spore formation was never observed. The guanine-plus-cytosine content of the DNA was determined to be 64.2 ± 1.0 mol%.

Cells of strain LuHBu1 were long, straight rods, 1.2–1.5 × 5.5–10 µm in size, with rounded ends (Fig. 1b). Heat resistant spherical spores were formed in the terminal to subterminal region (Fig. 1c). Especially with fructose, lactate, or pyruvate, spores were formed very soon after reaching the stationary growth phase. Sporulation medium (Hollauss and Sleytr 1972) did not accelerate spore formation. Cells grown with fructose or lactate formed round dark inclusions, probably polyphosphates. Poly- β -hydroxybutyrate could not be detected. Growing cells were slowly motile but lost motility very soon in

Fig. 1a–c. Phase contrast photomicrograph of 2-hydroxybutyrate-utilizing isolates. **a** Strain FMHBu1; **b** vegetative cells of strain LuHBu1; **c** sporulated cells of strain LuHBu1. Bar equals 10 µm for all panels

ageing cultures. Flagella staining demonstrated 4–6 peritrichously inserted flagella. Cells stained Gram negative, however, the gram type determined by the KOH test was positive. The guanine-plus-cytosine content of the DNA was determined to be 32.0 ± 1.0 mol%. No cytochromes were detected by redox difference spectroscopy in crude cell extracts of fructose-grown cells.

Physiology and stoichiometry of fermentation

Both strains were obligately anaerobic. Strain FMHBu1 grew in mineral medium with a least 0.5% NaCl. Growth was possible in a temperature range between 4°C and 32°C, with an optimum at 28°C; the pH-range was 6.0–8.5 with an optimum at 7.0. Phosphate concentrations up to 50 mM did not inhibit growth. Neither nitrate, nitrite, nor sulfate, thiosulfate, sulfite, or sulfur was reduced. With 2-hydroxybutyrate as substrate, a growth rate of 0.238 h^{-1} ($t_d = 3 \text{ h}$) was reached. Strain FMHBu1 fermented 2- and 3-hydroxybutyrate and crotonate to acetate and butyrate, pyruvate to acetate and H_2 , and succinate to propionate.

Strain LuHBu1 grew in freshwater and in brackish water medium containing 1% NaCl and 0.15% $\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$, but not in saltwater medium. However, growth in brackish water medium was delayed, and cell yields were lower. Phosphate was tolerated at concentrations up to 50 mM. Growth was possible at temperatures between 20°C and 40°C, the optimum was 37°C; the pH range was 5.6–8.3 with an optimum at 7.2. No inorganic electron acceptors such as nitrate, sulfate, thiosulfate, sulfite, or sulfur were reduced. The cells could use molecular nitrogen as sole nitrogen source. The doubling time with 10 mM 2-hydroxybutyrate was 8 h. A growth curve with 2-hydroxybutyrate is presented in Fig. 2. Strain LuHBu1 grew well with 2-, 3-, or 4-hydroxybutyrate, crotonate, vinylacetate, fructose, pyruvate, lactate and, with lower growth rates, also with 4-chlorobutyrate and acrylate at low concentrations (2.5 mM). Fermentation products of the hydroxybutyrate, 4-chlorobutyrate, vinylacetate, crotonate, and pyruvate were acetate and butyrate. If cells grew in culture vessels with a gas head space also hydrogen was produced from pyruvate.

Fructose was fermented to acetate, butyrate, butanol and H_2 , while lactate and acrylate were fermented to acetate and propionate. The stoichiometry of substrate

Fig. 2. Time course of growth and product formation by strain LuHBu1 with 2-hydroxybutyrate as substrate. Experiments were carried out in 100-ml serum bottles. OD₅₄₆: optical density at 546 nm. Symbols: ○ cell density, □ acetate, △ butyrate

utilization, product formation, and growth yields are presented in Table 1.

Induction experiments

To check for possible common step in degradation of the hydroxybutyrate isomers, induction experiments were carried out with strain LuHBu1. The results are summarized in Table 2. 3-hydroxybutyrate was degraded rapidly in all assays, no matter on which substrate cells were pregrown, and chloramphenicol had no effect. Addition of chloramphenicol to 2-hydroxybutyrate-grown cell suspensions inhibited not only degradation of 4-hydroxybutyrate, but also of 2-hydroxybutyrate. Degradation of lactate by lactate-grown cells was slightly inhibited by chloramphenicol. Lactate-pregrown cells fermented 2-hydroxybutyrate to acetate, propionate, and butyrate according to the following fermentation equation:

These cells did not degrade propanol or propionate, neither in pure culture nor in coculture with *Methanospirillum hungatei*.

Enzyme activities of strain LUHBu1

Phosphate acetyl transferase (EC 2.3.1.8) activity in fructose-grown cells was 1.13 U/mg protein. Acetate kinase

Table 1. Stoichiometry of fermentation and growth yields of strain LuHBu1

Substrate	Substrate added [μmol]	Cell dry mass formed ^a [mg]	Products formed [μmol]					Electron-recovery [%]	Molar growth yield [g/mol]
			Acetate	Butyrate	Propionate	Butanol	H ₂		
2-Hydroxybutyrate	200	0.73	179	103	—	—	n.d. ^b	98	3.65
3-Hydroxybutyrate	200	0.87	184	107	—	—	n.d.	104	4.35
4-Hydroxybutyrate	200	0.87	182	90	—	—	n.d.	107	4.35
4-Chlorobutyrate	100	0.35	94	46	—	—	n.d.	96	3.50
Crotonate	200	0.95	203	94	—	—	n.d.	102	4.75
Vinylacetate	200	0.89	190	100	—	—	n.d.	102	4.45
Pyruvate	200	1.23	109	42	—	—	n.d.	100	6.15
Lactate	200	0.87	63	—	94	—	n.d.	79.5	4.35
Acrylate	50	0.22	16	—	33	—	n.d.	104	4.40
Fructose	100	0.49	73	41	—	11	135	103	31.20

Experiments were carried out in 22 ml tubes completely filled with mineral medium, or in 100 ml infusion bottles with head space in case of fructose as substrate.

^a Cell dry matter was calculated from turbidity at 546 nm using the conversion factor $0.1 \text{ OD}_{546} = 33.0 \text{ mg/l}$, which was obtained by direct determinations in 500 ml cultures grown with fructose

^b not determined

Table 2. Degradation of various substrates by cell suspensions of strain LuHBu1 pregrown with 2-, 3-, 4-hydroxybutyrate, or lactate

Substrate	Cells pregrown with			
	2-hydroxybutyrate	3-hydroxybutyrate	4-hydroxybutyrate	lactate
2-Hydroxybutyrate	++ ^a	+	+	+
2-Hydroxybutyrate + CA ^c	— ^d	—	—	—
3-Hydroxybutyrate	++	++	++	++
3-Hydroxybutyrate + CA	++	++	++	++
4-Hydroxybutyrate	+	+	++	+
4-Hydroxybutyrate + CA	—	—	++	—
Lactate	+	+	+	++
Lactate + CA	—	—	—	+

^a Rapid degradation within 5 h or less

^b Delayed degradation starting after 20 h or more

^c Chloramphenicol

^d No degradation

(EC 2.7.2.1) was found at 0.56 U/mg protein. NAD-independent lactate dehydrogenase (EC 1.1.2.3) activity was 0.46 U/mg protein. NAD-dependent lactate-dehydrogenase (EC 1.1.1.27) activity could not be detected.

Discussion

Physiology

Two bacterial strains are described in this paper which were isolated with 2-hydroxybutyrate as sole carbon and energy source from anoxic sewage sludge or marine sediment. Strain FMHBu1 fermented 2- and 3-hydroxybutyrate and crotonate to acetate and butyrate, pyruvate to acetate and H₂, and succinate to propionate. With these properties, as well as by its cytological characters, it resembles the 3-hydroxybutyrate-fermenting *Ilyobacter polytropus* (Stieb and Schink 1984), however, differs from this strain by its ability to use 2-hydroxybutyrate, and by its comparably high guanine-plus-

cytosine content of the DNA ($32.2 \pm 0.5 \text{ mol\%}$ for *I. polytropus*, as opposed to $64.2 \pm 1 \text{ mol\%}$ for strain FMHBu1). This strain was not characterized in further detail.

More interesting was the freshwater isolate from sewage sludge, strain LuHBu1. It fermented the isomers 2-, 3- and 4-hydroxybutyrate, as well as 4-chlorobutyrate, crotonate, vinylacetate and pyruvate, to acetate and butyrate. Fructose was converted to acetate, butyrate, traces of butanol, and H₂, whereas lactate and acrylate were degraded to acetate and propionate. Induction experiments revealed that 3-hydroxybutyrate was degraded by an enzyme system which was present as well in cells pregrown with either 2-hydroxybutyrate, 4-hydroxybutyrate, or lactate.

Obviously, dismutation of 3-hydroxybutyrate via crotonate to acetate and butyrate plays a central role in the metabolism of this strain (Fig. 3). 2-hydroxybutyrate could enter this pathway by elimination of the 2-hydroxy group, analogous to lactyl-CoA conversion to acrylyl-CoA by *Clostridium propionicum* (Cardon and Barker

Fig. 3. Hypothetical pathway of fermentation of 2-, 3- and 4-hydroxybutyrate by strain LuHBU1. A possible direct reduction of vinylacetate to butyrate is indicated by the dashed line

1947). The reversible dehydration of 2-hydroxy acids to 2-enoic acids is of considerable interest in biochemistry since the eliminated hydrogen is not activated and the hydroxyl group is a very poor leaving group. The reaction probably proceeds through a radical mechanism, similar to reversible dehydration of 2-hydroxyglutaryl-CoA to glutaconyl-CoA during glutamate fermentation by *Acidaminococcus fermentans* (Schweiger and Buckel 1984). This reaction is inhibited by chloramphenicol (Buckel, personal communication) as was observed also for 2-hydroxybutyrate degradation by strain LuHBU1, indicating that similar enzyme systems are involved in these α -dehydration reactions. Degradation of 4-hydroxybutyrate or 4-chlorobutyrate poses similar chemical problems. The hydrogen at C-atom 3 is not activated and therefore a radical mechanism must be postulated for these elimination reactions, too. Conversion of 4-hydroxybutyrate or 4-chlorobutyrate to butyrate could include a direct reduction of vinylacetate or could require an isomerization of vinylacetate to crotonate, a reaction observed also for *C. aminobutyricum* (Hardman and Stadtman 1963). All these transformations occur probably with the respective coenzyme A-esters as future research will have to elucidate. The results obtained by the induction experiments are summarized in a hypothetical pathway (Fig. 3).

To our surprise, lactate-grown cells in dense suspension converted 2-hydroxybutyrate to butyrate, acetate and considerable amounts of propionate. Obviously, propionate and butyrate are formed from 2-hydroxybutyrate in a similar manner as lactate is fermented to acetate and propionate. Although a net conversion of butyrate to propionate could not be established as was originally intended in this study, it is evident that the enzyme apparatus of strain LuHBU1 can mediate between C_3 - and C_4 -compounds.

Strain LuHBU1 also grew with 4-chlorobutyrate as sole energy and carbon source but did not use 3-chlorobutyrate, 3-chloropropionate or 2-chloropropionate as substrate, as described for a recently isolated anaerobic bacterium with a similar fermentation pattern (Matsuda et al., Abstract, FEMS Symposium on Microbiology and Biochemistry of Strict Anaerobes Involved in Interspecies Hydrogen Transfer, Marseille, France, September 1989).

Strain LuHBU1 degraded the various substrates according to the following fermentation equations (calculations of free energy changes after Thauer et al. 1977).

$$\Delta G'^{\circ} = -59.4 \text{ kJ/mol substrate}$$

$$\Delta G'^{\circ} = -51.1 \text{ kJ/mol substrate}$$

$$\Delta G'^{\circ} = -72.4 \text{ kJ/mol substrate}$$

$$\Delta G'^{\circ} = -54.9 \text{ kJ/mol substrate}$$

$$\Delta G'^{\circ} = -49.4 \text{ kJ/mol substrate}$$

$$\Delta G'^{\circ} = -325.7 \text{ kJ/mol substrate.}$$

Degradation of the hydroxybutyrates, crotonate, vinylacetate, and 4-chlorobutyrate allows a net synthesis of one mol ATP per 2 mol substrate fermented (Fig. 3). Sufficient activities of phosphate acetyl transferase and acetate kinase for ATP formation via substrate level phosphorylation were detected. The cell yields measured (3.65–4.75 g per mol substrate) agree with formation of 0.5 mol ATP per mol substrate, assuming a yield coefficient of about 10 g cell dry weight per mol ATP (Stouthamer 1979). Fermentation of lactate via the acrylate pathway is coupled with formation of only one mol ATP per three mol lactate degraded. The cell yields determined with lactate (4.35 g/mol) and acrylate (4.4 g/mol) are slightly higher than expected from this concept. An additional ATP gain by electron transport phosphorylation has been proposed for alanine degradation by *Megasphaera elsdenii* (Anderson and Wood 1969), but was never proven.

Taxonomy

The new isolate described in this study, strain LuHBU1, is an obligately anaerobic spore-forming rod and has therefore to be assigned to the genus *Clostridium* (Cato et al. 1986). The cells stained gram-negative, however, it is known that some species of the genus *Clostridium* stain gram-variable to gram-negative (Cato et al. 1986). Strain LuHBU1 fermented fructose via a butyrate-butanol fermentation similar to *C. butylicum* or *C. acetobutylicum* (Davies and Stephenson 1941), and lactate to acetate and propionate similar to *C. propionicum* (Cardon and Barker 1947). The guanine-plus-cytosine content of the DNA of strain LuHBU1 was $32.0 \pm 1.0\%$, and was similar to that

of *C. propionicum* ($35.3 \pm 0.5\%$). Growth tests revealed that *C. propionicum* cannot grow with 2- or 4-hydroxybutyrate as substrate. To our knowledge there is no species of the genus *Clostridium* described so far that combines all major fermentation patterns of our new isolate, and therefore a new species, *C. homopropionicum* is established.

Species description

C. ho.mo.pro.pi.o'ni.cum sp. nov. *homoios*, Gr adj. similar; *acidum propionicum*, M. L. n. propionic acid; *homo-propionicum* referring to metabolic analogy to *C. propionicum*.

Straight rods with rounded ends, $1.2-1.5 \times 5.5-10 \mu\text{m}$ in size, single or in pairs. Motile in young cultures by peritrichously inserted flagella. Spherical spores formed terminally to subterminally in ageing cultures, preferentially after growth with pyruvate or lactate, or in agar medium. Dark inclusions formed after growth with lactate. Strictly anaerobic chemoorganotroph. Fructose fermented to acetate, butyrate, butanol, and H_2 . 2-, 3-, and 4-hydroxybutyrate, 4-chlorobutyrate, crotonate, vinylacetate and pyruvate fermented to acetate and butyrate. Lactate and acrylate fermented to acetate and propionate. No growth with glucose, arabinose, xylose, ethanol, ethanol + acetate, glycerol, 2-aminobutyrate, 4-aminobutyrate, α -alanine, β -alanine, β -hydroxypropionate, serine, aspartate, phenylalanine, malate, fumarate, succinate, mandelate, glycolate, ethylene glycol, trimethoxybenzoate, acetoin, or H_2/CO_2 . Gelatin is not hydrolyzed.

Nitrate, nitrite, sulfite, sulfur, or thiosulfate not reduced. Fixation of molecular nitrogen. Optimal growth only in freshwater medium. Total growth inhibition at 1% NaCl. 50 mM phosphate did not inhibit growth.

Selective enrichment in freshwater medium with 2-hydroxybutyrate as substrate. pH-range: 5.6–8.3, temperature optimum at 37°C . No cytochromes detectable. DNA base ratio: $32.0 \pm 1.0\%$ guanine plus cytosine (thermal denaturation).

Habitats: anoxic digester sludge. Type strain: LuHBu1, DSM 5847, deposited at: Deutsche Sammlung für Mikroorganismen, Braunschweig.

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