Systematic and Applied Microbiology 44 (2021) 126225

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: www.elsevier.com/locate/syapm

Pelorhabdus rhamnosifermentans gen. nov., sp. nov., a strictly anaerobic rhamnose degrader from freshwater lake sediment

Fabian Gräßle [a,b], Caroline Plugge [b,c], Paolo Franchini [a], Bernhard Schink [a,b], David Schleheck [a,b], Nicolai Müller [a,b,*]

[a] Department of Biology, University of Konstanz, Konstanz, Germany
[b] Research Training Group R3 – Resilience of Lake Ecosystems, University of Konstanz, Konstanz, Germany
[c] Laboratory of Microbiology, Wageningen University & Research, Wageningen, Netherlands

ARTICLE INFO

Article history:
Received 16 January 2021
Revised 14 May 2021
Accepted 8 June 2021

Keywords:
Rhamnose
Anaerobic metabolism
Sugar fermentation

ABSTRACT

A rhamnose-degrading bacterium, strain BoRhaA^T, was isolated from profundal sediment of Lake Constance in agar dilution series with L-rhamnose as substrate and with a background lawn of *Methanospirillum hungatei*. The isolated strain was a motile rod that stained Gram positive. Growth was observed within a pH range of 4.0–7.5 and a temperature range of 15–30°C. Fermentation products of rhamnose or glucose were acetate, propionate, ethanol, butyrate, and 1-propanol. The G+C content was 40.6% G+C. The dominant fatty acids are $C_{16:1}\omega9c$, i-$C_{13:0}$3OH, $C_{16:0}$ and $C_{17:1}\omega8c$ with 8–21% relative abundance. Polar lipids were glycolipids, phosphatidylethanolamine, phosphoaminolipid and other lipids, of which phosphatidylethanolamine was most abundant. The sequence of the 16S rRNA gene of the new isolate matches the sequence of its closest relative *Anaerosporomusa subterranea* to 92.4%. A comparison of the genome with this strain showed 60.2% genome-wide average amino acid identity (AAI), comparisons with other type strains showed a maximum of 62.7% AAI. Thus, the definition of a new genus is justified for which we propose the name *Pelorhabdus*. For strain BoRhaA^T, we propose the name *Pelorhabdus rhamnosifermentans* gen. nov., sp. nov., with strain BoRhaA^T (DSM 111565^T = JCM 39158^T) as the type strain.

Introduction

The deoxy-sugar rhamnose is a ubiquitous constituent of cell walls of plants and algae, and occurs also in fungi [19]. Rhamnose was found to be present in the microalga *Prymnesium parvum* [63], in the blue alga *Agmenellum quadruplicatum* [11], and in the extracellular polymeric substance (EPS) of the diatoms *Cylindrotheca closterium* and *Navicula salinarum* [53]. Rhamnose can therefore be considered as an abundant sugar in lake ecosystems that are rich in algae. It is conceivable that parts of the bacterial community have specialized on the degradation of rhamnose and its polymer precursors such as pectin or bacterial lipopolysaccharides [19,42,46]. In aquatic sediments of oligotrophic freshwater lakes, organic substrates are supplied to bacteria only at very low rate. In a sediment core from Upper Lake Constance, Germany, lactate, acetate, propionate, succinate, ethanol, caproate, isopropanol and formate were present in a concentration range of 6–50 μM [28]. Also glucose was detected at concentrations below 10 μM. Tracer experiments with radioactively labelled glucose revealed that these sediments degrade 11 μM glucose completely in less than one hour, and that radioactively labeled carbon was incorporated into fermentation products [28]. Thus, the fermenting microbial community of oligotrophic sediments contributes to fast turnover of organic monomers, especially glucose. In sediments of eutrophic aquatic ecosystems, e.g. the hard-water lake Blue Lake, Minnesota, USA, sugars are generally much more abundant and diverse and include glucose, xylose, arabinose, ribose, mannose, galactose and rhamnose [47]. In another study, the abundance of monosaccharides was compared at different water depths in two eutrophic lakes and a dystrophic mountain lake, and rhamnose appeared to be abundant mainly in the profundal zone of all three lakes (23.9–25.8% of the monosaccharide composition) [40]. This might in part be explained by the fact that rhamnose is known as a "fairly stable" monosaccharide in aquatic sediments [47]. It is conceivable that increased primary production in eutrophic aquatic systems leads to an increase of sugar concentrations in sediments [24,52].

* Corresponding author at: Department of Biology, University of Konstanz, Konstanz, Germany (N. Müller).
E-mail address: Nicolai.Mueller@uni-konstanz.de (N. Müller).

https://doi.org/10.1016/j.syapm.2021.126225
0723-2020/

Easily degradable sugars such as glucose vanish at high turnover rates, while more stable sugars such as rhamnose might accumulate. Rhamnose, in contrast to glucose, is found in comparatively high concentrations (0.26–2.19 μg/mg dry weight) in sediment traps and surface sediment of Lake Itasca, which is consistent with its relative stability described before [21,47]. Since pectin is also a component of plant material, rhamnose-utilizing bacteria like the butyrogen *Anaerostipes rhamnosivorans* are important constituents of the human digestive system [9]. In a co-culture with the formate/hydrogen consuming *Methanobrevibacter smithii,* butyrate production during glucose fermentation by *A. rhamnosivorans* was higher than in the axenic culture [10]. Different from most known sugar-fermenting bacteria, *Mesobacillus stamsii* (formerly *Bacillus stamsii*) could be isolated only under anoxic conditions and from anoxic sediment if a hydrogen- or formate-scavenging partner was supplied [37]. This partner was *Methanospirillum hungatei* strain M1h, a methanogenic archaeon isolated from sediment of Lake Constance and able to grow on formate or H_2/CO_2.

In the present study on anaerobic saccharolytic bacteria in the profundal sediment of Lake Constance, Germany, we isolated a novel sugar-degrading bacterium in agar shake series with rhamnose as substrate on a background lawn of *Methanospirillum hungatei*. If supplied with yeast extract axenic growth was reproducible after the methanogen partner organism was removed from the co-culture. In the absence of yeast extract, no growth was observed in axenic cultures, indicating that rhamnose utilization by the *Sporomusaceae* bacterium strain BoRhaA^T obligately depends on either a methanogenic partner or complex supplements.

Materials and methods

Source of organism

Profundal sediment was collected from a depth of 80 m with a gravity core from Upper Lake Constance near the island Mainau (Germany). Samples were taken from the sulfidic layer about 30 mm below the sediment surface of an undisturbed gravity core and used to inoculate agar shake dilution series with 5 mM rhamnose as sole carbon and energy source and a background lawn of *Methanospirillum hungatei* strain M1h as syntrophic partner. *M. hungatei* M1h was from our own collection and can be made available upon request. *Anaerosporomusa subterranea* strain RU4 DSM 29728^T and *Clostridium phytofermentans* ISDg DSM 18823^T were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). After 4 weeks, colonies developed in the culture tubes down to a 10^{-7} dilution. Colonies were picked aseptically with needles and syringes or with a Pasteur pipette connected to a suction tube operated with a pipetting aid. Picked colonies were inoculated into liquid mineral medium described below with 5 mM rhamnose. The isolation procedure was repeated until the strain was pure as judged by light microscopy. As product patterns were the same in co-cultures and axenic cultures, only the axenic culture was investigated further. Yeast extract was supplied at 0.05% concentration to achieve reproducible growth. The new isolate was defined as strain BoRhaA^T and was deposited at the DSMZ as DSM 111565^T and at the Japan Collection of Microorganisms (JCM) as JCM 39158^T.

Cultivation conditions

Cultivation, isolation, and growth experiments were performed in anoxic bicarbonate-buffered freshwater mineral medium as described previously [69]. For strictly anoxic, reduced media, Na_2S*9 H_2O at a concentration of 1 mM was added after autoclaving from a separately autoclaved stock solution. For preparation of non-reduced media, 0.5 mM Na_2SO_4 was added as a sulfur source before autoclaving [69]. The medium (excluding Na_2S*9 H_2O and $NaHCO_3$) was autoclaved at 121°C and 1 bar overpressure for 25 min and cooled under an oxygen-free N_2/CO_2 (80:20) gas mixture. $NaHCO_3$ was added to a final concentration of 2.5 g/l from stock solutions autoclaved separately under 100% CO_2. Trace element solution SL-13 [37,68], selenate tungstate [62] and seven-vitamin solution [43] were added from 1000-fold concentrated stocks. The initial pH of the medium was adjusted to 7.3 ± 0.1 with sterile Na_2CO_3 or HCl. Cultivation was performed under N_2/CO_2 (80:20) atmosphere in the dark at 30°C. *Methanospirillum hungatei* was cultivated with 5 mM acetate under a CO_2/H_2 (20:80) headspace.

Morphological characterization

The isolate was examined by phase-contrast microscopy (Axiophot Zeiss) and photographs were taken using the agar slide technique [44]. Gram staining was carried out with a staining kit (Difco Laboratories) according to the manufacturer's instructions, and by the KOH test [20].

Growth experiments

Substrates were added to a final concentration of 5 mM unless indicated otherwise. Aerobic growth was tested in LB medium and the oxic minimal medium described previously [38]. The temperature range for growth was studied at 5, 15, 25, 30, 37, 55 and 80°C. The pH range of growth was determined over the pH range 4–9 at intervals of 0.5 pH units in basal mineral medium containing a mix of 10 mM 2-(N-morpholino) ethanesulfonic acid (MES), (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (HEPES), tris(hydroxymethyl) aminomethane (Tris) and N-Cyclohexyl-2-aminoethane sulfonic acid (CHES). Dependence of growth on the salt (NaCl plus $MgCl_2$) concentration was determined in freshwater medium (1 and 0.4 g/l), brackish water medium (7 and 1 g/l) and seawater medium (20 and 3 g/l). The ability to reduce sulfate (20 mM) was studied with rhamnose (5 mM) as electron donor in sulfide-reduced medium; nitrate (20 mM) reduction was tested in non-reduced medium and compared to a control without electron acceptor. Since strain BoRahA survived pasteurization at 80°C for 20 min, survival at 100°C or 121°C for 20 min and 20°C for 24 h was also tested. Substrates tested for utilization are listed in the results section. Growth with carbon monoxide was tested by adding 2 ml to about 10 ml headspace (corresponding to a theoretical concentration of 8 mM in the headspace). Benzoate, phenol, and lactate were tested as electron donors with sulfate as acceptor. As it was required for reproducible growth with L-rhamnose, yeast extract (0.05%) was supplied in all growth experiments and compared to a negative control with 0.05% yeast extract only. Utilization of each electron acceptor or donor was analyzed via turbidity. Sugars, alcohols, and organic acids were identified and quantified by ion-exchange chromatography using an HPLC system as described before [39,41]. Samples were separated on a Rezex RHM-monosaccharide H^+ 300 × 7.80 mm 8-micron ion exchange column (Phenomenex, Aschaffenburg, Germany) operated at 40°C, with 30 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 ml/min, and the eluted compounds were detected with a refractive index detector as described before using the Lab Solutions software (Shimadzu) [38,41]. Growth experiments were carried out in triplicates and terminated after 2 weeks of incubation. Cultures were transferred three times to rule out any carry-over of substrate from the pre-culture. For comparison with BoRhaA^T, reference strains were only tested in sulfide-reduced medium.

Chemotaxonomic analysis

Analyses of G+C content [13,32,58], fatty acids [29,33], respiratory quinones, and polar lipids [5,60-61] were carried out by the Identification Service, Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

16S-rRNA gene amplification and sequencing

Crude genomic DNA of strain BoRhaA^T was prepared by centrifugation of 1 ml culture at 12,000×g for 5 min. The resulting pellet was suspended in 100 µl autoclaved dd H_2O, incubated at 100°C for 15 min and subsequently cooled on ice. From this solution, 1 µl was used in a PCR mixture for amplification of the 16S rRNA gene by PCR as described by Müller et al. [37] with bacterial universal primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) [18] and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) [67]. PCR products were purified with the DNA Clean & Concentrator™-5 kit (Zymo Research, Orange, California, USA) and Sanger sequenced using the primers 27F, 1492R, 533F (5′-GTG CCA GCA GCC GCG GTA A-3′) [67] and 533R (5′-TTA CCG CGG CTG CTG GCA C-3′) [67] in contract with Eurofins-GATC (Konstanz, Germany). Obtained sequences were trimmed and assembled with genious (Biomatters, Auckland, New Zealand) aiming at a sequence of 1477 bp which was published under the accession number MW010209 at NCBI. EzBioCloud's identification service was used for the phylogenetic classification [17] and close relatives were identified using the BLASTN search program [1] (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

DNA extraction and genome sequencing

Genomic DNA of the culture was extracted according to the JGI-protocol "Bacterial genomic DNA isolation using CTAB" [70]. Genome sequencing was performed by Eurofins-GATC (Constance, Germany) using an Illumina NovaSeq 6000 platform, resulting in 15358756 paired reads (2x151bp). Trimmomatic v0.39 [6] was used to remove adapters from the reads with default settings, filter the reads by quality (LEADING:3 TRAILING:3 SLIDINGWINDOW:4:20) and to discard those shorter than 50 bp. The resulting 14057080 paired reads were *de novo* assembled with SPAdes v3.14.1 [4] using the "isolate" option and kmer size of 21, 33, 55 and 77. The assembly procedure generated 341 scaffolds. Finally, the scaffolds were sorted in descending order according to their length and annotated by GOLD (Genomes OnLine Database) [15,36] (GOLD Analysis ID: Ga0466249). The genome sequence and its annotation is publicly available at, and can be downloaded from, JGI's IMG platform (https://img.jgi.doe.gov) under accession number 2901394022. As no genes for the subunits of methylmalonyl-CoA carboxyltransferase were identified in the genome, the amino acid sequences of these enzymes in *Propionibacterium freudenreichii* subsp. *freudenreichii* were aligned to the genome of BoRhaA^T using BLAST [1,31].

Phylogenetic analyses

The alignment of the 16S rRNA gene sequence of BoRhaA^T with related strains was calculated using the SINA Aligner online tool (https://www.arb-silva.de/aligner) removing bases remaining unaligned at the ends [45]. As no 16S-rRNA gene sequence could be detected in the incomplete genome sequence, the sequence obtained by Sanger-sequencing was added as additional contig for the phylogenetic analyses. The Genome Taxonomy Database toolkit (gtdb-tk) was used to calculate an alignment of marker genes from the genome sequences [14]. Phylogenetic trees were calculated with iqtree [39]. The trees were edited and Bootstrap values were visualized with the online tool of itol.embl.de [30] and in inkscape (inkscape.org). AAI values were obtained with the software toolkit CompareM (https://github.com/dparks1134/CompareM) using DIAMOND to perform sequence similarity searches [8] and Prodigal for gene calling [23].

Enzyme assay

Activity of NADPH-dependent methylglyoxal reductase was determined in photometric assays in a Jasco Photometer V-630 (Tokyo, Japan) at 30°C. Assays were carried out in cuvettes with 10 mm light path sealed with rubber stoppers and gassed with 100% N_2. Additions were made with syringes. The assay mix consisted of 950 µl of anoxic 50 mM Tris-HCl buffer, pH 7.5, with 3 mM DTT. Then, 20 µl of cell-free extract and 20 µl of NADPH to a final concentration of 0.2 mM were added. The protein contents were 8.4 mg/ml and 10.4 mg/ml in glucose- and rhamnose-grown cells, respectively. The unspecific decrease of absorption of NADPH was followed at 365 nm for 2 min. Then, methylglyoxal was added from a 1 M anoxic stock solution to a final concentration of 10 mM. One unit was defined as 1 µmol NADPH oxidized per min at 30°C and at pH 7.5. Specific activities were expressed as units per mg protein (U/mg). Protein concentrations were estimated with the Bradford assay [7].

Results

Enrichment and isolation

Rhamnose-degrading anaerobic bacteria were selected in direct dilution series from profundal sediment material in agar-solidified mineral medium containing rhamnose as sole source of carbon and energy and a background lawn of *M. hungatei*. After incubation for 4 weeks, colonies developed down to the 5th–7th dilution tube, corresponding to a cell number of about 10^6 cells per ml of inoculum material. These colonies were surrounded by satellite colonies. Microscopic examination of the colony material revealed rod-shaped cells of about 3–5 µm length accompanied by long filamentous spirilloid cells of ca. 10 µm length or longer which were most likely cells of *M. hungatei*. Rhamnose-degrading cells grew also without the partner organism after pasteurization of the cell material. In the absence of the partner, the rhamnose-degrading strain grew reliably only in the presence of 0.05% yeast extract. Product patterns of dissolved fermentation products with and without *M. hungatei* were almost identical, yet the formation of hydrogen was not investigated. It was concluded that *M. hungatei* might use hydrogen formed by strain BoRhaA^T but strain BoRhaA^T did not depend on *M. hungatei*. Therefore, only the axenic culture was investigated further.

Cell morphology

Cells of strain BoRhaA^T were motile and rod-shaped, 2.9–5.3 µm long and 0.7–1.1 µm wide (Fig. 1). Cells occurred as single cells or in pairs. In older cultures chains of several cells and thicker cells were observed. Although the culture survived pasteurization for 10 min at 80°C spores could not be detected by microcopy in the culture at any time. Genes involved in sporulation were identified in the genome and are listed in Table S1.

Chemotaxonomic characterization

Cells of strain BoRhaA^T stained Gram-positive as determined both by staining with crystal violet and by the KOH test. Catalase and oxidase tests were negative, indicating a strictly anaerobic life-

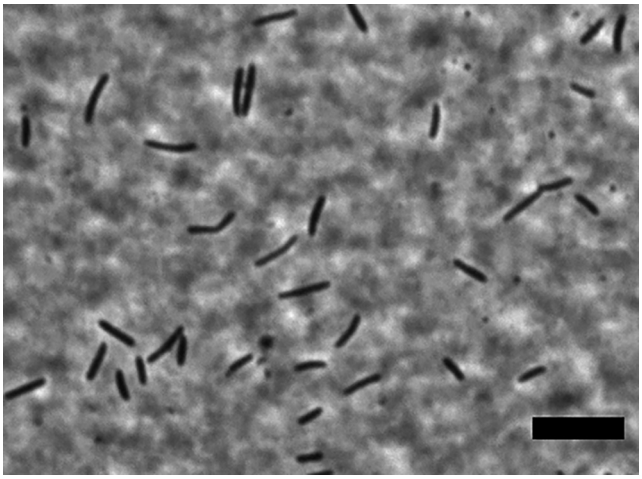

Fig. 1. Phase contrast micrograph of cells of strain BoRhaA^T grown on rhamnose. Bar length equals 10 μm.

style (Table 1). No quinones were detected, confirming a strictly fermentative lifestyle. The experimental G+C content was 40.6%; the one predicted by the genome sequence was 40.8. The most dominant fatty acids were $C_{16:1}\omega9c$, i-$C_{13:0}$3OH, $C_{16:0}$ and $C_{17:1}\omega8c$ with 8–21% (Table S2). Other members of the family Sporomusaceae outlined in Table S2 vary highly in their fatty acid composition and a common trend can hardly be seen which is similar to previous observations in *Methylomusa anaerophila* and *Lucifera butyrica* [2,48]. The only abundant (≥10%) fatty acid that strain BoRhaA has in common with other close relatives is $C_{16:1}$ ω9c which is only abundant in *Lucifera butyrica* and *Methylomonas anaerophila* (Table S2).

Polar lipids were composed of glycolipids, phosphatidylethanolamine, phosphoaminolipid and other lipids, of which phosphatidylethanolamine was most abundant (Fig. S1).

Growth characteristics

Growth was not observed under oxic conditions but observed in non-reduced anoxic freshwater medium. In anoxic, sulfide-reduced medium, strain BoRhaA^T degraded L-rhamnose, glucose, arabinose, fructose, gluconate, mannitol, xylose, and citrate (Table 2). No growth was observed with pectin, L-fucose, lactate, acetate, CO, 1,2-propanediol, glycerol, H_2/CO_2, maleate, pyruvate, and succinate (Table 2). For comparison of the growth behavior of strain BoRhaA^T with the growth behavior of closely related strains and other rhamnose utilizers, additional growth tests were also done with *Anaerosporomusa subterranea* and *Clostridium phytofermentans* in this study. Table 1 shows growth of the latter two strains in sulfide-reduced medium, even though the medium for *C. phytofermentans* was described as anoxic with cysteine as

Table 1
Protologue description of the genus and the species of strain BoRhaA^T.

	Genus	Species
Genus name	*Pelorhabdus*	*Pelorhabdus*
Species name		*Pelorhabdus rhamnosifermentans*
Genus status	gen. nov.	–
Genus etymology	Pe.lo.rhab'dus. Gr. masc. n. *pelos*, mud; Gr. fem. n. *rhabdos*, staff; fem. n. *Pelorhabdus* a staff from mud	–
Type species of the genus	*Pelorhabdus rhamnosifermentans*	
Specific epithet		*rhamnosifermentans*
Species status		sp. nov.
Species etymology		rham.no.si.fer.men'tans N.L. neut. n. *rhamnosum*, rhamnose; L. pres. part, *fermentans*, fermenting; N.L. part. adj. *rhamnosifermentans*, fermenting rhamnose
Description of the new taxon and diagnostic traits	Strictly anaerobic, Gram-positive, motile bacterium. Cells are catalase-negative and oxidase negative after anaerobic growth with 5 mM rhamnose. Cells are rod-shaped and 0.7–1.1 μm wide and 2.9–5.3 μm long. Growth depends on sulfide as reducing agent and is observed with L-rhamnose, glucose, arabinose, fructose, gluconate, mannitol, xylose, and citrate as sole sources of carbon and energy. No growth is observed with L-fucose, lactate, acetate, CO, 1,2-propanediol, glycerol, H_2/CO_2, maleate, pyruvate, and succinate. The bacterium grows at a temperature of 15–30°C and at pH 4–7.5. Growth mode is restricted to mixed product fermentation yielding acetate, propionate, ethanol, butyrate, and 1-propanol as major fermentation products. Oxygen and nitrate are not utilized as external electron acceptors. Addition of small amounts of yeast extract allows reproducible growth of laboratory cultures. The G+C content was 40.6% G+C. The most dominant fatty acids were $C_{16:1}\omega9c$, i-$C_{13:0}$3OH, $C_{16:0}$ and $C_{17:1}\omega8c$ with 8–21% (Table S1). Polar lipids were composed of glycolipids, phosphatidylethanolamine, phosphoaminolipid and other lipids, of which phosphatidylethanolamine was most abundant.	
Country of origin	Germany	
Region of origin	Upper Lake Constance near Mainau island	
Date of isolation	22/03/2018	
Source of isolation	profundal sediment (80 m below water surface)	
Sampling date	23/01/2018	
Latitude	47°41′43.7″N	
Longitude	9°12′50.8″E	
Altitude (meters above sea level)	~311 m (sediment surface)	
16S rRNA gene accession nr.	MW010209 (NCBI)	
Genome accession number	2901394022 (IMG), ASM1883558v1 (NCBI)	
Genome status	draft	
Genome size	5251 kbp	
GC mol%	40.8 (genome based)	
Number of strains in study	1	
Designation of the Type Strain	*Pelorhabdus rhamnosifermentans*	*Pelorhabdus rhamnosifermentans* strain BoRhaA^T
Strain Collection Numbers	DSM 111565^T = JCM 39158^T	

Table 2
Morphological and physiological characteristics of 1, strain BoRhaA^T; 2, *Anaerosporomusa subterranea* strain RU4 (taken from [16]); 3, *Lucifera butyrica* (taken from [48]); 4, *Dendrosporobacter quercicolus* (taken from [55,57]); 5, *Pelosinus fermentans* (taken from [51]; 6, *Anaerosinus glycerini* DSM 5192 (taken from [49,56]); 7, *Methylomusa anaerophila* (taken from [2]); 8, *Anaerostipes rhamnosivorans* (taken from [9]); 9 *Clostridium phytofermentans* strain ISDg (taken from [64]) ("+" = observable growth, "–" = no observable growth, "nd" = no data, [1] data from this study). Additional substrate tests in this study were done with sulfide-reduced media for comparability.

strain	1	2	3	4	5	6	7	8	9
Oxygen	strictly anaerobic	anaerobic	obligate anaerobe	anaerobic	anaerobic	strictly anaerobic	strictly anaerobic	strictly anaerobic	anaerobic
Gram type	+	–	+	–	–	–	–	–/+	+
Shape	straight rods	curved rods	rod-shaped	straight rods	Slightly curved rods	vibrioid	curved rods	curly rod-shaped	straight rods
Motility	+	+	+	+	+	-	+	-	+
Spores	not visible	+	+	+	+	-	+	+	+
GC [%]	40.6	52	47.0	48.5	41	34.3	46.6	44.5	35.9
temp spec	15–30	24–30	25–40	20–45	4–36	10–42	30–37	15–45	
temp opt	30		37	25–30	22–30	37		37	37
growth rate	0.15/h @30°C pH 7.2 with rhamnose					0.12/h @ 25°C; 0.43/h @ 37°C			
pH range	4–7.5	5–7.5	3.5–7	nd	5.5–8	5.0–8.5	5.9–6.9	5.5–8.0	6.0–9.0
pH opt			5.5	nd	7	6.5–7.5		6.5–7.0	
Substrates									
1,2-Propane-diol	–	–[1]	nd	nd	nd	nd	nd	nd	–[1]
Acetate	–	–	nd	nd	nd	nd	nd	nd	–[1]
Arabinose	+	–[1]	nd	nd	nd	nd	nd	nd	+
Citrate	+	–	+	nd	nd	nd	nd	nd	–[1]
CO	–	–[1]	nd	nd	nd	nd	nd	nd	–[1]
Formate	–	–	nd	nd	nd	nd	nd	nd	–[1]
Fructose	+	–[1]	–	+	+	–	–	nd	+
L-Fucose	–	–[1]	nd	nd	nd	nd	nd	nd	+
Fumarate	+	+	nd	nd	+	–	–	nd	–[1]
Glucose	+	–	+	nd	+	–	nd	nd	+
Gluconate	+	–[1]	nd	nd	nd	nd	nd	nd	–[1]
Glycerol	–	–	+	+	–	+	–	nd	–[1]
H_2/CO_2	–	–	nd	–	–	–	–	nd	–[1]
Lactate	–	–	–	nd	+	–	+	nd	–[1]
Maleate	–	+	nd	nd	nd	nd	nd	nd	–[1]
Mannitol	+	–[1]	nd	–	+	–	nd	+	–[1]
Mannose	+	–[1]	+	nd	nd	nd	nd	nd	+
Pectin	–	+[1]	nd	nd	nd	nd	nd	nd	+
Pyruvate	–	–	+	nd	+	–	nd	nd	–[1]
Ribose	–	–[1]	+	nd	nd	nd	nd	+	+
Succinate	–	–	+	nd	+	–	nd	nd	–[1]
Sucrose	–	–[1]	–	nd	nd	nd	nd	nd	–
Trehalose	–	–[1]	nd	nd	nd	nd	nd	+	–
Xylose	+	–[1]	+	nd	nd	nd	nd	–	+
L–Rhamnose	+	–[1]	+	nd	nd	nd	nd	+	+

reducing agent [64]. Yet, in the positive control with 10 mM L-rhamnose and 0.05% yeast extract a final OD_{600} of 0.171 was observed, indicating that growth of *C. phytofermentans* in the presence of sulfide is possible. *C. phytofermentans* as a distant phylogenetic relative of strain BoRhaA exclusively utilizes monosaccharides and pectin, while the close relative *A. subterranea* only uses fumarate, maleate and pectin (Δ OD_{600} with pectin = 0.1 after 35 days, Table 1). Strain BoRhaA is more versatile and utilizes a wide range of the above-mentioned sugars, organic acids, and alcohols. When grown with rhamnose or glucose, the main fermentation products of strain BoRhaA^T were acetate, propionate, ethanol, butyrate and propanol as shown in Table 2. Fermentation of glucose produced more ethanol, fermentation of rhamnose more propionate. No growth was observed with sulfate as electron acceptor, neither with rhamnose nor lactate as electron donor. Growth with nitrate as electron acceptor and rhamnose as electron donor was lower compared to cultures with rhamnose alone. Nitrite was not formed in nitrate-supplied cultures. The genome contains transporters for nitrate and sulfate, but at first sight no genes for nitrate or sulfate reduction. Strain BoRhaA^T grew only in freshwater medium at 15, 25 and 30°C. Growth was observed with initial pH between 4 and 7.5 with similar growth rates. Cells survived –20°C for 24 h and 80°C for 20 min but neither 100°C nor 121°C for 20 min. When vitamin B_{12} (adenosylcobalamin) was added to cultures with rhamnose, 1,2-propanediol was transiently formed as an intermediate. Growth with 1,2-propanediol and 0.05% yeast extract and in the presence of adenosylcobalamin was not observed. The fermentation patterns in Table 2 did not suggest that strain BoRhaA^T is an acetogen, yet genes of the Wood-Ljungdahl-pathway can be found in the genome sequence (see below).

Taxonomic classification

Phylogenetic analysis of the almost complete 16S rRNA gene identified strain BoRhaA^T as a member of the family Sporomusaceae (ezbiocloud.net) within the order Selenomonadales, belonging to the Negativicutes. Sporomusaceae were described as primarily spore-forming, Gram-negative, straight or slightly curved rods [12]. The closest identified relatives were *Anaerosporomusa subterranea* strain RU4 16S (92.41%), *Dendrosporobacter quercicolus* strain DSM 1736 (91.80%), *Anaerosinus glycerini* strain DSM 5192 (91.68%) and *Pelosinus fermentans* (91.48%). Illumina sequencing and assembly of the genome of strain BoRhaA^T yielded a total of 5250691 bp on 341 scaffolds. Annotation identified 5629 genes (2901394022 to 2901399650); 5436 of them were protein-coding genes. The genome tree is shown in Fig. 2 and the 16S tree in Fig. 3. AAI values of strain BoRhaA^T to all stains used in the trees were pairwise calculated using the software toolkit CompareM and are listed in Table 3.

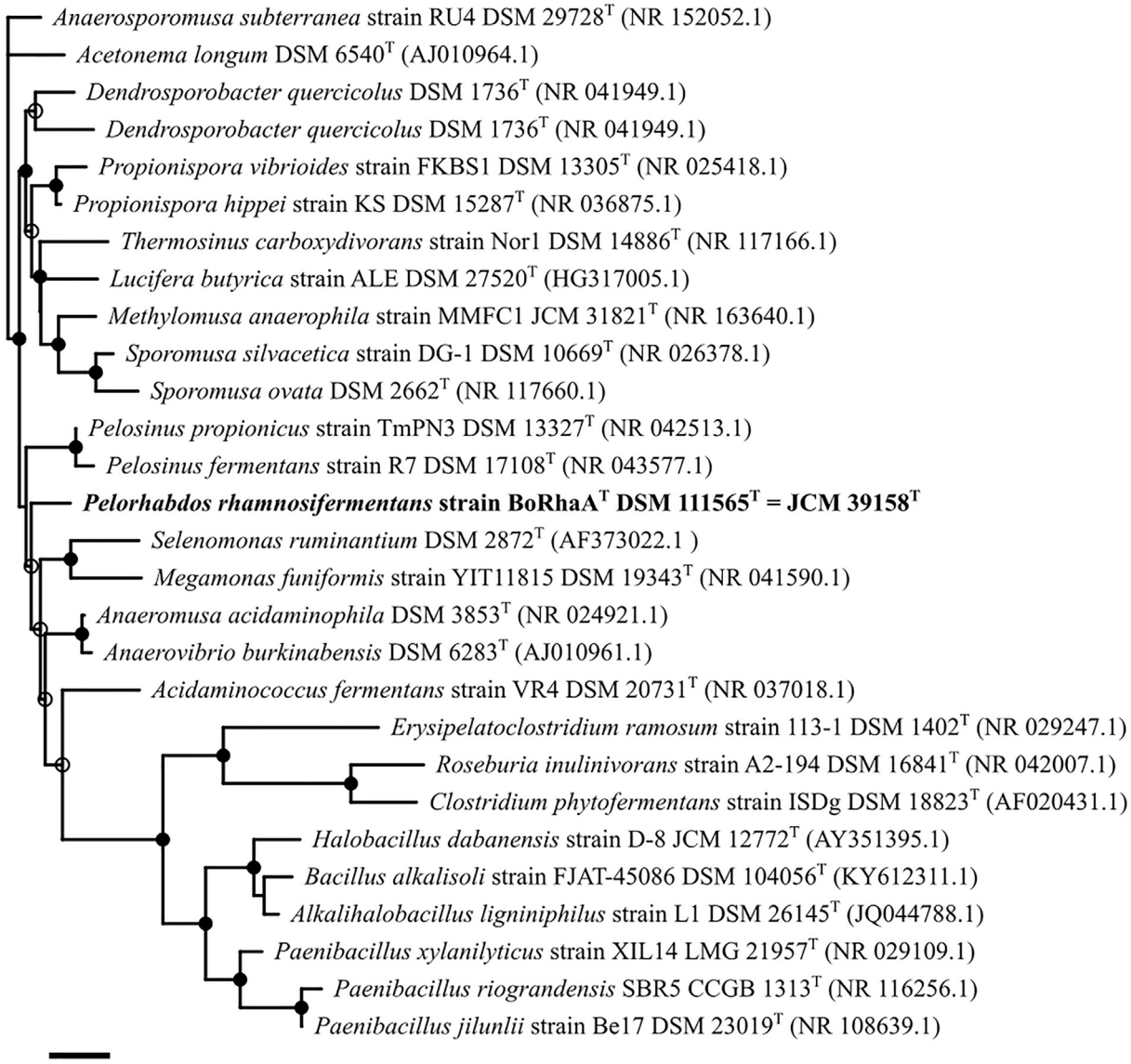

Fig. 2. Phylogenetic tree of strain BoRhA^T based on the 16S rRNA gene sequence comparison. Empty circles indicate a Bootstrap support of >70%, filled circles >90% derived form 1000 samples. Scale bar indicates 1 nucleotide substitution per side. The alignment was calculated using the SINA Aligner and the tree was created using iqtree. The tree was visualized in the online tool of https://itol.embl.de/ and Inkscape.

We propose *Pelorhabdus rhamnosifermentans* strain BoRhaA^T as a novel bacterial species and genus (Table 1). According to the 16S rRNA gene sequence, strain BoRhaA^T belongs to the family Sporomusaceae (ezbiocloud.net) and the closest relative is *A. subterranea* with an identity of 92.4%. As the currently recommended thresholds for the classification of new species and genera by 16S rRNA gene similarity are 98.7% [54] and 94.5% [71], respectively, a classification into a novel genus is indicated. Analysis of the genome confirms this concept as the AAI threshold for the same genus is 65% [27] and the highest values calculated for the reference type strain in this study is 62.7% (Table 4).

Glycolysis genes

All genes for glycolysis enzymes except for phosphoglycerate kinase were identified and are listed in Table S3. Four genes were identified as 6-phosphofructokinase 1, seven as fructose-bisphosphate aldolase class II, and two as glyceraldehyde 3-phosphate dehydrogenase.

Rhamnose degradation genes

All genes for rhamnose degradation according to Petit et al. [42] were identified and are listed in Table S4. The presence of genes for microcompartment shell proteins indicates that propionaldehyde derived from the degradation of rhamnose is processed in microcompartments (Table S4). The enzymes whose genes in the genome sequence of strain BoRhaA^T are annotated as lactaldehyde reductase, acetyltransferase, and acetate kinase are predicted to fulfill the same functions as the enzymes whose genes in *C. phytofermentans* are annotated as 1,2-propanediol oxidoreductase, propionyl-CoA-transferase and acetate/propionate kinase according to the annotation of Petit et al. [42].

Methylmalonyl-CoA pathway genes

Genes of the TCA cycle that are potentially involved in the conversion of pyruvate to succinyl-CoA and further fermentation to propionic acid are listed in Table S5. There are four genes coding

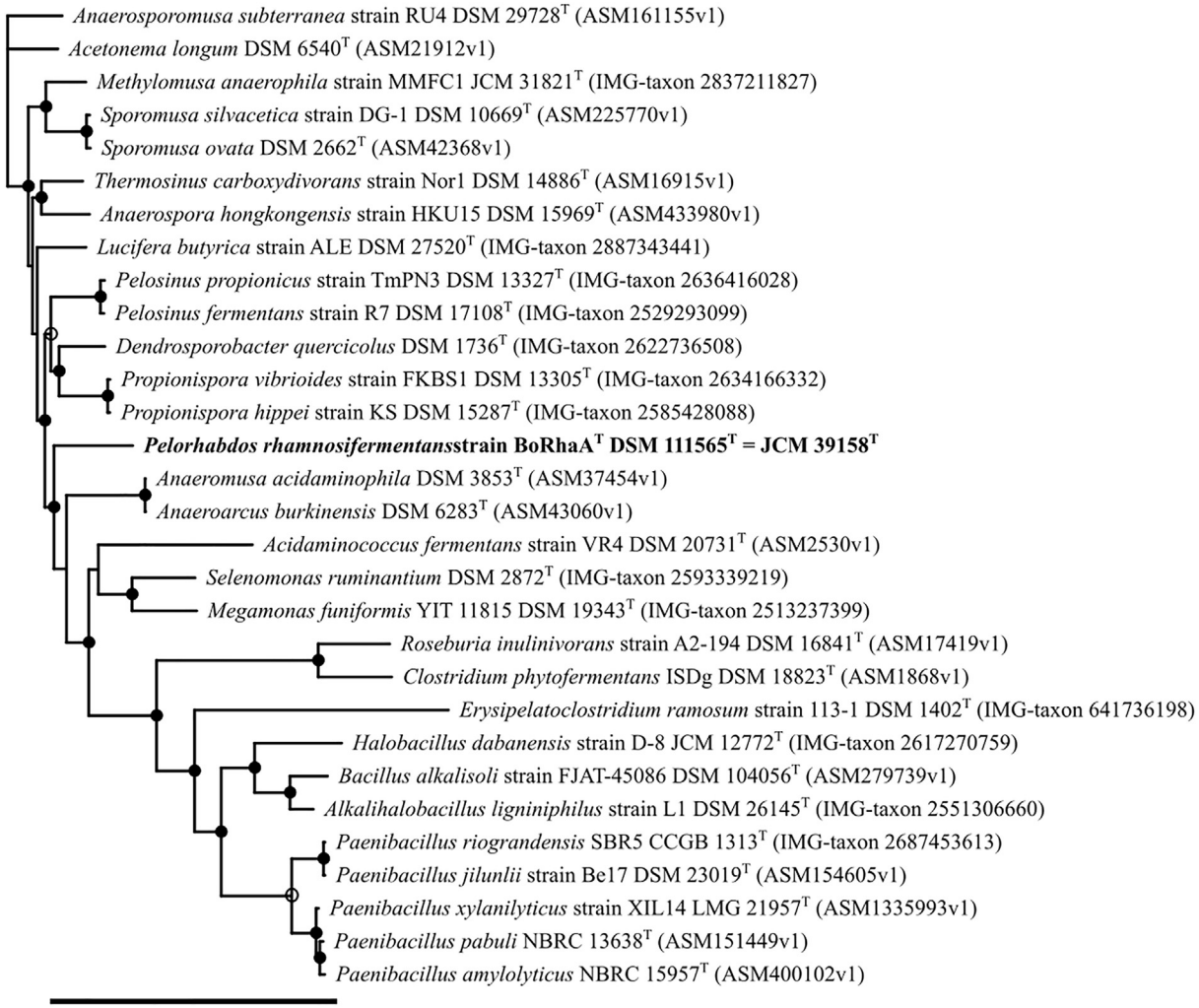

Fig. 3. Phylogenetic tree of strain BoRhA^T based on the genome sequence comparison. Empty circles indicate a Bootstrap support of >90%, filled circles >95% derived form 1000 samples. Scale bar indicates 1 nucleotide substitution per side. The alignment was calculated using gtdb-tk and the tree was created using iqtree. The tree was visualized in the online tool of https://itol.embl.de/ and Inkscape.

Table 3
Stoichiometry of rhamnose and glucose fermentation by strain BoRhaA^T. Mean values from three cultures.

Substrate	Substrate concentration (mM)	Cell dry mass formed (mg/l)[a]	Substrate assimilated (mM)[b]	Substrate dissimilated (mM)	Products formed (mM)					Electron recovery [%]
					Acetate	Propionate	Ethanol	Butyrate	Propanol	
L-rhamnose	4.87 ± 0.01	65.7 ± 4.28	0.42 ± 0.03	4.34 ± 0.12	2.37 ± 0.08	3.18 ± 0.16	1.15 ± 0.14	0.37 ± 0.13	1.21 ± 0.06	94.4 ± 3.7
D-glucose	5.55 ± 0.10	82.4 ± 7.5	0.57 ± 0.05	2.89 ± 0.35	1.94 ± 0.26	0.19 ± 0.05	3.32 ± 0.74	0.56 ± 0.08	0.47 ± 0.03	111.7 ± 9.4

a) Calculated from an experimentally determined conversion factor of 250 mg/l at OD_{600} = 1 as in [33].
b) Assimilation equation:
L-rhamnose: 34 $C_6H_{12}O_5$ + 12 H_2O+4 HCO_3^- → 52 < $C_4H_7O_3$> + 4 OH^-.
D-glucose: 17 $C_6H_{12}O_6$ → 24<$C_4H_7O_3$>+ 6HCO_3^- + 12 H_2O + 6H^+.

for an aconitate hydratase and three sets of the alpha and beta subunit of the 2-oxoglutarate ferredoxin oxidoreductase clustered pairwise, and an additional gene for the alpha subunit. Two clustered sets of methylmalonyl-CoA mutase were annotated and two genes for methylmalonyl-CoA/ethylmalonyl-CoA epimerase. No genes for the subunits of methylmalonyl-CoA carboxyltransferase were identified by the IMG-annotations. Yet, a BLAST-search with the amino-acid sequences of the subunits of methylmalonyl-CoA carboxyltransferase from *P. freudenreichii* subsp. *freudenreichii* against the genome of strain BoRhaA^T revealed several potential candidate genes for each of the 1.3S, 5S, and 12S subunits with a maximum identity of 44, 49, and 57%, respectively, and are listed in Table S6.

Wood-Ljungdahl-pathway genes

Genes of the Wood-Ljungdahl-pathway are found in one apparent consecutive gene cluster and include CO-dehydrogenase, acetyl-CoA synthase, methylene-tetrahydrofolate reductase, methylene-tetrahydrofolate dehydrogenase, formate tetrahydrofolate ligase, carbonmonoxide dehydrogenase catalytic subunit, acetyl-CoA synthase, acetyl-CoA decarbonylase/synthase complex

Table 4
Pairwise amino acid identity (%AAI) values of related genomes to the genome of strain BoRhaA^T. Values were calculated using the software toolkit CompareM.

	Assembly accession	%AAI with BoRhaA^T
Sporomusa silvacetica DSM 10669	GCA_002257705	62.7
Pelosinus fermentans DSM 17108	GCA_000271485	62.3
Thermosinus carboxydivorans Nor1	GCA_000169155	62.1
Pelosinus propionicus DSM 13327	GCA_900114615	62.0
Sporomusa ovata DSM 2662	GCF_000423685	62.0
Anaerospora hongkongensis	GCA_004339805	61.7
Propionispora vibrioides	GCA_900110485	61.2
Propionispora hippei DSM 15287	GCA_900141835	61.1
Dendrosporobacter quercicolus	GCA_900104455	61.1
Acetonema longum DSM 6540	GCA_000219125	60.4
Anaerosporomusa subterranea	GCA_001611555	60.2
Anaeroarcus burkinensis DSM 6283	GCA_000430605	59.3
Anaeromusa acidaminophila	GCA_000374545	59.2
Megamonas funiformis YIT 11815	GCA_000245775	54.7
Selenomonas ruminantium	GCA_900107615	54.6
Acidaminococcus fermentans DSM 20731	GCA_000025305	52.0
Paenibacillus jilunlii	GCA_001546055	49.0
Paenibacillus riograndensis SBR5	GCA_000981585	49.0
Paenibacillus pabuli NBRC 13638	GCA_001514495	48.8
Paenibacillus xylanilyticus	GCA_013359935	48.7
Paenibacillus amylolyticus	GCA_004001025	48.6
Alkalihalobacillus ligniniphilus	GCA_000334155	48.5
Bacillus sp. FJAT-45086	GCA_002797395	48.3
Clostridium phytofermentans ISDg	GCA_000018685	47.8
Halobacillus dabanensis	GCA_900114165	47.6
Roseburia inulinivorans	GCA_000174195	47.5
Erysipelatoclostridium ramosum	GCA_900660185	46.2

subunit gamma, acetyl-CoA decarbonylase/synthase complex subunit delta, 5-methyltetrahydrofolate corrinoid/iron sulfur protein methyltransferase and 5-methyltetrahydrofolate-homocysteine methyltransferase (Table S7).

Enzyme assay

For the identification of a biochemical pathway that links glycolysis with lactaldehyde and 1,2-propanediol degradation, activities of methylglyoxal reductase were assayed. Activities of NADPH-dependent methylglyoxal reductase were detected in crude extracts of cells grown with glucose (33.3 ± 3.6 U/mg) or rhamnose (19.6 ± 0.5 U/mg).

Discussion

Various bacteria such as *Escherichia coli*, *Bacillus subtilis,* and *Clostridium phytofermentans* are known for their ability to ferment rhamnose via a phosphorylation pathway which splits rhamnose into dihydroxyacetone phosphate and lactaldehyde using ATP [22,36,42]. Lactaldehyde is then converted via 1,2-propanediol to propionate and propanol inside microcompartments, while dihydroxyacetone phosphate goes through the EMP pathway finally to the mixed-acid fermentation. Furthermore, two pathways of non-phosphorylating oxidative rhamnose dissimilation using NAD(P)$^+$ in bacteria have been described, the aldolase pathway producing pyruvate and lactaldehyde [25,26,65] and the diketohydrolase pathway producing pyruvate and lactate [3,66]. It is likely that rhamnose accumulates due to the faster degradation of the much larger supply of glucose, while rhamnose is utilized slowly by specialists. As strain BoRhaA^T also has genes for the methylmalonyl-CoA pathway, propionate could theoretically be formed also through this route. However, production of 1-propanol as a fermentation end-product cannot be explained in this case. Moreover, succinate cannot be utilized and converted to propionate by strain BoRhaA^T, thus the expression of the genes of the methylmalonyl-CoA pathway is unlikely [31]. Possibly, methylmalonyl-CoA pathway genes are expressed only under anaerobic, non-reducing conditions, as the methylmalonyl-CoA operon is under the control of a redox sensing transcriptional regulator. Moreover, the non-phosphorylating, oxidative degradation pathway yielding pyruvate and lactate from rhamnose is not used by strain BoRhaA^T, as lactate is not among the fermentation products. Therefore, we conclude that strain BoRhaA^T most likely degrades rhamnose via the phosphorylation pathway and thus through microcompartments that generate 1,2-propanediol and finally propionate and 1-propanol. Besides the latter two products, butyrate was formed as well during growth of strain BoRhaA^T with rhamnose or glucose. Butyrate was not reported as product of rhamnose fermentation in *C. phytofermentans* [42], but has been reported as a product of rhamnose fermentation in the intestinal rhamnose degrader *A. rhamnosivorans* [9]. In *C. phytofermentans*, uptake of rhamnose or fucose depends on two separate transporter systems [42]. Strain BoRhA uses rhamnose, but not the structurally related fucose, even though a gene annotated as FHS family L-fucose permease-like MFS transporter (IMG locus-tag Ga0466249_047_5000_6346) was identified in the genome sequence. Interestingly, glucose fermentation yields the same products as rhamnose fermentation, though at different concentrations, and the production of propionate and propanol indicates that similar biochemical routes are used for both rhamnose and glucose. Degradation of rhamnose in *C. phytofermentans* occurs through lactaldehyde and 1,2-propanediol. The latter is frequently formed as reduced end product in other organisms [9], but in *C. phytofermentans* it is further dehydrated to propionaldehyde and subsequently disproportionated to propionate and propanol [42]. The same pathway seems to be used by strain BoRhaA^T as these genes were identified in the genome of strain BoRhaA^T (Table S4). Moreover, glucose is most likely converted to dihydroxyacetone phosphate (DHAP). Then, DHAP is likely converted to methylglyoxal (2-oxopropanal), which is reduced to lactaldehyde as judged by our enzyme assays. Genes of enzymes catalyzing this reaction were also identified in the genome (Table S8). Further degradation of lactaldehyde could follow the same pathway as in the degradation of rhamnose, although the ethanol-forming branch of the metabolic pathway is used more intensively in glucose-grown cells to reoxidize redox carriers (Table 2). The reactions of lactaldehyde and 1,2-propanediol conversion occur in *C. phytofermentans* inside microcompartments, and the latter reaction was dependent on vitamin B_{12} [42]. We observed transient 1,2-propanediol accumulation in cultures with additional B_{12} indicating an involvement of microcompartment-resident enzymes during rhamnose degradation in strain BoRhaA^T. On the other hand, the combination of glycolytic enzymes with the enzymes of 1,2-propanediol utilization connected through the methylglyoxal pathway could pose a novel mechanism of glucose degradation. The redundancy of genes coding for glycolysis enzymes and their distribution over the genome might be due to their involvement in more branched degradation pathways. The fermentation patterns and our enzyme test indicate that the metabolites glyceraldehyde 3-phosphate and dihydroxyacetone phosphate from glycolysis are also degraded via methylglyoxal to propionate and propanol. The ability to degrade both glucose and rhamnose through similar pathways might render strain BoRhaA^T more competitive towards other rhamnose-non-utilizers, as the strain can use the same enzymatic machinery for both substrates via the connection between DHAP, methylglyoxal, and lactaldehyde. Yet, even strain BoRhaA^T might have a lower affinity for rhamnose than for glucose due to its biochemically difficult degradation, with lactaldehyde as toxic intermediate that has to be metabolized in

microcompartments. We did not find any indications that strain BoRhaA^T degrades polysaccharides. For instance, neither did strain BoRhaA^T grow with pectin nor were pectin degradation genes found in the genome. The strain therefore possibly depends on polymer-degrading microorganisms such as the pectin degrader *Clostridium butyricum* strain 4P1 to release rhamnose as an intermediate from pectin breakdown [50]. Moreover, in pore water samples of sediment cores that were 20-fold concentrated by freeze-drying, we did not detect rhamnose by HPLC-RID, possibly because the tight cooperation of polysaccharide degraders with rhamnose degraders might not lead to an accumulation of rhamnose in sediments of Lake Constance (data not shown). It is conceivable that strain BoRhaA^T contains many more to-date unknown modes of growth. For instance, even though genes of the Wood-Ljungdahl-pathway are present in the genome, these genes were likely not expressed under any of the investigated growth conditions, as judged also from the fermentation patterns. In acetogens, acetate would be expected as the sole fermentation product of sugar degradation, which was not the case for BoRhaA^T. On the other hand, acetogens should be able to utilize H_2 and CO_2 or formate, but also these substrates did not promote growth of strain BoRhaA^T. Hence, under native growth conditions, strain BoRhaA^T might act as an acetogen, which could potentially extend its substrate range. By such metabolic flexibility of bacteria, the microbial community is able to respond to changes in the food supply that could be caused by both seasonal and trophic fluctuations. With their broad substrate spectrum such bacteria ensure the adaptation of the ecosystem to external influences and ensure the reversibility of the ecosystem after disturbances have subsided. On the other hand, after disturbing events or during repeated fluctuations of the trophic state of a lake, these bacteria could act as pioneers to re-initiate the microbial community.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the Research Training Group (GRK 2272) R3 – Responses to biotic and abiotic Changes, Resilience and Reversibility of Lake Ecosystems and the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, for funding. This study was funded also by research funds of the University of Konstanz. The Joint Genome Institute is acknowledged for the annotation of the genome sequence. The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. We gratefully acknowledge guidance and recommendations by Joana Thiel and Franziska Klotz on phylogenetic analyses, technical help by Julia Schmidt, Sylke Wiechmann, and Antje Wiese, and help with sediment coring by Alfred Sulger and Josef Halder.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.syapm.2021.126225.

References

[1] Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. (1990) Basic local alignment search tool. J. Mol. Biol. 215, 403–410.
[2] Amano, N., Yamamuro, A., Miyahara, M., Kouzuma, A., Abe, T., Watanabe, K. (2018) *Methylomusa anaerophila* gen. nov., sp. nov., an anaerobic methanol-utilizing bacterium isolated from a microbial fuel cell. Int. J. Syst. Evol. Microbiol. 68, 1118–1122.
[3] Bae, J., Kim, S.M., Lee, S.B. (2015) Identification and characterization of 2-keto-3-deoxy-L-rhamnonate dehydrogenase belonging to the MDR superfamily from the thermoacidophilic bacterium *Sulfobacillus thermosulfidooxidans*: implications to L-rhamnose metabolism in archaea. Extremophiles 19 (2), 469–478.
[4] Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19 (5), 455–477.
[5] Bligh, E.G., Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37 (8), 911–917.
[6] Bolger, A.M., Lohse, M., Usadel, B. (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120.
[7] Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
[8] Buchfink, B., Xie, C., Huson, D.H. (2014) Fast and sensitive protein alignment using DIAMOND. Nat. Methods 12 (1), 59–60.
[9] Bui, T.P.N., de Vos, W.M., Plugge, C.M. (2014) *Anaerostipes rhamnosivorans* sp. nov., a human intestinal, butyrate-forming bacterium. Int. J. Syst. Evol. Microbiol. 64 (PART 3), 787–793.
[10] Bui, T.P.N., Schols, H.A., Jonathan, M., Stams, A.J.M., de Vos, W.M., Plugge, C.M. (2019) Mutual metabolic interactions in co-cultures of the intestinal *Anaerostipes rhamnosivorans* with an acetogen, methanogen, or pectin-degrader affecting butyrate production. Front. Microbiol. 10 (November), 1–12.
[11] Buttke, T.M., Ingram, L.O. (1975) Comparison of lipopolysaccharides from *Agmenellum quadruplicatum* to *Escherichia coli* and *Salmonella typhimurium* by using thin layer chromatography. J. Bacteriol. 124 (3), 1566–1573.
[12] Campbell, C., Adeolu, M., Gupta, R.S. (2015) Genome-based taxonomic framework for the class Negativicutes: Division of the class Negativicutes into the orders Selenomonadales emend., Acidaminococcales ord. nov. and Veillonellales ord. nov. Int. J. Syst. Evol. Microbiol. 65 (9), 3203–3215.
[13] Cashion, P., Holder-Franklin, M.A., McCully, J., Franklin, M. (1977) A rapid method for the base ratio determination of bacterial DNA. Anal. Biochem. 81 (2), 461–466.
[14] Chaumeil, P.A., Mussig, A.J., Hugenholtz, P., Parks, D.H. (2020) GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics 36, 1925–1927.
[15] Chen, I.M.A., Chu, K., Palaniappan, K., Pillay, M., Ratner, A., Huang, J., Huntemann, M., Varghese, N., White, J.R., Seshadri, R., Smirnova, T., Kirton, E., Jungbluth, S.P., Woyke, T., Eloe-Fadrosh, E.A., Ivanova, N.N., Kyrpides, N.C. (2019) IMG/M vol 5.0: An integrated data management and comparative analysis system for microbial genomes and microbiomes. Nucleic Acids Res. 47 (D1), 666–677.
[16] Choi, J.K., Shah, M., Yee, N. (2016) *Anaerosporomusa subterranea* gen. nov., sp. nov., a spore-forming anaerobe belonging to the class Negativicutes isolated from saprolite. Int. J. Syst. Evol. Microbiol. 66 (10), 3848–3854.
[17] Chun, J., Lee, J.H., Jung, Y., Kim, M., Kim, S., Kim, B.K., Lim, Y.W. (2007) EzTaxon: A web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int. J. Syst. Evol. Microbiol. 57 (10), 2259–2261.
[18] Edwards, U., Rogall, T., Blöcker, H., Emde, M., Böttger, E.C. (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res. 17, 7843–7853.
[19] Giraud, M.F., Naismith, J.H. (2000) The rhamnose pathway. Curr. Opin. Struct. Biol. 10, 687–696.
[20] Gregersen, T. (1978) Rapid method for distinction of Gram-negative from Gram-positive bacteria. Eur. J. Appl. Microbiol. Biotechnol. 5 (2), 123–127.
[21] Hicks, R.E., Owen, C.J., Aas, P. (1994) Deposition, resuspension, and decomposition of particulate organic matter in the sediments of Lake Itasca, Minnesota, USA. Hydrobiologia 284 (1), 79–91.
[22] Hirooka, K., Kodoi, Y., Satomura, T., Fujita, Y. (2016) Regulation of the rhaEWRBMA operon involved in L-rhamnose catabolism through two transcriptional factors, RhaR and CcpA, *Bacillus subtilis*. J. Bacteriol. 198 (5), 830–845.
[23] Hyatt, D., Chen, G.-L., LoCascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J. (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinform. 11 (1), 119.
[24] Kawasaki, N., Komatsu, K., Kohzu, A., Tomioka, N., Shinohara, R., Satou, T., Watanabe, F.N., Tada, Y., Hamasaki, K., Kushairi, M.R.M., Imai, A. (2013) Bacterial contribution to dissolved organic matter in eutrophic lake Kasumigaura. Japan. Appl. Environ. Microbiol. 79 (23), 7160–7168.
[25] Koivistoinen, O.M., Hilditch, S., Voutilainen, S.P., Boer, H., Penttilä, M., Richard, P. (2008) Identification in the yeast *Pichia stipitis* of the first L-rhamnose-1-dehydrogenase gene. FEBS J. 275 (10), 2482–2488.
[26] Koivistoinen, O.M., Arvas, M., Headman, J.R., Andberg, M., Penttilä, M., Jeffries, T.W., Richard, P. (2012) Characterisation of the gene cluster for L-rhamnose catabolism in the yeast *Scheffersomyces (Pichia) stipitis*. Gene 492 (1), 177–185.
[27] Konstantinidis, K.T., Rosselló-Móra, R., Amann, R. (2017) Uncultivated microbes in need of their own taxonomy. ISME J. 11 (11), 2399–2406.

[28] Krumböck, M., Conrad, R. (1991) Metabolism of position-labelled glucose in anoxic methanogenic paddy soil and lake sediment. FEMS Microbiol. Lett. 85 (3), 247–256.
[29] Kuykendall, L.D., Roy, M.A., O'Neill, J.J., Devine, T.E. (1988) Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. Int. J. Syst. Bacteriol. 38 (4), 358–361.
[30] Letunic, I., Bork, P. (2007) Interactive tree of life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinform. 23 (1), 127–128.
[31] Liu, B., Popp, D., Müller, N., Sträuber, H., Harms, H., Kleinsteuber, S. (2020) Three novel *Clostridia* isolates produce *n*-caproate and *iso*-butyrate from lactate: Comparative genomics of chain-elongating bacteria. Microorganisms 8 (12), 1–23.
[32] Mesbah, M., Premachandran, U., Whitman, W.B. (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. Int. J. Syst. Bacteriol. 39 (2), 159–167.
[33] Miller, L.T. (1982) Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. J. Clin. Microbiol. 16 (3), 584–586.
[34] Moe, W.M., Stebbing, R.E., Rao, J.U., Bowman, K.S., Nobre, M.F., da Costa, M.S., Rainey, F.A. (2012) *Pelosinus defluvii* sp. nov., isolated from chlorinated solvent-contaminated groundwater, emended description of the genus *Pelosinus* and transfer of *Sporotalea propionica* to *Pelosinus propionicus* comb. nov. Int. J. Syst. Evol. Microbiol. 62, 1369–1376.
[35] Moralejo, P., Egan, S.M., Hidalgo, E., Aguilar, J. (1993) Sequencing and characterization of a gene cluster encoding the enzymes for L-rhamnose metabolism in *Escherichia coli*. J. Bacteriol. 175 (17), 5585–5594.
[36] Mukherjee, S., Stamatis, D., Bertsch, J., Ovchinnikova, G., Katta, H.Y., Mojica, A., Chen, I.M.A., Kyrpides, N.C., Reddy, T.B.K. (2019) Genomes OnLine database (GOLD) vol 7: Updates and new features. Nucleic Acids Res. 47 (D1), 649–659.
[37] Müller, N., Griffin, B.M., Stingl, U., Schink, B. (2008) Dominant sugar utilizers in sediment of Lake Constance depend on syntrophic cooperation with methanogenic partner organisms. Environ. Microbiol. 10 (6), 1501–1511.
[38] Müller, N., Scherag, F.D., Pester, M., Schink, B. (2015) *Bacillus stamsii* sp. nov., a facultatively anaerobic sugar degrader that is numerically dominant in freshwater lake sediment. Syst. Appl. Microbiol. 38 (6), 379–389.
[39] Nguyen, L.T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32 (1), 268–274.
[40] Ochiai, M., Hanya, T. (1980) Vertical distribution of monosaccharides in lake water. Hydrobiologia 70 (1–2), 165–169.
[41] Patil, Y., Junghare, M., Müller, N. (2017) Fermentation of glycerol by *Anaerobium acetethylicum* and its potential use in biofuel production. Microb. Biotechnol. 10, 203–217.
[42] Petit, E., LaTouf, W.G., Coppi, M.V., Warnick, T.A., Currie, D., Romashko, I., Deshpande, S., Haas, K., Alvelo-Maurosa, J.G., Wardman, C., Schnell, D.J., Leschine, S.B., Blanchard, J.L. (2013) Involvement of a bacterial microcompartment in the metabolism of fucose and rhamnose by *Clostridium phytofermentans*. PLoS One 8 (1), 1–12.
[43] Pfennig, N. (1978) *Rhodocyclus purpureus* gen. nov. and sp. nov., a ring-shaped, vitamin B_{12}-requiring member of the family Rhodospirillaceae. Int. J. Syst. Bacteriol. 6, 283–288.
[44] Pfennig, N., Wagener, S. (1986) An improved method of preparing wet mounts for photomicrographs of microorganisms. J. Microbiol. Methods 4 (5–6), 303–306.
[45] Pruesse, E., Peplies, J., Glöckner, F.O. (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28, 1823–1829.
[46] Reinhardt, A., Johnsen, U., Schönheit, P. (2019) L-rhamnose catabolism in archaea. Mol. Microbiol. 111 (4), 1093–1108.
[47] Rogers, M.A. (1965) Carbohydrates in aquatic plants and associated sediments from two Minnesota lakes. Geochim. Cosmochim. Acta 29 (3), 183–200.
[48] Sánchez-Andrea, I., Florentino, A.P., Semerel, J., Strepis, N., Sousa, D.Z., Stams, A.J.M. (2018) Co-culture of a novel fermentative bacterium, *Lucifera butyrica* gen. nov. sp. nov., with the sulfur reducer *Desulfurella amilsii* for enhanced sulfidogenesis. Front. Microbiol. 9, 3108. https://doi.org/10.3389/fmicb.2018.03108.
[49] Schauder, R., Schink, B. (1989) *Anaerovibrio glycerini* sp. nov., an anaerobic bacterium fermenting glycerol to propionate, cell matter, and hydrogen. Arch. Microbiol. 152 (5), 473–478.
[50] Schink, B., Ward, J.C., Zeikus, J.G. (1981) Microbiology of wetwood: importance of pectin degradation and *Clostridium* species in living trees. Appl. Environ. Microbiol. 42 (3), 526–532.
[51] Shelobolina, E.S., Nevin, K.P., Blakeney-Hayward, J.D., Johnsen, C.V., Plaia, T.W., Krader, P., Woodard, T., Holmes, D.E., VanPraagh, C.G., Lovley, D.R. (2007) *Geobacter pickeringii* sp. nov., *Geobacter argillaceus* sp. nov. and *Pelosinus fermentans* gen. nov., sp. nov., isolated from subsurface kaolin lenses. Int. J. Syst. Evol. Microbiol. 57 (1), 126–135.
[52] Song, K., Wen, Z., Xu, Y., Yang, H., Lyu, L., Zhao, Y., Fang, C., Shang, Y., Du, J. (2018) Dissolved carbon in a large variety of lakes across five limnetic regions in China. J. Hydrol. 563, 143–154.
[53] Staats, N., De Winder, B., Mur, L.R., Stal, L.J. (1999) Isolation and characterization of extracellular polysaccharides from the epipelic diatoms *Cylindrotheca closterium* and *Navicula salinarum*. Eur. J. Phycol. 34 (2), 161–169.
[54] Stackebrandt, E., Ebers, J. (2006) Taxonomic parameters revisited: tarnished gold standards. Microbiol. Today 8, 6–9.
[55] Stankewich, J.P., Cosenza, B.J., Shigo, A.L. (1971) *Clostridium quercicolum* sp. n., isolated from discolored tissues in living oak trees. Antonie Van Leeuwenhoek 37, 299–302.
[56] Strömpl, C., Tindall, B.J., Jarvis, G.N., Lünsdorf, H., Moore, E.R.B., Hippe, H. (1999) A re-evaluation of the taxonomy of the genus *Anaerovibrio*, with the reclassification of *Anaerovibrio glycerini* as *Anaerosinus glycerini* gen. nov., comb. nov., and *Anaerovibrio burkinabensis* as *Anaeroarcus burkinensis* [corrig.] gen. nov., comb. nov. Int. J. Syst. Evol. Microbiol. 49, 1861–1872.
[57] Strömpl, C., Tindall, B.J., Lünsdorf, H., Wong, T.Y., Moore, E.R.B., Hippe, H. (2000) Reclassification of *Clostridium quercicolum* as *Dendrosporobacter quercicolus* gen. nov., comb. nov. Int. J. Syst. Evol. Microbiol. 50, 101–106.
[58] Tamaoka, J., Komagata, K. (1984) Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microbiol. Lett. 25 (1), 125–128.
[59] Tindall, B.J. (1990) Lipid composition of *Halobacterium lacusprofundi*. FEMS Microbiol. Lett. 66 (1–3), 199–202.
[60] Tindall, B.J. (1990) A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. Syst. Appl. Microbiol. 13 (2), 128–130.
[61] Tindall, B.J., Sikorski, J., Smibert, R.A., Krieg, N.R. (2007) Phenotypic characterization and the principles of comparative systematics. Methods for General and Molecular Microbiology, Third Edition, American Society of Microbiology, pp. 330–93.
[62] Tschech, A., Pfennig, N. (1984) Growth yield increase linked to caffeate reduction in *Acetobacterium woodii*. Arch. Microbiol. 137 (2), 163–167.
[63] Wagstaff, B.A., Rejzek, M., Kuhaudomlarp, S., Hill, L., Mascia, I., Nepogodiev, S. A., Dorfmueller, H.C., Field, R.A. (2019) Discovery of an RmlC/D fusion protein in the microalga *Prymnesium parvum* and its implications for NDP-L-rhamnose biosynthesis in microalgae. J. Biol. Chem. 294 (23), 9172–9185.
[64] Warnick, T.A., Methe, B.A., Leschine, S.B. (2002) *Clostridium phytofermentans* sp. nov., a cellulolytic mesophile from forest soil. Int. J. Syst. Evol. Microbiol. 52 (4), 1155–1160.
[65] Watanabe, S., Piyanart, S., Makino, K. (2008) Metabolic fate of L-lactaldehyde derived from an alternative L-rhamnose pathway. FEBS J. 275 (20), 5139–5149.
[66] Watanabe, S., Makino, K. (2009) Novel modified version of non-phosphorylated sugar metabolism - an alternative L-rhamnose pathway of *Sphingomonas* sp. FEBS J. 276 (6), 1554–1567.
[67] Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J. (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 173 (2), 697–703.
[68] Widdel, F., Kohring, G.W., Mayer, F. (1983) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids.III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov. sp. nov., and *Desulfonema magna* sp. nov. Arch. Microbiol. 134, 286–294.
[69] Widdel, F., Bak, F. Gram-negative mesophilic sulfate-reducing bacteria. In: Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H. (Eds.), The Prokaryotes. Springer Verlag, Berlin, pp. 3352–3378.
[70] William, S., Feil, H., Copeland, A. (2012) Bacterial genomic DNA isolation using CTAB. Sigma 50 (6876).
[71] Yarza, P., Yilmaz, P., Pruesse, E., Glöckner, F.O., Ludwig, W., Schleifer, K.H., Whitman, W.B., Euzéby, J., Amann, R., Roselló-Móra, R. (2014) Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat. Rev. Microbiol. 12 (9), 635–645.