Supplemental material

# Comparative analysis of *Brucepastera parasyntrophica* gen. nov., sp. nov. and *Teretinema zuelzerae* gen. nov., comb. nov. (*Treponemataceae*) reveals the importance of interspecies hydrogen transfer in the energy metabolism of spirochetes

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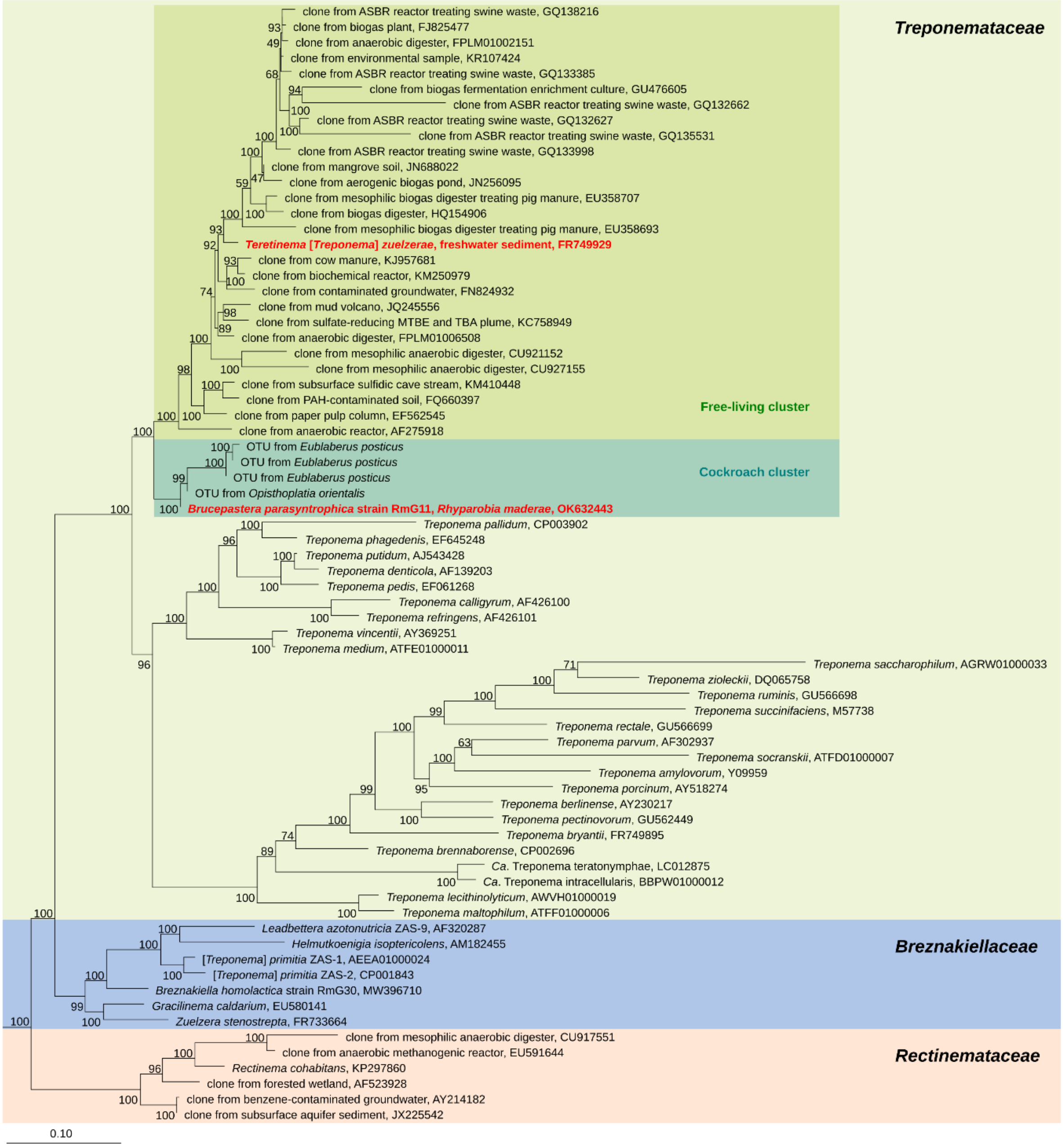

**Fig. S1.** 16S rRNA gene tree illustrating the relationship of strain RmG11 and *Treponema zuelzerae* to their closest relatives in public datasets. Members of *Breznakiellaceae* and *Rectinemataceae* were used as outgroup.

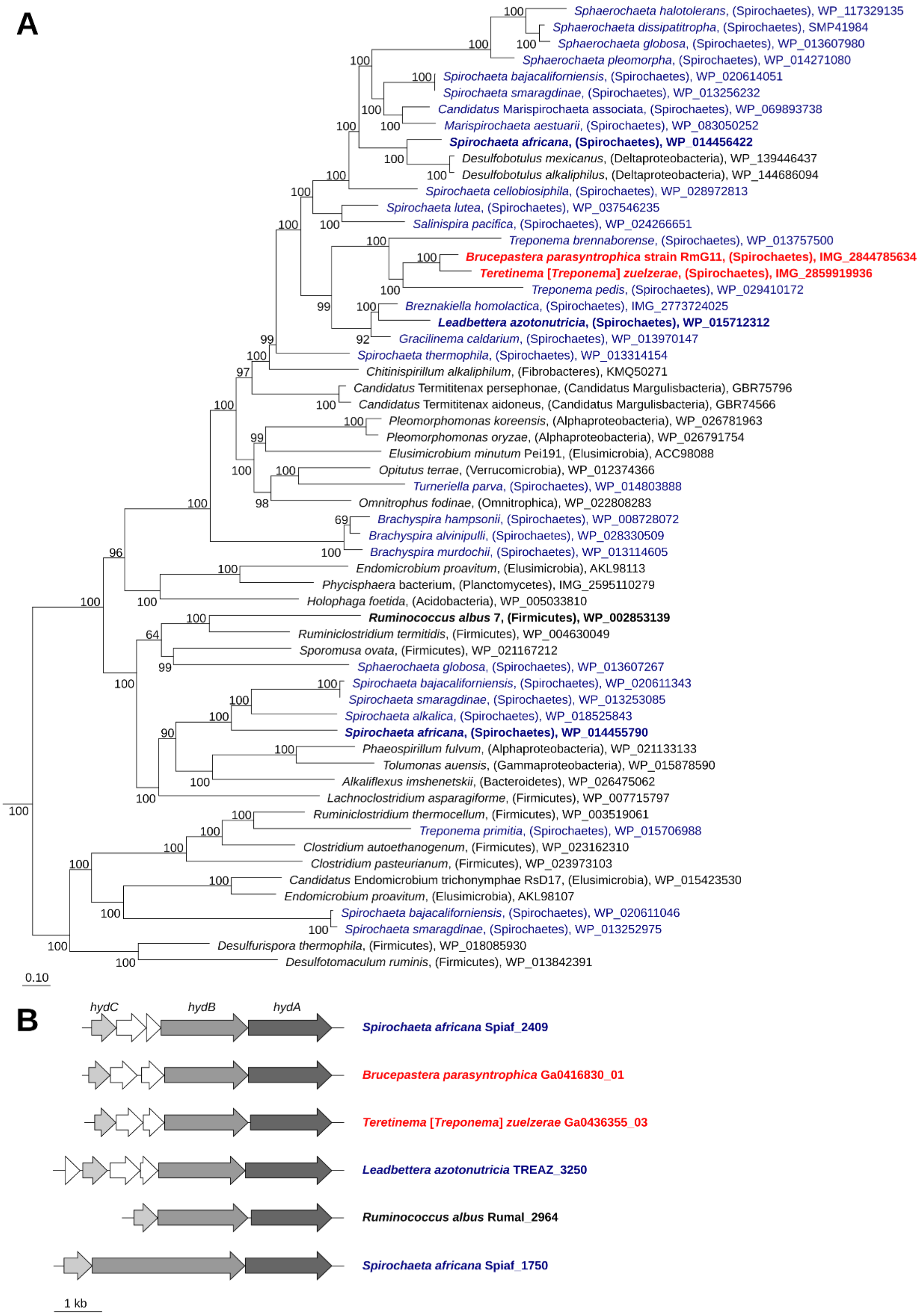

**Fig. S2.** (A) Phylogenetic analysis of the catalytic subunit HydA of the ferredoxin- and $NAD^+$-dependent electron-confurcating [FeFe]-hydrogenases (group A3) of strain RmG11 and *Treponema zuelzerae*. The maximum-likelihood tree is based on the comprehensive dataset reported by Greening *et al.* (1) and rooted with representatives of group C. Homologs from spirochetes are set in blue. (B) Organization of the gene sets encoding the subunits of selected orthologs (set in boldface in A).

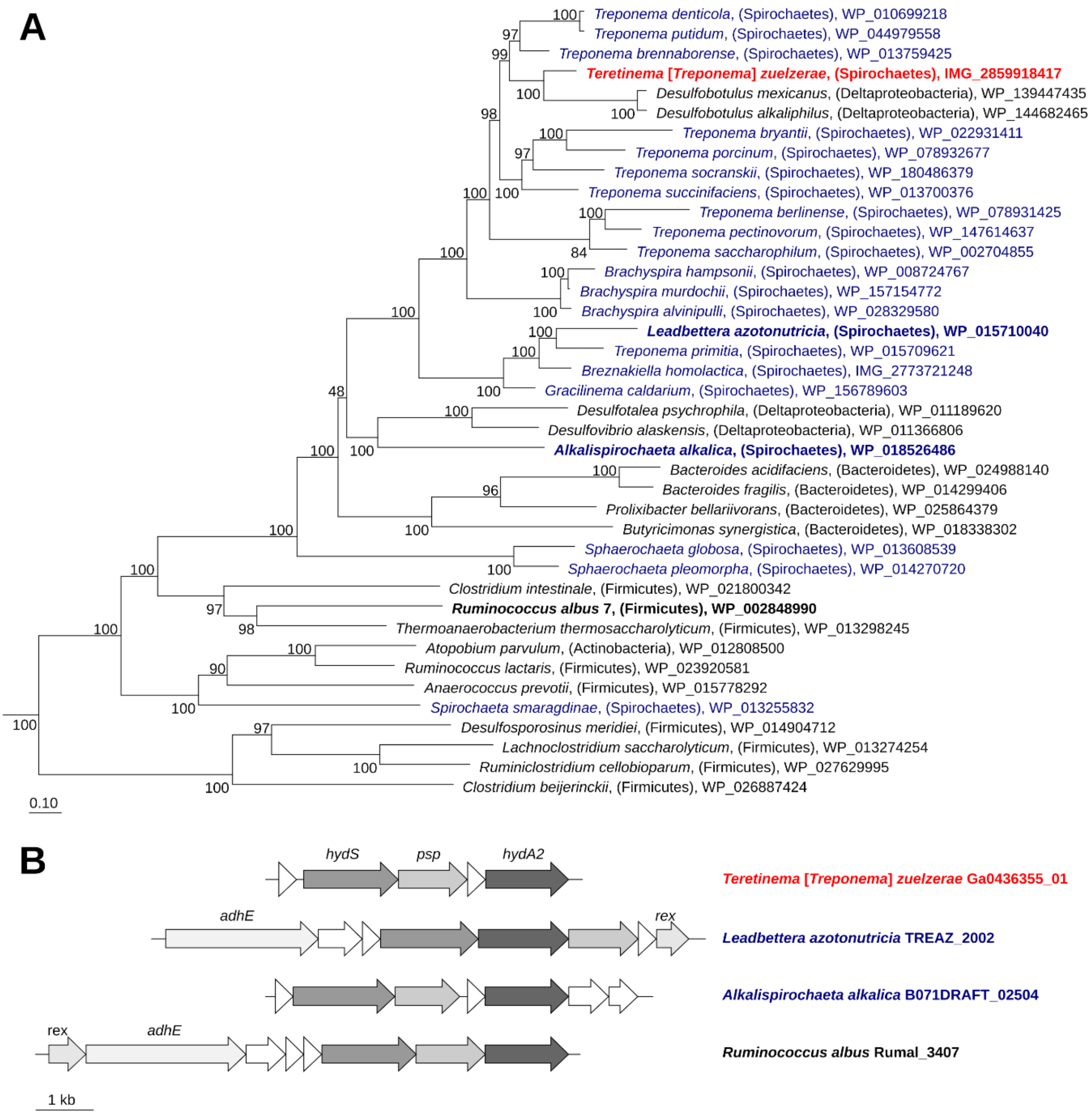

**Fig. S3.** (A) Phylogenetic analysis of the monomeric ferredoxin-dependent [FeFe]-hydrogenases HydA2 (group B) of *Treponema zuelzerae*. The maximum-likelihood tree is based on the comprehensive data set reported by Greening *et al.* (2016) and rooted with representatives of group C. Homologs from spirochetes are set in blue. (B) Organization of the gene sets encoding selected orthologs (set in boldface in A). Gene abbreviations: *hydS*, $H_2$-sensing [FeFe]-hydrogenase (group C3); *psp*, protein serine/threonine phosphatase; *rex*, redox-sensing transcriptional repressor; *adhE*, bi-functional alcohol/aldehyde dehydrogenase.

**Table S1.** The influence of $H_2$ partial pressure ($P[H_2]$) on the growth and fermentation products of strain RmG11 and *Treponema zuelzerae*. Values are means of results obtained with replicate cultures (less than 10% deviation).

| Initial $P[H_2]$ (bar) | Glucose consumed (mM) | Turbidity ($OD_{578}$) | Growth yield (g/mol) | Glucose assim. (mM) | Products formed (mM) | | | | | | Final $P[H_2]$ (bar) | Carbon recovery (%) | Electron recovery (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Ethanol | Acetate | $H_2$[a] | Lactate | Pyruvate | Succinate | | | |
| Strain RmG11 (*n* = 3) | | | | | | | | | | | | | |
| 0 | 7.4 | 0.163 | 12.9 | 0.7 | 8.6 | 1.4 | 6.1 | 0.3 | 0.9 | – | 0.066 | 81 | 84 |
| 0.045 | 6.6 | 0.163 | 14.5 | 0.7 | 7.0 | 0.8 | 5.1 | 0.3 | 1.1 | – | 0.101 | 75 | 78 |
| 0.091 | 4.8 | 0.113 | 13.9 | 0.5 | 3.6 | 0.3 | 3.0 | 0.3 | 1.1 | – | 0.124 | 59 | 62 |
| *Treponema zuelzerae* (n = 2) | | | | | | | | | | | | | |
| 0 | 8.9 | 0.460 | 29.4 | 1.8 | – | 14.5 | 30.3 | 1.0 | – | 0.3 | 0.330 | 105 | 107 |
| 0.800 | 8.9 | 0.459 | 29.3 | 1.8 | – | 13.4 | 30.4 | 1.9 | – | 0.3 | 1.130 | 104 | 108 |

Growth parameters were calculated as described in Table 1. Turbidity and products formed in basal medium without substrate were subtracted in the subsequent calculations.

[a] $H_2$ concentration is expressed as if $H_2$ were completely dissolved in the liquid phase to facilitate comparison with other products.

**Table S2.** The effect of $H_2$ removal from the culture headspace of strain RmG11 on growth and fermentation products from glucose (8 mM). In the flushed group, the headspace gas ($N_2/CO_2$) was exchanged every 24 h. Values are means of results obtained with *n* replicates (± standard deviation).

| Headspace | Glucose consumed | Turbidity | Growth yield | Glucose assim. | Products formed (mM) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (mM) | ($OD_{578}$) | (g/mol) | (mM) | Ethanol | Acetate | $H_2$[a] | Lactate | Pyruvate |
| Not flushed (*n* = 3) | 6.7 ± 0.1 | 0.147 ± 0.002 | 12.9 ± 0.3 | 0.6 ± 0.0 | 6.3 ± 0.2 | 1.9 ± 0.0 | 6.5 ± 0.3 | 0.3 ± 0.0 | 1.6 ± 0.0 |
| Flushed (*n* = 6) | 5.7 ± 0.2 | 0.163 ± 0.004 | 16.9 ± 1.0 | 0.7 ± 0.0 | 3.9 ± 0.3 | 3.2 ± 0.1 | ND | 0.3 ± 0.0 | 1.2 ± 0.1 |

ND, not determined. Growth parameters were calculated as described in Table 1. Turbidity and products formed in basal medium without substrate were subtracted in the subsequent calculations.

[a] $H_2$ concentration is expressed as if $H_2$ were completely dissolved in the liquid phase to facilitate comparison with other products.

**Table S3.** The growth parameters and fermentation products of strain RmG11 in pure culture and in coculture with *Methanospirillum hungatei*. Values are means of results obtained with triplicated cultures (± standard deviation).

|  | Glucose consumed | Turbidity | Growth yield | Relative cell | Glucose assim. | Products formed (mM) |  |  |  |  | Carbon recovery | Electron recovery |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | (mM) | ($OD_{578}$) | (g/mol) | density[a] | (mM) | Ethanol | Acetate | $H_2$[b] | Lactate | Pyruvate | (%) | (%) |
| Pure culture | 4.1 ± 0.1 | 0.067 ± 0.003 | 9.2 ± 0.4 | 1.0 ± 0.4 | 0.3 ± 0.0 | 3.6 ± 0.3 | 1.5 ± 0.4 | 1.1 ± 0.2 | 0.5 ± 0.4 | 0.2 ± 0.0 | 72.2 ± 6.2 | 68.1 ± 3.6 |
| Coculture | 4.0 ± 0.1 | 0.103 ± 0.006 | 15.0 ± 0.9[c] | 2.9 ± 1.0 | 0.4 ± 0.0 | – | 6.2 ± 0.2 | 9.3 ± 0.9[d] | – | – | 84.5 ± 2.0 | 76.9 ± 2.8 |

Growth parameters were calculated as in Table 1. Turbidity and products formed in basal medium without substrate were subtracted in the subsequent calculations.

[a] Cell counts of strain RmG11 were determined by phase-contrast microscopy at the end of growth. The cell counts of *M. hungatei* did not increase significantly in the coculture.

[b] $H_2$ concentration is expressed as if $H_2$ were completely dissolved in the liquid phase to facilitate comparison with other products. The formation of 1 mM $H_2$ corresponds to a partial pressure of 0.011 bar.

[c] The increase in turbidity contributed by *M. hungatei* was not corrected but assumed to be negligible due to its low growth yield on hydrogen.

[d] $H_2$ formed by strain RmG11 in the coculture was estimated from $CH_4$ production, using a stoichiometry of 4:1.

**Table S4.** Genes encoding metabolic pathways of strain RmG11 and *Treponema zuelzerae*. The genes are sorted by functional groups. Homologs detected in the respective genomes are labeled with their IMG gene IDs.

| **Enzyme** | **Strain RmG11** | ***Treponema zuelzerae*** |
|---|---|---|
| **Monosaccharide and disaccharide utilization** | | |
| Fructokinase [EC:2.7.1.4] | 2844785895 | 2859918318 |
| Mannose-6-phosphate isomerase [EC:5.3.1.8] | - | 2859917266 |
| Galactose mutarotase/aldose 1-epimerase [EC:5.1.3.3] | - | 2859918872 |
| Galactokinase [EC:2.7.1.6] | 2844786647 | 2859918921 |
| Glucosamine-6-phosphate deaminase [EC:3.5.99.6] | 2844785501 | 2859918342 |
| N-Acetylglucosamine-6-phosphate deacetylase [EC:3.5.1.25] | - | 2859918343 |
| D-Xylose isomerase [EC:5.3.1.5] | - | 2859917701 |
| D-Xylulose kinase [EC:2.7.1.17] | - | 2859917700 |
| L-Arabinose isomerase [EC:5.3.1.4] | - | 2859919999 |
| L-Ribulokinase [EC:2.7.1.16] | - | 2859920000 |
| L-Ribulose 5-phosphate 4-epimerase [EC:5.1.3.4] | - | 2859919998 |
| D-Ribokinase [EC:2.7.1.15] | - | - |
| Cellobiose phosphorylase [EC:2.4.1.20] | - | 2859918084;<br>2859918087;<br>2859918338;<br>2859918341 |
| Trehalase [EC:3.2.1.28] | 2844788064 | 2859918269 |
| Trehalose phosphorylase [EC:2.4.1.64] | - | - |
| Maltase [EC:3.2.1.20] | - | - |
| Maltose phosphorylase [EC:2.4.1.8] | - | - |
| Maltose alpha-D-glucosyltransferase/trehalose synthase [EC:5.4.99.16] | 2844786167 | - |
| Lactase [EC:3.2.1.108] | - | - |
| **Glycolysis** | | |
| Hexokinase [EC:2.7.1.1] | 2844787036 | 2859918746 |
| Glucose-6-phosphate isomerase [EC:5.3.1.9] | 2844786390 | 2859917494 |
| 6-Phosphofructokinase [EC:2.7.1.11] | 2844785449;<br>2844785718 | 2859918378;<br>2859919869 |
| Diphosphate—fructose-6-phosphate 1-phosphotransferase. [EC:2.7.1.90] | 2844785875 | 2859917748 |
| Fructose-bisphosphate aldolase class I [EC:4.1.2.13] | 2844787493 | - |
| Fructose-bisphosphate aldolase class II [EC:4.1.2.13] | 2844787172 | 2859917259 |
| Triosephosphate isomerase [EC:5.3.1.1] | 2844787389 | 2859917335 |
| Glyceraldehyde-3-phosphate dehydrogenase, type I [EC:1.2.1.12] | 2844787391 | 2859917344 |
| 3-Phosphoglycerate kinase [EC:2.7.2.3] | 2844787390 | 2859917336 |
| Phosphoglycerate mutase (BPG-dependent) [EC:5.4.2.11] | 2844786469 | - |

| | | |
|---|---|---|
| Phosphoglycerate mutase (BPG-independent) [EC:5.4.2.12] | 2844787713 | 2859919460 |
| Enolase/phosphopyruvate hydratase [EC:4.2.1.11] | 2844785699 | 2859919564 |
| Pyruvate kinase [EC:2.7.1.40] | 2844785927 | 2859917756 |
| **Pentose phosphate pathway** | | |
| Glucose-6-phosphate dehydrogenase [EC:1.1.1.49 1.1.1.363] | - | 2859917476 |
| 6-Phosphogluconolactonase [EC:3.1.1.31] | - | - |
| 6-Phosphogluconate dehydrogenase [EC:1.1.1.44 1.1.1.343] | - | 2859919236 |
| Ribose 5-phosphate isomerase [EC:5.3.1.6] | 2844788125 | 2859917890 |
| Ribulose-phosphate 3-epimerase [EC:5.1.3.1] | 2844786804 | 2859918831 |
| Transketolase [EC:2.2.1.1] | 2844788052 | 2859918260 |
| Transaldolase [EC:2.2.1.2] | - | - |
| Phosphoketolase [EC:4.1.2.9] | - | - |
| Pyruvate metabolism | | |
| Pyruvate:ferredoxin oxidoreductase (PFOR) [EC:1.2.7.1] | 2844785656 | 2859918444 |
| Pyruvate formate lyase [EC:2.3.1.54] | - | - |
| Pyruvate carboxylase [EC:6.4.1.1] | - | - |
| Pyruvate decarboxylase [EC:4.1.1.1] | - | - |
| Malate dehydrogenase (oxaloacetate-decarboxylating) [EC:1.1.1.38] | 2844786484 | 2859919074 |
| **Fermentation** | | |
| D-Lactate dehydrogenase [EC:1.1.1.28] | 2844787271 | 2859919199 |
| Phosphate acetyltransferase [EC:2.3.1.8] | 2844786273 | 2859917513 |
| Acetate kinase [EC:2.7.2.1] | 2844786037 | 2859917900 |
| Bifunctional aldehyde dehydrogenase/alcohol dehydrogenase (AdhE) [EC:1.2.1.10 1.1.1.1] | 2844786755 | - |
| Acetaldehyde dehydrogenase [EC:1.2.1.10] | - | - |
| Alcohol dehydrogenase [EC:1.1.1.1] | 2844785670 | 2859920275 |
| **Hydrogenase (and gene neighborhood)** | | |
| HydA, [FeFe] hydrogenase (group A3) | 2844785634 | 2859919936 |
| HydB | 2844785633 | 2859919935 |
| Hypothetical protein | 2844785632 | 2859919934 |
| Hypothetical protein | 2844785631 | 2859919933 |
| HydC | 2844785630 | 2859919932 |
| HydA2, [FeFe] hydrogenase (group B) | - | 2859918417 |
| Hypothetical protein | - | 2859918418 |
| Hypothetical protein | - | 2859918419 |
| HydS, $H_2$-sensing [FeFe]-hydrogenase (group C3) | - | 2859918420 |
| **Redox balance** | | |
| Rnf complex | 2844787908,<br>2844787909,<br>2844787910, | 2859917892,<br>2859917893,<br>2859917894, |

|  |  |  |
|---|---|---|
|  | 2844787911,<br>2844787912,<br>2844787913 | 2859917895,<br>2859917896,<br>2859917897 |
| Ferredoxin—NADP+ reductase [EC:1.18.1.2] | 2844785133 | 2859917171 |
|  |  |  |
| **TCA cycle** |  |  |
| Citrate synthase [EC:2.3.3.1] | 2844786213 | 2859917460 |
| Aconitase/aconitate hydratase [EC:4.2.1.3] | 2844787135 | 2859917225 |
| Isocitrate dehydrogenase [EC:1.1.1.42] | 2844786218 | 2859919060 |
| 2-Oxoglutarate:ferredoxin oxidoreductase complex [EC:1.2.7.3 1.2.7.11] | - | 2859919203,<br>2859919204,<br>2859919205,<br>2859919206 |
| 2-oxoglutarate dehydrogenase (NAD(P)$^+$-dependent) [EC:1.2.4.2] | - | - |
| Succinyl-CoA synthetase [EC:6.2.1.49 1.2.7.11] | - | - |
| Fumarate reductase [EC:1.3.5.4] | - | 2859918090 |
| Fumarase/fumarate hydratase [EC:4.2.1.2] | 2844786546 | 2859918995 |
|  |  |  |
| **Wood–Ljungdahl pathway** |  |  |
| Formate dehydrogenase [EC:1.17.1.9] | - | - |
| Formyl-THF synthetase/Formate-THF ligase [EC:6.3.4.3] | - | - |
| Formyl-THF cyclohydrolase/methylene-THF dehydrogenase [EC:1.5.1.5 3.5.4.9] | 2844787700 | 2859919434 |
| Methylene-THF reductase [EC:1.5.1.20] | 2844787607 | 2859919483 |
| 5,10-Methenyl-THF synthetase [EC:6.3.3.2] | 2844787413 | 2859917368 |
|  |  |  |
| **Gluconeogenesis** |  |  |
| Phosphoglucomutase [EC:5.4.2.2] | 2844786217;<br>2844787190 | 2859917276;<br>2859917314 |
| ADP-glucose pyrophosphorylase/glucose-1-phosphate adenylyltransferase [EC:2.7.7.27] | 2844786869 | 2859918754 |
| Glycogen synthase [EC:2.4.1.21] | 2844786916;<br>2844786988 | 2859918641;<br>2844786988 |
| 1,4-Alpha-glucan branching enzyme [EC:2.4.1.18] | 2844785123;<br>2844786001 | 2859917304 |

**Table S5.** Genes encoding carbohydrate-active enzymes of strain RmG11 and *Treponema zuelzerae* that putatively participate in the degradation of polysaccharides. The genes are sorted by functional groups. Homologs are labeled with their respective IMG gene IDs; genes that encode a signal peptide (SP) are set in boldface.

| **Enzyme** | **CAZy family** | **Strain RmG11** | ***Treponema zuelzerae*** |
|---|---|---|---|
| **Starch and glycogen** | | | |
| Glycogen phosphorylase [EC:2.4.1.1] | GT35 | 2844788247 | 2859917425;<br>2859918179 |
| Pullulanase/glycogen debranching enzyme [EC:3.2.1.41] | GH13 | **2844786880**$^{SP}$;<br>**2844788122**$^{SP}$;<br>2844787709;<br>2844788248;<br>2844785769;<br>2844785693;<br>2844785692 | **2859919897**$^{SP}$;<br>**2859919901**$^{SP}$;<br>2859918180;<br>2859919567 |
| Alpha-amylase [EC:3.2.1.1] | GH13 | **2844786638**$^{SP}$;<br>**2844786879**$^{SP}$;<br>2844787561;<br>2844787707;<br>2844788171 | - |
| Oligo-1,6-glucosidase [EC:3.2.1.10] | GH13 | 2844787561 | 2859919985 |
| Alpha-glucosidase [EC:3.2.1.20] | GH13 | **2844788123**$^{SP}$;<br>2844785312;<br>2844786917 | **2859919900**$^{SP}$;<br>2859918430;<br>2859919899 |
| **Cellulose** | | | |
| Endocellulase/endo-1,4-beta-glucanase [EC:3.2.1.4] | GH5 | 2844785437 | **2859917102**$^{SP}$;<br>**2859917392**$^{SP}$;<br>**2859919167**$^{SP}$;<br>2859917698;<br>2859919966 |
| Beta-glucosidase [EC:3.2.1.21] | GH3 | - | **2859919841**$^{SP}$;<br>**2859917880**$^{SP}$;<br>2859917688;<br>2859919169;<br>2859919833 |
| Beta-glucosidase [EC:3.2.1.21] | GH1 | - | 2859919653;<br>2859919683 |
| **Hemicelluloses** | | | |
| Endo-1,4-beta-xylanase [EC:3.2.1.8] | GH10 | - | **2859920045**$^{SP}$ |
| Endo-1,4-beta-xylanase [EC:3.2.1.8] | GH11 | - | **2859917769**$^{SP}$ |
| Oligosaccharide reducing-end xylanase [EC:3.2.1.156] | GH8 | - | 2859919133 |
| Xylan 1,4-alpha-D-xylosidase [EC:3.2.1.177] | GH31 | - | 2859919984 |

| | | | |
|---|---|---|---|
| Xylan 1,4-beta-D-xylosidase [EC:3.2.1.37] | GH43 | - | **2859917859**$^{SP}$;<br>2859917693;<br>2859917694;<br>2859920011 |
| Mannan endo-1,4-beta-mannosidase [EC:3.2.1.78] | GH5 | - | **2859919965**$^{SP}$ |
| 4-O-Beta-D-mannosyl-D-glucose phosphorylase [EC:2.4.1.281] | GH130 | - | 2859919970 |
| Beta-1,4-mannooligosaccharide/beta-1,4-mannosyl-N-acetylglucosamine phosphorylase [EC:2.4.1.319 2.4.1.320] | GH130 | - | 2859919972 |
| Beta-mannosidase [EC:3.2.1.25] | GH2 | - | 2859919464 |
| Arabinan endo-1,5-alpha-L-arabinosidase [EC:3.2.1.99] | GH43 | - | **2859920010**$^{SP}$;<br>**2859920012**$^{SP}$;<br>2859920005;<br>2859920013 |
| Alpha-L-arabinofuranosidase [EC:3.2.1.55] | GH51 | - | **2859920016**$^{SP}$;<br>2859918045;<br>2859918046 |
| Arabinogalactan endo-1,4-beta-galactosidase [EC:3.2.1.89] | GH53 | 2844786471 | **2859919238**$^{SP}$ |
| Beta-galactosidase [EC:3.2.1.23] | GH2 | - | 2859919671;<br>2859918044 |
| Alpha-galactosidase [EC:3.2.1.22] | GH4 | - | 2859917133 |
| Alpha-galactosidase [EC:3.2.1.22] | GH36 | - | 2859919670 |
| **Other substrates** | | | |
| Endo-1,3(4)-beta-glucanase [EC:3.2.1.6/3.2.1.39] | GH16 | - | **2859917455**$^{SP}$;<br>**2859917923**$^{SP}$;<br>**2859918088**$^{SP}$;<br>**2859918403**$^{SP}$;<br>**2859919837**$^{SP}$;<br>**2859919839**$^{SP}$;<br>**2859919848**$^{SP}$;<br>**2859918065**$^{SP}$ |
| Exo-beta-1,3-glucanase [EC:3.2.1.-] | GH17 | - | **2859919840**$^{SP}$ |
| Beta-N-acetylhexosaminidase [EC:3.2.1.52] | GH3 | 2844785934;<br>2844787186 | **2859917272**$^{SP}$ |
| Hexosaminidase [EC:3.2.1.52] | GH20 | - | 2859918351 |
| Glucosylceramidase [EC:3.2.1.45] | GH30 | - | 2859918275;<br>2859918280 |
| Poly(3-hydroxybutyrate) depolymerase [EC:3.1.1.75] | CE1 | - | 2859919088 |
| Peptidoglycan/xylan/chitin deacetylase [EC:3.5.1.-] | CE4 | **2844786202**$^{SP}$ | **2859920042**$^{SP}$ |

**Table S6** GTDB-tk classification of strain RmG11, *Treponema zuelzerae*, and other members of *Spirochaetales*. The table is available as a separate file (Supplemental File 2).

## Reference

1. Greening C, Biswas A, Carere CR, Jackson CJ, Taylor MC, Stott MB, Cook GM, Morales SE. 2016. Genomic and metagenomic surveys of hydrogenase distribution indicate $H_2$ is a widely utilised energy source for microbial growth and survival. ISME J 10:761–777. https://doi.org/10.1038/ismej.2015.153