Supplemental material:

Hydrogen Formation and its Regulation in *Ruminococcus albus*: Involvement of an Electron-Bifurcating [FeFe]-Hydrogenase, of a Non Electron-bifurcating [FeFe]-hydrogenase and of a Putative Hydrogen-Sensing [FeFe]-Hydrogenase

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TABLE S1 Primers used for transcriptional analysis of genes *hydB*, *hydA2,* and *hydS* (Fig. 2) and co-transcriptional analysis of the rumal_3398-3408 gene cluster (Fig. 5) in *R. albus* 7.

Primer	Sequence (5'→3')	Application
hydB-forward	AAGGAAACAGGCTACCGCAAG	Amplification of a 1,459 bp intragenic region of *hydB*
hydB-reverse	GATCCTCCATCTCGCCCTTG	
hydA2-forward	CGGCAGGATATGAGGTCACGG	Amplification of a 532 bp intragenic region of *hydA2*
hydA2-reverse	CACAGGAACATGGCAGGAGTTC	
hydS-forward	TAATCGCGTGGGTGTGACTGTC	Amplification of a 954 bp intragenic region of *hydS*
hydS-reverse	GGCAGCAGGACGATATCACAAC	
3399-forward	GAGCGTATGTACCGTCAGGG	Amplification of a 1,433 bp intergenic region between *lysR* and *rstR*
3400-reverse	CCAGTGAAAGCGCCAGATTC	
3400-forward	TTGTTATTGTCGGTGCGGGA	Amplification of a 1,979 bp intergenic region between *rstR* and the gene encoding bifunctional acetaldehyde/ethanol dehydrogenase
3401-reverse	ATAGCCTTTACGCCCTCCAC	
3401-forward	TTCATGGGGTGGAAACTCGG	Amplification of a 2,164 bp intergenic region between the gene encoding bifunctional acetaldehyde/ethanol dehydrogenase and the gene encoding Ser/Thr protein kinase
3402-reverse	ACGCTCTTTAGCATTGCCCT	
3402-forward	CGGTAAGACCCTTGCACAGA	Amplification of a 2,238 bp intergenic region between Ser/Thr protein kinase gene and *hydS*
3405-reverse	TTTCAGGAAAGGCAGGCACA	
3405-forward	ATCTTCTGCCCATGCTTGCT	Amplification of a 1,871 bp intergenic region between *hydS* and the gene encoding Ser/Thr protein phosphatase
3406-reverse	CGGATAACGCAGGCAGTAGT	

3406-forward	ATGTTCGGTGAGGGGAGTTG	Amplification of a 2,010 bp intergenic region between the gene encoding Ser/Thr protein phosphatase and *hydA2*
3407-reverse	GGCTGTCAAAAAGTGCCTGG	
3407-forward	ATGTATGTCCCAGAGGGGCT	Amplification of a 1,649 bp intergenic region between *hydA2* and *rumal_3408*
3408-reverse	AACAAGCAGAGACGGCACAA	
3408-forward	CATTTACCGATTTCTATCAGCCG	Amplification of a 2,153 bp intergenic region between *rumal_3408* and *rumal_3409*
3409- reverse	ATAGCATAGCACCGCAGCTT	

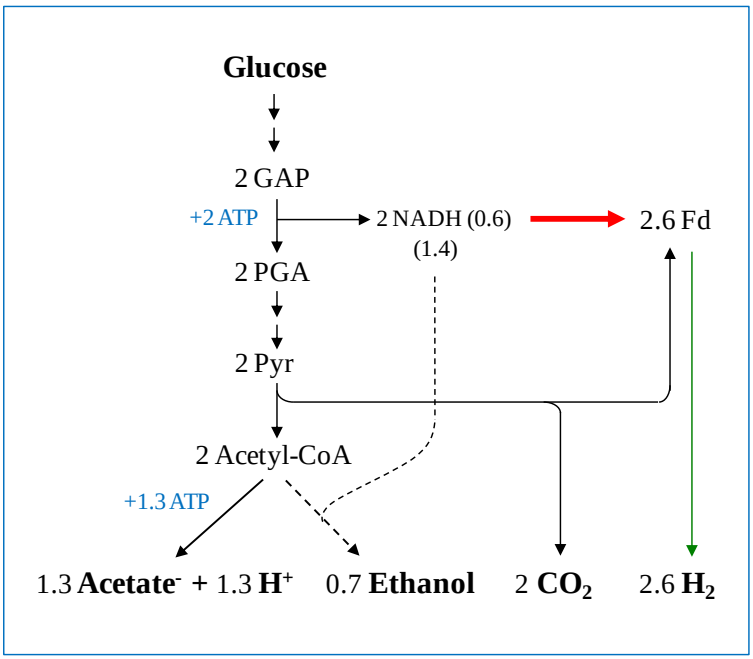
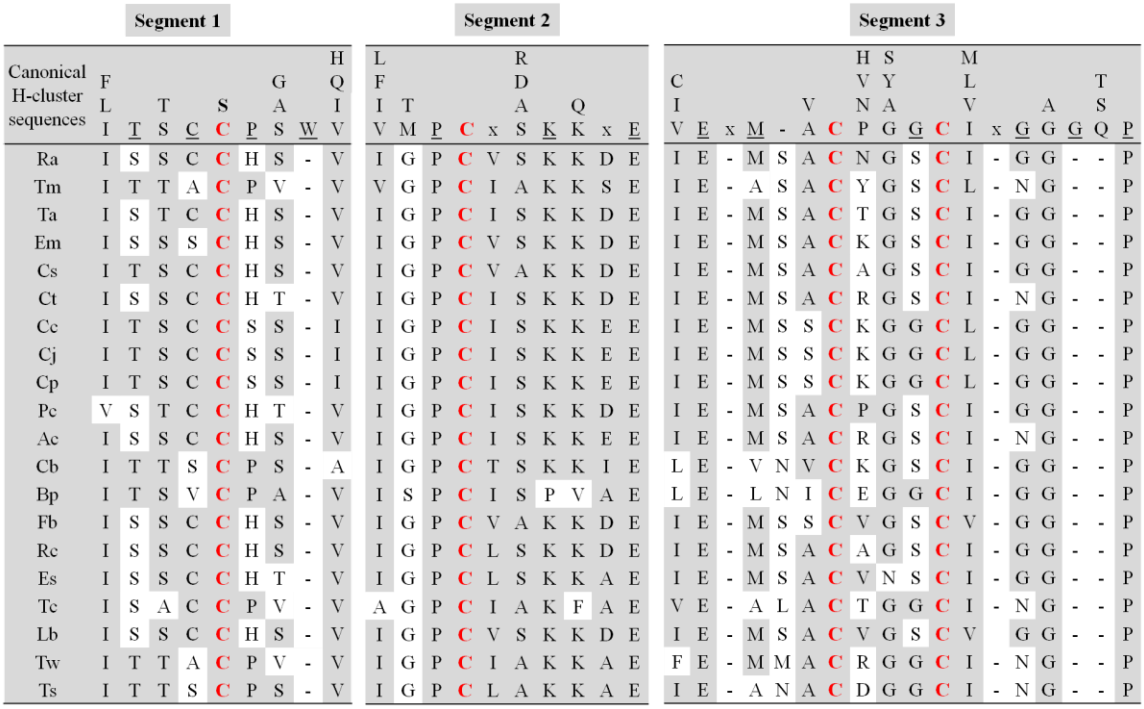


FIG S1 Fermentation scheme of *R. albus* growing in batch culture on glucose as proposed in 1977 by Thauer *et al.* (1). It assumes a reduction of ferredoxin with NADH (red arrow), which is thermodynamically only feasible if coupled to an exergonic reaction. The corrected fermentation scheme is shown in Fig. 6. G-6-P, glucose-6-phosphate; GAP, glyceraldehyde-3-phosphate; PGA, phosphoglycerate; Pyr, pyruvate.



Canonical H-cluster sequences	Segment 1									Segment 2									Segment 3																
	F L I		T		S			G A	H Q I	L F I V	T M	P	C	x	R D A S	K	Q K	x	E	C I V	E	x	M	-	V A	C	H V N P	S Y A G	G	C	M L V I	x	A G	G	T S Q	P
Ra	I	S	S	C	C	H	S	-	V	I	G	P	C	V	S	K	K	D	E	I	E	-	M	S	A	C	N	G	S	C	I	-	G	G	-	-	P
Tm	I	T	T	A	C	P	V	-	V	V	G	P	C	I	A	K	K	S	E	I	E	-	A	S	A	C	Y	G	S	C	L	-	N	G	-	-	P
Ta	I	S	T	C	C	H	S	-	V	I	G	P	C	I	S	K	K	D	E	I	E	-	M	S	A	C	T	G	S	C	I	-	G	G	-	-	P
Em	I	S	S	S	C	H	S	-	V	I	G	P	C	V	S	K	K	D	E	I	E	-	M	S	A	C	K	G	S	C	I	-	G	G	-	-	P
Cs	I	T	S	C	C	H	S	-	V	I	G	P	C	V	A	K	K	D	E	I	E	-	M	S	A	C	A	G	S	C	I	-	G	G	-	-	P
Ct	I	S	S	C	C	H	T	-	V	I	G	P	C	I	S	K	K	D	E	I	E	-	M	S	A	C	R	G	S	C	I	-	N	G	-	-	P
Cc	I	T	S	C	C	S	S	-	I	I	G	P	C	I	S	K	K	E	E	I	E	-	M	S	S	C	K	G	G	C	L	-	G	G	-	-	P
Cj	I	T	S	C	C	S	S	-	I	I	G	P	C	I	S	K	K	E	E	I	E	-	M	S	S	C	K	G	G	C	L	-	G	G	-	-	P
Cp	I	T	S	C	C	S	S	-	I	I	G	P	C	I	S	K	K	E	E	I	E	-	M	S	S	C	K	G	G	C	L	-	G	G	-	-	P
Pc	V	S	T	C	C	H	T	-	V	I	G	P	C	I	S	K	K	D	E	I	E	-	M	S	A	C	P	G	S	C	I	-	G	G	-	-	P
Ac	I	S	S	C	C	H	S	-	V	I	G	P	C	I	S	K	K	E	E	I	E	-	M	S	A	C	R	G	S	C	I	-	N	G	-	-	P
Cb	I	T	T	S	C	P	S	-	A	I	G	P	C	T	S	K	K	I	E	L	E	-	V	N	V	C	K	G	S	C	I	-	G	G	-	-	P
Bp	I	T	S	V	C	P	A	-	V	I	S	P	C	I	S	P	V	A	E	L	E	-	L	N	I	C	E	G	G	C	I	-	G	G	-	-	P
Fb	I	S	S	C	C	H	S	-	V	I	G	P	C	V	A	K	K	D	E	I	E	-	M	S	S	C	V	G	S	C	V	-	G	G	-	-	P
Rc	I	S	S	C	C	H	S	-	V	I	G	P	C	L	S	K	K	D	E	I	E	-	M	S	A	C	A	G	S	C	I	-	G	G	-	-	P
Es	I	S	S	C	C	H	T	-	V	I	G	P	C	L	S	K	K	A	E	I	E	-	M	S	A	C	V	N	S	C	I	-	G	G	-	-	P
Tc	I	S	A	C	C	P	V	-	V	A	G	P	C	I	A	K	F	A	E	V	E	-	A	L	A	C	T	G	G	C	I	-	N	G	-	-	P
Lb	I	S	S	C	C	H	S	-	V	I	G	P	C	V	S	K	K	D	E	I	E	-	M	S	A	C	V	G	S	C	V		G	G	-	-	P
Tw	I	T	T	A	C	P	V	-	V	I	G	P	C	I	A	K	K	A	E	F	E	-	M	M	A	C	R	G	G	C	I	-	N	G	-	-	P
Ts	I	T	T	S	C	P	S	-	V	I	G	P	C	L	A	K	K	A	E	I	E	-	A	N	A	C	D	G	G	C	I	-	N	G	-	-	P

FIG S2 The three segments encompassing the four cysteine ligands (in red) involved in H-cluster iron binding in the putative H_2-sensing [FeFe]-hydrogenase HydS of different bacteria (not complete). The consensus sequence in the three segments of metabolic [FeFe]-hydrogenases is based on the review by Vignais *et al.* in 2001 (2) and Lubitz *et al.* 2014 (3). Underlining indicates fully conserved residues; x indicates that more than four different residues are found at that position. Ra, *Ruminococcus albus*; Tm, *Thermotoga maritima*; Ta, *Treponema azotonutricium*; Em, *Elusimicrobium minutum*; Cs *Clostridium stercorarium*; Ct, *C. thermocellum*; Cc, *C. cellulolyticum*; Cj, *C. josui*; Cp, *C. papyrosolvens*; Pc, *Pseudoflavonifractor capillosus*; Ac, *Acetivibrio cellulolyticus*; Cb, *Clostridiaceae bacterium*; Bp, *Blautia producta*; Fb, *Firmicutes bacterium*; Rc, *Ruminococcus champanellensis*; Es, *Eubacterium siraeum*; Tc, *Thermosinus carboxydivorans*; Lb, *Lachnospiraceae bacterium*; Tw, *Thermoanaerobacter wiegelii*; Ts, *Thermoanaerobacterium saccharolyticum*.

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