

Supplementary Table S1. List of the [NiFe] hydrogenases identified in the sequenced genomes of members of the genus *Clostridium*

NA, Not analysed.

Protein GenBank accession no.	Group*	No. of subunits	Protein length (aa)	Predicted localization†	Subunit	Remarks
<i>Clostridium acetobutylicum</i> ATCC 824						
NP_149304	I	3	291	CM (1 IH)	Small	[NiFe] hydrogenase
NP_149305			471	C	Large	
NP_149307			143	U	Cytochrome b5	
<i>Clostridium beijerinckii</i> NCIMB 8052						
YP_001310107	I	2	463	C	Large	[NiFe] hydrogenase
YP_001310108			291	CM (1 IH)	Small	
<i>Clostridium botulinum</i> A3 str. Loch Maree						
YP_001787161			171	U	Cytochrome b5	[NiFe] hydrogenase
YP_001787163	I	3	471	C	Large	
YP_001787164			290	CM (1 IH)	Small	
<i>Clostridium carboxidivorans</i> P7						
ZP_05392032	I	3	466	CM	Large	[NiFe] hydrogenase
ZP_05392033			277	CM	Small	
ZP_05392034			140	U	Cytochrome b5	
ZP_05392428	NA	7	242	CM		Putative [NiFe] hydrogenase complex
ZP_05392429			526	C		
ZP_05392430			186	CM (5IH)		Hydrogenase complex
ZP_05392431			297	CM (9IH)		
ZP_05392432			203	CM (7IH)		
ZP_05392433			309	CM (8IH)		
ZP_05392434			594	CM (14IH)		
<i>Clostridium cellulolyticum</i> H10						
YP_002505408	I	3	149	U	Cytochrome b5	[NiFe] hydrogenase
YP_002505409			299	CM	small	
YP_002505410			470	C	large	
YP_002507635	IV	6	124	C	EchF	Ech hydrogenase complex
YP_002507636			359	C	EchE	

YP_002507637			126	C	EchD	
YP_002507638			149	C	EchC	
YP_002507639			283	CM (10 IH)	EchB	
YP_002507640			661	CM (18 IH)	EchA	
YP_002506017	NA	6	266	CM		Putative [NiFe] hydrogenase complex
YP_002506018			530	C		
YP_002506019			495	CM (13 IH)		
YP_002506020			220	CM (7 IH)		
YP_002506021			312	CM (8 IH)		
YP_002506022			688	CM (18 IH)		
<i>Clostridium cellulovorans</i> 743B						
ZP_04804852	I	2	281	CM	Small	[NiFe] hydrogenase
ZP_04804853			466	C	Large	
<i>Clostridium papyrosolvens</i> DSM 2782						
ZP_05496689	I	3	470	C	Large	NiFe] hydrogenase
ZP_05496690			299	CM	Small	
ZP_05496691			149	U	Cytochrome b5	
ZP_05495139	IV	6	124	U	EchF	Ech hydrogenase complex
ZP_05495140			359	C	EchE	
ZP_05495141			127	U	EchD	
ZP_05495142			149	CM	EchC	
ZP_05495143			285	CM (8 IH)	EchB	
ZP_05495144			661	CM (18 IH)	EchA	
ZP_05495464	NA	6	266	CM		Putative [NiFe] hydrogenase complex
ZP_05495465			530	C		
ZP_05495466			495	CM (13 IH)		
ZP_05495467			220	CM (7 IH)		
ZP_05495468			312	CM (8 IH)		
ZP_05495469			688	CM (15 IH)		
<i>Clostridium phytofermentans</i> ISDg						
YP_001558841	IV	6	637	CM (18IH)	EchA	Ech hydrogenase complex
YP_001558842			286	CM (8IH)	EchB	
YP_001558843			144	U	EchC	
YP_001558844			117	C	EchD	

YP_001558845			359	C (1IH)	EchE	
YP_001558846			120	U	EchF	
<i>Clostridium thermocellum</i> ATCC 27405						
YP_001039408	IV	6	128	C	EchF	Ech hydrogenase complex
YP_001039409			359	C (1 IH)	EchE	
YP_001039410			119	U	EchD	
YP_001039411			145	U	EchC	
YP_001039412			291	CM (8 IH)	EchB	
YP_001039413			636	CM (18 IH)	EchA	
<i>Clostridium sporogenes</i> ATCC 15579						
ZP_02996037	I	3	306	C	Small	[NiFe] hydrogenase
ZP_02996038			471	U (2 IH)	Large	
ZP_02996040			150	U	Cytochrome b5	

*According to their localization in the distance tree of the [NiFe] hydrogenases, see Fig. 2.

†PSORTb v.2.0. predicted results (Gardy *et al.*, 2005); C, cytoplasmic; CM, cytoplasmic membrane (number of internal membrane helices); U, unknown.

Supplementary Table S2. List of the [FeFe] hydrogenases and their maturation genes identified in the sequenced genomes of *Clostridium* spp.

For *C. botulinum* strains: E3 str. Alaska E43, B str. Eklund 17B, C str. Eklund, only hydrogenases used in the alignment analysis are indicated in the table. Catalytic subunits are indicated in bold characters.

GenBank accession no.	Cluster*	No. of subunits	Protein length (aa)	Predicted localization†	Modular structure‡	Maturation protein name – accession no. (size; aa)
Complete genomes						
<i>Clostridium acetobutylicum</i> ATCC 824						
NP_346675	A2	1	582	C	M3	HydE – NP_348258 (350)
NP_349825	B2	1	450	C	M2a	HydF – NP_348277 (411) HydG – NP_347984 (472)
<i>Clostridium beijerinckii</i> NCIMB 8052						
YP_001307471	B3	1	496	C	M3a	HydE – YP_001307445 (348)
YP_001308901	A5	1	644	C	M2c	HydF – YP_001310328 (412)
YP_001309028	B2	1	449	C	M2a	HydG – YP_001311504 (472)
YP_001310868	A3	3	189	C	TR(M2)	
YP_001310869			461	C		
YP_001310860			184	C		
YP_001311066	None	1	530	C	M2b	
YP_001311176	A8	3	567	C	TR(M3)	
YP_001311177			626	C		

YP_001311178			164	C		
<i>Clostridium botulinum</i>						
A3 str. Loch Maree						
YP_001787172	A6	3	577	C	TR(M3)	HydE – YP_001788925 (348)
YP_001787173			631	C		HydF – YP_001788927 (397)
YP_001787174			159	C		HydG – YP_001788926 (458)
YP_001788618	B3	1	497	C	M3a	
YP_001788890	B2	1	449	C	M2a	
YP_001787154	C	1	579	C	M2f	
YP_001787152	C	2	574	C	D(M2f)	
YP_001787151			80	C		
<i>Clostridium botulinum</i>						
E3 str. Alaska E43						
YP_001921030	A7	2	582	C	D(M3)	
YP_001921031			611	C		
YP_001920992	A5	1	646	C	M2c	
<i>Clostridium botulinum</i>						
B str. Eklund 17B						
YP_001884306	A2	1	576	C	M3	
YP_001884615	B3	1	494	C	M3a	
YP_001885900		1	451	C		
YP_001886496	A8	3	565	C	TR(M3)	
YP_001886497			626	C		
YP_001886498			163	C		
<i>Clostridium botulinum</i>						
C str. Eklund						

ZP_02621445	A2	1	579	C	M3	
<i>Clostridium cellulolyticum</i> H10						
YP_002506620	A8	3	562	C	TR(M3)	HydE - YP_002507459 (345)
YP_002506621			623	C		HydF - YP_002507375 (396)
YP_002506622			157	C		HydG - YP_002507376 (470)
YP_002506617	C	2	574	C	D(M2f)	
YP_002506618			86	C		
YP_002506776	A1	1	574	C	M3	
YP_002506554	A1	3	582	C	TR(M3)	
YP_002506555			597	C		
YP_002506556			123	C (1IH)		
<i>Clostridium difficile</i> 630						
YP_001087367	B3	1	509	C	M3a	HydE – YP_001088668 (363)
YP_001087368	B3	1	498	C	M3a	HydF – YP_001088667 (402)
YP_001089774	B2	1	478	C	M2a	HydG – YP_001088669(458)
YP_001089829	A3	3	171	C	TR(M2)	
YP_001089830			461	C		
YP_001089831			183	CM		
YP_001089925	A6	3	165	C	TR(M3)	
YP_001089926			628	C		
YP_001089927			593	C		
<i>Clostridium kluyveri</i>						

DSM 555						
YP_001393559	B2	1	447	C	M2a	HydE – Y P_001394007 (347)
YP_001394237	A3	4	320	C	TE(M2)	HydF – YP_001395713 (413)
YP_001394238			253	C		HydG – YP_001397177 (475)
YP_001394239			178	CM		
YP_001394240			457	C		
YP_001395640	A2	1	580	C	M3	
<i>Clostridium novyi</i> NT						
YP_877103	B2	1	448	C	M2a	HydE – YP_877565 (348)
YP_877778	A6	1	443	C	M2	HydF – YP_877567 (396)
YP_878332	A2	1	579	C	M3	HydG – YP_877566 (458)
YP_878935	B3	1	494	U	M3a	
<i>Clostridium perfringens</i> ATCC 13124						
YP_694731	B2	1	449	C	M2a	HydE – YP_697148 (347)
YP_695522	A4	1	696	C	M2d	HydF – YP_694567 (440)
YP_697035	A2	1	572	C	M3	HydG – YP_694570 (473)
YP_697264	B3	1	490	C	M3a	
<i>Clostridium</i> <i>phytofermentans</i> ISDg						
YP_001557216	A8	3	567	C	TR(M3)	HydE – YP_001559190 (372)

YP_001557217			628	C(3IH)		HydF – YP_001559188 (416)
YP_001557218			161	C		HydG – YP_001559189 (472)
YP_001557219	B3	1	484	C	M3a	
YP_001557221	C	1	577	C	M2f	
YP_001559163	A5	1	644	C	M2c	
YP_001560889	A1	3	123	C(1IH)	TR(M3)	
YP_001560890			595	C		
YP_001560891			582	C		
<i>Clostridium tetani</i> E88						
NP_782947	B3	1	494	C	M3a	HydE – NP_781954 (349)
NP_780897	B2	1	448	C	M2a	HydF – NP_781952 (396) HydG – NP_781953 (478)
<i>Clostridium thermocellum</i> ATCC 27405						
YP_001036766	D	1	448	C	M2e	HydE – YP_001038253 (353)
YP_001036771	A1	3	122	C(1IH)	TR(M3)	HydF – YP_001036476 (400)
YP_001036772			597	C		HydG – YP_001037082 (458)
YP_001036773			582	C		
YP_001036859	A8	3	165	C	TR(M3)	
YP_001036860			624	C(3IH)		
YP_001036861			566	C		

YP_001036857	C	2	556	C	D(M2f)	
YP_001036856			81	C		
YP_001039392		2	644	C	D(M2 g)	
YP_001039393			493	C		
Genomes in progress						
<i>Clostridium</i>						
<i>asparagiforme</i> DSM						
15981						
ZP_03760851	A1	3	597	C	TR(M3)	HydE – ZP_03759890 (362)
ZP_03760852			1048	C		HydF – ZP_03759888 (435)
ZP_03760853			170	C		HydG – ZP_03759889 (472)
ZP_03761376	A4	1	683	C (1IH)	M2d	
ZP_03760837		3	350	C(1IH)		Truncated protein; not used in phylogenetic analysis
ZP_03760838			218	C(1IH)		
ZP_03760839			419	C		
ZP_03760911	B3	1	484	C	M3a	
ZP_03760913	C	2	641	C	D(M2f)	
ZP_03760914			104	C		
ZP_03760831	D	1	471	C	M2e	
<i>Clostridiales</i> bacterium						
1_7_47_FAA						
ZP_036510999	A1	3	125	C	TR(M3)	HydE – ZP_04672030 (358)
ZP_036511000			596	C		HydF – ZP_04669283 (393)

ZP_036511001			596	C		HydG – ZP_04668728 (409)
ZP_036511002	A1	3	602	C	TR(M3)	
ZP_036511003			1031	C		
ZP_036511004			171	C		
ZP_03651544	A4	1	461	C		
ZP_03652433	B1	1	507	C	M3a'	
ZP_03651292	B3	1	483	C	M3a	
ZP_03651294	C	2	695	C	D(M2f)	
ZP_03651295			79	C		
ZP_03651094	D	1	466	C	M2e	
<i>Clostridium bartlettii</i> DSM 16795						
ZP_02212773	C	1	573	C	M2f	HydE – ZP_02210998 (351)
ZP_02212776	C	1	589	C	M2f	HydF – ZP_02211233 (407)
ZP_02210685	A6	3	598	C	TR(M3)	HydG – ZP_02211340 (476)
ZP_02210686			628	C		
ZP_02210687			166	C		
ZP_02212110	A6	3	166	C	TR(M3)	
ZP_02212111			628	C		
ZP_02212112			598	C		
ZP_02210701	A3	3	184	C	TR(M2)	
ZP_02210702			455	C		
ZP_02210703			183	CM		
ZP_02210839	A5	1	646	C	M2c	
<i>Clostridium bolteae</i>						

ATCC BAA-613						
ZP_02088081	C	2	677	C	D(M2f)	HydE – ZP_02087032 (358)
ZP_02088082			79	C		HydF – ZP_02087803 (411)
ZP_02088079	B3	1	505	C	M3a	HydG – ZP_02087802 (473)
ZP_02083500	A1	3	581	C	TR(M3)	
ZP_02083501			574	C		
ZP_02083502			125	C		
ZP_0208347	A1	3	171	C	TR(M3)	
ZP_02083480			1033	C		
ZP_02083481			597	C		
ZP_02089734	A8	3	162	C	TR(M3)	
ZP_02089735			624	C		
ZP_02089736			568	C		
ZP_02089645	A4	2	451	C	D(M2)	
ZP_02089644			635	C		
ZP_02082663	B1	1	507	C	M3a'	
ZP_02083508	D	1	464	C	M2e	
<i>Clostridium butyricum</i>						
5521						
ZP_02948973	A8	3 [§]	593	C	Putative	HydE – ZP_02950586 (351)
ZP_02949040			656	C(1IH)	TR(M3)§	HydF – ZP_02948272 (400)
ZP_02950663	B3	1	495	C	M3a	HydG – ZP_02950303 (472)
ZP_02951109	B2	1	456	C	M2a	

ZP_02951233	A2	1	574	C	M3	
<i>Clostridium carboxidivorans</i> P7						
ZP_05393708	A6	3	574	C	TM(M3)	HydE – ZP_05390403 (346)
ZP_05393709			631	C		HydF – ZP_05392841 (411)
ZP_05393710			159	C		HydG – ZP_05391261(386)
ZP_05391258	A6	3	160	C	TM(M3)	
ZP_05391259			626	C		
ZP_05391260			580	C		
ZP_05394384	A3	3	184	CM	TM(M2)	
ZP_05394385			426	U		
ZP_05394386			195	CM		
ZP_05392646	B3	1	458	U	M3a	
ZP_05390990	B2	1	449	U	M2a	
ZP_05390528	C	2	84	U	D(M2f)	
ZP_05390529			573	U		
<i>Clostridium cellulovorans</i> 743B						
ZP_04803630	A2	1	574	C	M3	HydE – ZP_04807365 (346)
ZP_04805479	A6	3	579	C	TM(M3)	HydF – ZP_04804598 (411)
ZP_04805480			630	C		HydG – ZP_04806412 (469)
ZP_04805481			160	U		
ZP_04805685	B2	1	447	U	M2a	

<i>Clostridium hathewayi</i>						
DSM 13479						
ZP_06111961	A1	3	125	U	TM(M3)	HydE – ZP_06117908(359)
ZP_06111962			595	C		HydF – ZP_06118601 (407)
ZP_06111963			587	C		HydG – ZP_06118802 (343)
ZP_06112087	A4	1	457	U	M2d	
ZP_06111989	B3	1	483	U	M3a	
ZP_06111991	C	2	577	U	D(M2f)	
ZP_06111992			79	U		
ZP_06115111	D	1	466	U	M2e	
<i>Clostridium hiranonis</i>						
DSM 13275						
ZP_03292845	A7	2	578	C	D(M3)	HydE – ZP_03292564 (349)
ZP_03292844			611	C		HydF – ZP_03292566 (412)
ZP_03292196	B3	1	501	C	M3a	HydG – ZP_03292565 (479)
<i>Clostridium hylemonae</i>						
DSM 15053						
ZP_03778055	A1	3	583	C	TR(M3)	HydE – ZP_03778460 (346)
ZP_03778056			595	C(1IH)		HydF – ZP_03778462 (404)
ZP_03778057			126	C		HydG – ZP_03778461 (473)
ZP_03778515	A4	1	719	C	M2d	

ZP_03779629	A8	3	157	C	TR(M3)	
ZP_03779630			628	C		
ZP_03779631			560	C		
ZP_03778211	B2	1	412	C	M2a	
ZP_03777938	C	2	571	C	D(M2f)	
ZP_03777939			81	C		
<i>Clostridium leptum</i> DSM 753						
ZP_02080483	C	2	569	C	D(M2f)	HydE – ZP_02081236 (317)
ZP_02080482			63	C		HydF – ZP_02081234 (396)
ZP_02080485	B3	1	546	C	M3a	HydG – ZP_02081235 (470)
ZP_02082401	A1	3	123	C	TR(M1)	
ZP_02082402			571	C(1IH)		
ZP_02082403			366	C		
ZP_02081315	B2	1	443	C	M2a	
ZP_02082395	D	1	431	C	M2e	
<i>Clostridium methylpentosum</i> DSM 5476						
ZP_03705348		1	338	U		HydE – ZP_03708102 (341)
ZP_03705331		1	490	U		HydF – ZP_03708100 (397)
						HydG – ZP_03708101 (470)
<i>Clostridium nexile</i> DSM 1787						

ZP_03290965	A1	3	594	C	TR(M3)	HydE – ZP_03290619 (350)
ZP_03290966			626	C		HydF – ZP_03290617 (396)
ZP_03290967			184	C		HydG – ZP_03290618 (473)
ZP_03288284	A7	3	590	C		
ZP_03288285			209			
ZP_03288286			202			
ZP_03290755	B1	1	502	C	M3a'	
ZP_03288257	B2	1	408	C	M2a	
ZP_03290961	C	1	571	C	M2f	
<i>Clostridium</i> <i>papyrosolvens</i> DSM 2782						
ZP_05494541	A1	3	574	C	TM(M3)	HydE – ZP_05496763 (345)
ZP_05494542			429	C		HydF – ZP_05495266 (396)
ZP_05494543			156	C		HydG – ZP_05495267 (470)
ZP_05496564	A1	3	583	C	TM(M3)	
ZP_05496565			597	C		
ZP_05496566			123	U		
ZP_05495280	A6	3	592	C	TM(M3)	
ZP_05495281			575	C		
ZP_05495282			196	C		
ZP_05497653	A8	3	562	C	TM(M3)	
ZP_05497654			623	C		

ZP_05497655			157	C		
ZP_05497650	C	2	574	U	D(M2f)	
ZP_05497651			86	U		
<i>Clostridium ramosum</i>						
DSM 1402						
ZP_02427918	A1	3	582	C	TR(M3)	HydE – ZP_02428409 (352)
ZP_02427919			599	C		HydF – ZP_02428411 (395)
ZP_02427920			122	C		HydG – ZP_02428410 (473)
ZP_02428761	B1	1	507	C	M3a'	
ZP_02427924	D	1	387	C	M2e	
<i>Clostridium scindens</i>						
ATCC 35704						
ZP_02433352	A1	3	126	C	TR(M3)	HydE – ZP_02432053 (229)
ZP_02433353			595	C		HydF – ZP_02432055 (406)
ZP_02433354			583	C		HydG – ZP_02432054 (473)
ZP_02433356	C	2	90	C	DM(M2f)	
ZP_02433357			548	C		
ZP_02433347	D	1	468	C	M2e	
ZP_02430229	B1	1	503	C	M3a'	
ZP_02432701	B2	1	426	C	M2a	
ZP_02431953	A7	2	576	C	D(M3)	
ZP_02431954			610	C		
ZP_02432096	A4	1	708	C	M2d	

<i>Clostridium spiroforme</i>						
DSM 1552						
ZP_02868193	D	1	386	C	M2e	HydE – ZP_02867474 (362)
ZP_02867943	B1	1	507	C	M3a'	HydF – ZP_02867472 (396)
ZP_02868187	A1	3	582	C	TR(M3)	HydG – ZP_02867473 (475)
ZP_02868188			599	C		
ZP_02868189			122	C(1IH)		
<i>Clostridium sp.</i> L2-50						
ZP_02074529	A8	3	561	C	TR(M3)	HydE – ZP_02074795 (350)
ZP_02074530			661	C		HydF – ZP_02074771 (329)
ZP_02074531			160	C		HydG – ZP_02074773 (489)
ZP_02075337	B3	1	483	C	M3a	
<i>Clostridium sp.</i> SS2/1						
ZP_02440567	A7	2	610	C	D(M3)	HydE – ZP_02437903 (347)
ZP_02440568			570	C		HydF – ZP_02438347 (402)
ZP_02438749	B1	1	495	C	M3a'	HydG – ZP_02440252 (472)
ZP_02439799	B3	1	481	C	M3a	
<i>Clostridium sporogenes</i>						
ATCC 15579						
ZP_02996046	C	1	579	U	M2f	HydE – ZP_02993906 (352)

ZP_02996048	C	2	574	C	D(M2f)	HydF – ZP_02993904 (397)
ZP_02996049						HydG – ZP_02993905 (458)
ZP_02993941	B2	1	449	C	M2a	
ZP_02993254	B3	1	497	C	M3a	
ZP_02996023	A6	3	159	C	TR(M3)	
ZP_02996024			631	C		
ZP_02996025			577	C		

*According to the localization in the distance tree of the [FeFe] hydrogenases, see Fig. 5.

†PSORTb v.2.0. predicted results (Gardy *et al.*, 2005); C, cytoplasmic; CM, cytoplasmic membrane (number of internal membrane helices); U, unknown.

‡Refer to Fig. 4.

§From the two genomes of *C. butyricum*, that are under sequencing (strain E3 and 5521), there is no clear evidence for the presence of the third subunit. In the strain E3, it is split into two thioredoxin – like smaller subunits. These differences can come from genome sequencing and splicing errors.

Supplementary Reference

Gardy, J. L., Laird, M. R., Chen, F., Rey, S., Walsh, C. J., Ester, M. & Brinkman, F. S. L. (2005). PSORTb v.2.0: expanded prediction of bacterial protein subcellular localization and insights gained from comparative proteome analysis. *Bioinformatics* **21**, 617–623. [Medline](#)