

*Rectinema cohabitans* strain HM:

- May evolve H<sub>2</sub> during carbohydrate fermentation by two ferredoxin-coupled FeFe A1 hydrogenases
- May also enhance H<sub>2</sub> evolution using predicted ferredoxin-coupled FeFe B hydrogenase
- May reversibly bifurcate electrons from H<sub>2</sub> to NAD and ferredoxin by multimeric FeFe A3 hydrogenase
- May sense H<sub>2</sub> leading to hydrogenase regulation by FeFe C1 and C3 hydrogenases

SPBIB\_v1\_260013|ID:27163327|hndD| FeFe Group A3 hydrogenase catalytic subunit [Uncultured spirochete bib]  
MVNVKVNIPVQVAEGSTVLEAAKKANVKIPTLCYNPDLSPWASCGICVVKIEGSNKMLRSCCTPVSEGM  
SIISNDPDLVQTRKTVIELILSTHPDDCLACPRNQACELQTLAQEFGIREQPYKKMVRDIPQDTSTGSLI  
LNPSKCIRCGRCVEVCQEMQGVWAVEFLGRGESIRIAPAADV KLGDSPIKCGQCSAHCPVGAIIYENDQT  
KLVDALMKEGPEAKTCAVQIAPAVRVALAESFGLPPGTDLTGKIYTALRRLGFDAVFDTNFSADLTIME  
EGTEFVHRLTGALKAGMQQATADKSMPLITSCCPAVVDYMEKYYPDMIPNFSTAKSPQQMMGAMIKTYWA  
AKAKVDPAKIYSVSIMPCTAKKFENS RDESMYSSGYKDVDVTLTTRELARMIKQAGIDFLNLPESPDSP  
LGPYSGAGVIFGATGGVMEALRTAYFLVTKEELKDVNFTA VRGLSGIKEATVHINGIELRVA AAHQMG N  
IATVLDQVRKAREEGRETPWHFIEVMACRGGCIGGGGQPYGATDEV RKL RMRGIYDNDEKQEYRCSHDNP  
YIKQIYAEFLQKPASHKAHELLHTQYKERPLYLK\*

SPBIB\_v1\_20025|ID:27161852|hndD| FeFe Group A1 hydrogenase catalytic subunit [Uncultured spirochete bib]  
MEEQLIEITIDGQNV RVDPSANIVEACA HAGVKIPTLCYLKGISQNASCGVCVVEVEGAKSLVRSCVQKP  
VPGMKIRTSSPRVIRARKTAV ELLLANHPDDCLSCIRSDTCELHTIANILEVRADRFPGYKKYPMPDTTS  
EGIVRDDSKCILCGRCVAVCEETQGVHAI AFSGRGARTRVSTFLDRGLAQSACVQCGQCSVVCPTGAITE  
KDESREVFSAIEDPRLTVVVQTAPAIRASLGEALGLPAGSLVTGQMVAALRRLGFNRVFDTQFTADLTIM  
EEGSELLERLSKGGTLPMITSCSPGWINFIEGFYPDLLSHLSTCKSPQQMFGSVAKTYAKKAGLTPDHM  
RVVSIMPCTAKKYEARRKEMDGAWGWWEQDPGKVPARFFDVDWALTRELARMIKLAGIDISRLPEEE  
FDDPLGQSTGAGTIFGTTGGVMEALRTVYELVEKKPLENIEFTKVRGF EAIKTAEVVGGSPVRVAVAH  
GLANARVLLDEIRAGKSPYHFIEIMSCPGGCVGGGGQPV LADLEKKLARSQALYEEDRKLAIKSHENPA  
VRALYQEFGLGKPLGHLHELLHTSYKARVF\*

SPBIB\_v1\_210130|ID:27163022|hndD| FeFe Group A3 hydrogenase catalytic subunit [Uncultured spirochete bib]  
MKNVTLTINGKKISVPEHTTVFSAAEKAGISIPALCRHEDLEPKGACGMCIVKIEGQPGYKRSCVTAVEE  
GMEVLTSTAEIRDIRRGILEL VLAHPADCLQCIKHGKCELQTLAERFEIRD LRYDRYTRGLPVDRSSFG  
IVRDMNKCIGCGRCVQVCNEVQTVASIFFHGRG SNTIVSPAYGTTMGDSVCVNCGQCIVYCPV GALYEKE  
AIEEVWKAIDDPDKVVVAQIAPAVRVAIGEEFGLEPGELSIGKLYSALKALGIDVVFDTNFSADLTIVEE  
ATEFLERL KESGPFPLITSCSPGWIKFGETFYPELLENVSSCKSPQQMLGALIKTHYAESRGLDRNNIVS  
LSIMPCTAKKFEAGRPEMRSSGARDVDYVLT TREIARMIRQV GIDFRNLPDGTDPDLLSRYSGAATIFGA  
SGGVMEALRTAYELATGKALEKVDVFDVVRGMTGVKEAEIDVDGTPIKVAVTNGLANARKILDRVAEDKR  
RGRSSYHFIEIMACAGGCVGGGGQPIPN TLARRARRIEGLYREDRSLPLRKSHENPEIRALYAEFLGSPG  
SEKAHALLHTKYSPREYQFSE\*

SPBIB\_v1\_290056|ID:27163581| FeFe Group B hydrogenase catalytic subunit [Uncultured spirochete bib]  
VPEVFERMRGLYTPCTDIRRKIFVGVTRFVLAGKKPEDIDYLPFDIIMGKPT YRCCSYRELSIVKQRIR  
LAFGLPLIEERDNTPV SAGIGEAFDRKVISAPLVNVIRAACEKCPEDKVIVTDMCQSCMAHPCSIVCPV  
NAISFPNGGKA FIDQKKCVKCMKCVKACPYQSITRMVRPCA AACGVDAIHS DPDGYATIDQDKVNCGLC  
TVSCPFAAISDKTEL VQILHQLMGPEDRR PYAILAPSFVGGQFGR LASPGAIVAGLRAVGFGRVREVALGA  
DCDTLLLAKRLAEKSSPDNPQHKTFLGTSCCPSWVKTARRHFPEFADNIAESFTPMVETAKIIKDQDPSA  
RVVFIGPCIAKKA EALEPEMQPYVDHVLTFEELAAIFVAKDIDLAEITPVEFPDEASALGRGYAVAGGVA  
TAAIETARQKYGKENIAVARADTLRNCRAMLADIKSGKTSPELVEGMACPGGCVGGPGTLIGLLSARNEV  
GKFAGLAPKNPATLTK\*

SPBIB\_v1\_360012|ID:27164068| FeFe Group C3 hydrogenase catalytic subunit [Uncultured spirochete bib]  
MNRQVIYTETMRCQDCYKCVRECPV KAIQILDGHARVVEDICILCGHCVMVCPQSAKKVRSDVERVRLL  
ELKPVAVASLAPSF AAEFSGCTAGQLIASIRKLGFAAVSETALGADLVSSAMRNELGQLADNGRNFLIGA  
ACPAVVRYVSFYRPDLVPFLSENGSPMIAHARYLRSRFGENAL VVFIGPCIAKKKEADESGGLVNAVLT F  
AELRQLFEEENIDPSAEKPTSNDTFFPQRPSKGEL YPIEGGMIA SIKQHGSTDVPCMSFSGMGHIQAALR  
DLPDGIPETGIFLELLACEGGCINGPCSDASRGTVSKRMQILLYEKQGESEPFNTEIACQVKHSAAKPVQ

RQVREEEIRAALALVGGKKEPSDELNCSCGCGYDSCREFAKAIVIGHAEPTMCLSYMRNLAQKKANALLRAM  
PSAAVVVNADLKVIECNPFVVRILEKDAQFVAEAKPTLEGADLRKLLPFWERFNDVLHHSPGDAFIESDF  
RCGERILHGTIFSIEKQMIAGGLFQDITAPWIQKDRVIKEAKKVRQNVRTVQKIAYLLGENAAESEAL  
RSIIESFGGEVQG\*

SPBIB\_v1\_280015|ID:27163481| FeFe Group C1 hydrogenase catalytic subunit [Uncultured spirochete bib]  
VNNKTLQNQENNIRTFHVSQLDADLCVGGCTTCIKFCPTKAIRVRDGKAKIFEDRCIDCGECIRRCPKGAK  
KAISDPLFILDGYDLKVALPAPSLYAQFGTRYSDIFAALHHMGFDEVFDVAWGAIVATEITRSILAKP  
GPAPRISSACPVIVRLIQRFPSLIPNLMPIPPSEIAAREARKRLSEISRKIGIFFLSPCTAKVTAVRM  
PLGYSQSSIDAVISFSDIFLPLKRALEERKPLSPKKGFKSSVPQASSGHSPILPMDNLEPGMGWARSDG  
ELDALRIPDSVSDGISNVIDLFEAIENGNIESIQYIEALACPGGCVGGPMAVENPHIARSNMRQRCQKD  
NLAAEASSSPMKAIKPPSPKLPEQEELPSYIWTEQVSPKPVLVLDADLSKALEMAEKIDTIHAQLPGIDC  
GACGAPDCCLAEDIVRGFASIEDCRLLEHPYSKGTKENIHSEGLST\*

SPBIB\_v1\_360015|ID:27164071| FeFe Group B hydrogenase catalytic subunit [Uncultured spirochete bib]  
MPREDNTTRIRRELLVRLGRLALEGLTADSIDDVDFDMTAEWETMRCCVHHDRAILRLRSYALLGGDVR  
GMDDVRKPLSVLAEGFQNSRCQDGAELGESGRFPLQVLGEACNACAHSRYVVTDACQACVARPCKVNCPK  
GAVTVNGRSHIDPDKCVNCGLCCEKSCPFHAIKVPVPCVEVCPGTGAIKGEDGIARIDEAKCILCGKCLR  
ACPFGAPIEQTYLEAASWLREQARPLIALVAPATMAQFPVSSGKFMAGLYRLGFAA VA EVAEACATAE  
REAAELRERMHRSEGFLATSCCPSWVLASKALGDVAGHVLHTPSPMAIAAKWARKRYPEARIVFIGPCLA  
KRAEARGFPGEDGRKLVDVLSAEEIGALFTAQDIQLQALEEARAVQLAEQGSSLDLCYGRGFAQSGGVA  
ASVQSVLENENSRIEVGSSWTIRTLVIQGLSKQVLAALWLNKQAPDADLVEVMACEGGCIGGPLAIAQA  
KSAAVFLQRYMSEPPPEAKVASSSEAVEKAV\*

Uncultured spirochete bdmA 4:

May evolve H<sub>2</sub> during carbohydrate fermentation by ferredoxin-coupled FeFe A1 hydrogenases  
May reversibly bifurcate electrons from H<sub>2</sub> to NAD and ferredoxin by multimeric FeFe A3 hydrogenase  
May sense H<sub>2</sub> leading to hydrogenase regulation by FeFe C1 hydrogenase

SPBDM4\_v1\_80097|ID:27159873| FeFe Group A1 hydrogenase catalytic subunit (fragment) [Uncultured spirochete bdmA 4]  
VCEETQGVNAIAFTGRGARTRVATFMDRGLAQSACVQCGQCSVVCPTGAITEKDES RDVFDALRDSKLSV  
VVQTAPAIRASLGEALGLPAGSLVTGQMVAALRRLGFAKVFDTQFTADLTIMEEGSELLERLSHGTTTPM  
ITSCSPGWINFIEGFYPDLLGHVSTCKSPQQMFGAVAKTYAQAAGIAPDKMRVVSIMPCTAKKYEARRK  
EMDGAWGWWEQDPGKVPARPFVDVWALTTRELARMIKLAGIEIHLPEEDFDDPLGRSTGAATIFGTT  
GGVMEALRTVYEIVEKKPLENIEFTQVRGFESIKA EVVLGGSPVRVAVAHGLSNARILLDEIRAGKSP  
YQFIEIMSCPGGCIGGGQPVLADIEKKLARSKALYTEDRILPIRKSHENPAVNTLYKDFLGKPLGHLSH  
ELLHTSYRARVF\*

SPBDM4\_v1\_40494|ID:27157799| FeFe Group A3 hydrogenase catalytic subunit [Uncultured spirochete bdmA 4]  
MVNVKVNIPVQVAEGSTMLEAAKKAHVKIPTLCYNPDLSWAACGICVVKVEGSNKMLRSCCTPVSEGM  
SIITNDADLVQTRRTVIELILSTHPDDCLFCPRNQSCELQTLAQEFGIREQPYKKMVRDIPTDTSTNSLI  
LNPSKCIRCGRCVEVCQEMQGVWAIEFLGRGESIRIAPAADV KLG DSPICGQCSAHCPVGAIYENDQT  
SLVWDALMKEGPDAKICAVQIAPAVRVALAESFGLPPGTDLTGKIYALRRLGFDAVFDTNFSADLTIME  
EGTELVHRLTEALKGGMQQATADHKLPLITSCCPAWVDYMEKYYSMDIPNFSTAKSPQQMMGVMIKTYWA  
AKAKVDPKIYSVSIMPCTAKKFENGRDESMYSSGYKDVDVTLTTRELARMIKQAGIDFLNLPDSQPDSP  
LGPYSGAGVIFGATGGVMEALRTAYFLVTKEELKDVNFTAVRGLSGIKEATVHINGIELRVAAAHQMGN  
IATVLEEVRKARAEGRETPWHFIEVMACRGGCIGGGQPYGATDEV RKL RMRSIYDHDEKSEYRCSHDNP  
YIKQIYAEFLEKPGSHRSHELLHTHYVEKPLYLK\*

SPBDM4\_v1\_40759|ID:27158064| FeFe Group C1 hydrogenase catalytic subunit [Uncultured spirochete bdmA 4]  
VSFEPANQSPNIFQTFHVSLLDADLCVGGCTTCIKFCPTKAIRVRNGKAKIFEDRCIDCGECIRRCPKGAK  
KAVSDPLFMMDAYDIKVALPAPSLYAQFGTKYSQSDIFNAIHSAGFDEVFDVAWGALVTTMTRSILAQE  
ALRPRISSACPVIVRLIQRFPSLIPNLMPIPPSEIAAREARRRLGSLSQKVGIFLSPCTAKVTSVRT  
PLGYEQSAIDAVFSFGDIYASLKRALDPTPSSAPHIDAISSGNASTHLLPIMNNLEPGMGWARSDGELD  
ALHIENAVSVDGISNVIELFEAIENGNIDS IQYIEALACPGGCVGGPMAVENPHIARSTMRQRYQHPFSA  
YPGSKTAQDHHRTAKERSKSLSPESSTADKEYLAFKWTRPLPPNPVLVLDTDLSKALQMAEKIEQIRNRL

PGIDCGACGAPDCDAFAEDIVRGLSSIEDCRLLASPPPARNEEENPK\*

Uncultured spirochete SA-8:

May evolve H<sub>2</sub> during carbohydrate fermentation by ferredoxin-coupled FeFe B hydrogenases  
May reversibly bifurcate electrons from H<sub>2</sub> to NAD and ferredoxin by multimeric FeFe A3 hydrogenase  
May sense H<sub>2</sub> leading to hydrogenase regulation by FeFe C1 and C3 hydrogenases

JGI12104J13512\_100111416 FeFe Group A3 hydrogenase catalytic subunit [Uncultured spirochete SA-8]  
MINCKVNGIPVQVAEGATILEASKKANVKIPTLCYNPDLPWAACGICVAKIEGSNKMLRACCTPVAEGM  
NIITHDPDIVETRKTVIEMILSTHPDDCLACPRNQNCLEQLTAQEFGIREQAFPKMLHDLPIDDTTGSIV  
LNPEKCVRCGRCVTVCCQMQRVWVAIEFLGRGETIRIAPAADAKLGESPCIKCGQCSAHCPVGAIIYENDQT  
KIVWDALRKTGDDAKTCVVQIAPAVRVALGEAFGLQPGTDLTGKIYTALKRGLFDVVFDTNFAADLTIME  
EGTEFVKRLTSALQNLGTATREKSMPLITSCCPAWVDYMEKYFPDMIPNFSTAKSPQQMMGAMIKTYWA  
EKANVRPDKIFSVSIMPCTAKKFETHRDETMCSSGYQDQDVSITRELARMIKQAGIDLLNLPSEPDSP  
LGPYTGAGAIFGATGGVMEALRTAYYLVTGNELKDVNFTAVRGISGIKEASVHVNGVVLRVAVAHQMGN  
IEQVLNEVRKARDEGRDTPWHFIEVMACRGGCIGGGGQPYGATDEVKRLRIRGIYDHDEGKEYRCSHQNP  
YIKKVYNEFLEKPGSHKAHELLHHTYTERPLFLK\*

JGI12104J13512\_10045282 FeFe Group B hydrogenase catalytic subunit [Uncultured spirochete SA-8]  
MAKENNAVRLKRRLLICEVTSLVLEGLADEIDSIPYAMTNNWETIQCCIIHHRANLRLRLMSLLGYSTE  
GMLDVKPLRRTYAQEALSGKKPDAFPLSLMSAACNGCTKSRIVTNCVCGCLARPCVNCVPGAVSIINN  
QSHIDPELCVNCGLCEKSCPFHAIKIPVPCEEVCPVGAIQKGEDGIAKIDESKCILCGKCLSACPFGAP  
VEKSEIVHVLSALIHGKKLIAMVAPAAMVQFPFPKGFIAALEHFGFSKVMEEVAEADRVAEAEAREFAE  
RLHENNKVLATSCCPSWVRAAGSMGLEQILSDTPSPMLVAAKTAKAQDPDAFTVFIGPCLAKRWEAHRAS  
QNSPLVYAVLTSEEVGAMLMAAGIQVNEENEEDLATAGNASVYGRGFAATQGVTAAVKHALSPSAVEVNS  
CIVSGITKDTASQLKKVQESDKPLLIEVMACDGGCINGPCQLTNPKVAGAFLERYKAAAEHTTALKSA\*

JGI12104J13512\_10009791 FeFe Group C1 hydrogenase catalytic subunit [Uncultured spirochete SA-8]  
AAREAKKLVAADLPSLRVGMFFISPCTAKVTAIRMPLGYERSLVDAVFSFQDIFPALKKALSRPSETPSR  
FAPLQERIAMRRGHGMDWARSDGEIDGLGIEQAVSVDGIQNVIALLEEIDNGKFSSVPYIEALACPGGC  
VGGPMAVANPHVARAAMKHIAAAEKTAAAQSGQSEQPAQAGFGWERELQPKPVFVLDRLMLKALQLAEQM  
ELITSQLPGLDCGACGSPDCRALAEDIVKGVKAVIEDCLVMMRKKTAFNLTNE\*

JGI12104J13512\_10045285 FeFe Group C3 hydrogenase catalytic subunit [Uncultured spirochete SA-8]  
MDSAQVIYTELTCQDCYKCLRECPVKAIQIVRGHARVLEDRCIHCGNCVEICPQKAKKVRSDLERAKIL  
IRLRQKTVLSLAPSFIAEFGIPMQKLIAGCKKLGFSHVSETSVGADAVSESVSKTIAEQPPLMLSSAC  
PAVVQYVDKYLPEMSGFISTACSPMAHARIKQTLGPGTAVVFAGPCIAKKREADASEGAVDVALTFQE  
LRQWFCEEGIDPEDAAVRDEDSFFLNQAQDGILYPIEGGMVASIKHLKHSDAHCMYSGIHQIREAVRGI  
VDQYLENENNPQNQKTLFLEMLACEGGCVNGPMVQQSGGTVAKRLRVLSEKEKRTERGRVLSEPVSVQDI  
AADLTMPQSIRQKPVAAVAVSEEDIKASLASIGKYSRKDELNCSGCGYDSCQAFAAAMFLGKAEKTMCLS  
YTRKLAQKKANALLKAMPSAAVVVDASMHIVECNKPFHELLGTDVMELYALKPSLEGADLRKLLPFWEAF  
EQVLMPEQDIVASDFQCNGKIVHGSVFSIEKGLLAGGLFQDITAPWIQKDRVISQARKVMSQNLRTVQK  
IAYLLGENAAEAEAALTSIIESFQSERSGDSYKSNKE\*

Desulfobacterium naphthalenivorans strain N47:

May couple respiratory H<sub>2</sub> oxidation to sulfate reduction as with other Deltaproteobacteria. High sequence homology and conserved binding motifs to NiFe 1a hydrogenases. However, hydrogenase sequence is highly incomplete maybe due to genome assembly errors or incomplete sequencing.

NiFe 1b hydrogenase: Hydrogenotrophic respiration using sulfate, fumarate, nitrate, metals, and azo compounds as terminal electron acceptors. Enzyme transfers H<sub>2</sub>-liberated electrons through cytochromes to terminal reductase.

DESUN47\_v1\_100151|ID:27211809| NiFe Group 1a hydrogenase catalytic subunit (fragment) [Uncultured Desulfobacterium sp N47]  
MKVNLGPVTRIEGHLNIETTVENNKIVDARCMGEMFRGFVFLQGRSPLDAQQITQRICGVCPYAHAVAS

SYAQESVYKLVNPPNGRIMHNLIQGANHLYDYLLQFYQLAALDFVDITAILKYKG\*  
DESUN47\_v1\_100152|ID:27211810| NiFe Group 1b hydrogenase large subunit [Uncultured Desulfobacterium sp N47]  
MAKRITIDPITRIEHLRIEVEVADGKVVNAWSSGQMFRGIEMILKGRDPRDAPLFTQRS  
CGVCTYVHYLASVRAIEAAVGVQIPENARILRNLLHGTQYQHDHIIHFYHLHALDWVDIL  
SALKADPQKTAGLAENVCQARWGGTAYFKQVQDRIKTFVESGQLGPFNNAYWGHPAYVLS  
PEANLMAVSHYLEALRLQAKAAQMHAVFGAKNPHLQSLVVGGVTCAMDLPDRIAEFLYL  
WKETQTFVKNVYLPDVLAIKSFYKDWGALGGTSNFLAWGDFPEGEREPDSLFPMPRGLIMN  
RDISTVKQAEQDKITEHVAHSWYVGNADLHPFQGQTNPQHGDYNPDDRYSWIKAPRYEGE  
PCEVGPLARMLVAYGSGKSTARKLVDDTLTQLSIPVTALFSTLGRTAARALETVLVGDAM  
EGWIMKLVENLKSGQDNTYQWTMPDKAIGCGLNDVPRGSLGHWIEIEDKKIKNYQYVVP  
STWNLGPRCSNGKLGPEQALIGTPVADPKRPLEVLRTVHSFDPCIACAVHMIDPRSNEV  
YRIQVL\*