Supplementary Information for

The frequent occurrence and metabolic versatility of *Marinifilaceae* bacteria involved in organic matter mineralization a key member in global deep sea as

Jianyang Li[1, 2], Chunming Dong[1], Qiliang Lai[1], Guangyi Wang[2, *], Zongze Shao[1, 3, *]

Running head: The *Marinifilaceae* bacteria in deep sea

[1]*Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, Ministry of Natural Resources of PR China; State Key Laboratory Breeding Base of Marine Genetic Resources; Key Laboratory of Marine Genetic Resources of Fujian Province, Xiamen 361005, PR China.*

[2]*School of Environmental Science and Engineering, Tianjin University, Tianjin 300387, PR China*

[3]*Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai 519000, PR China*

*Corresponding authors:

Zongze Shao. Tel: +86-592-2195321. Fax: +86-592-2085376. E-mail: shaozz@163.com; Guangyi Wang. E-mail: gywang@tju.edu.cn.

Supplementary materials

Bacterial community succession incubated with lignin at laboratory

The compositions of artificial seawater medium are as follow: 30 $g \cdot L^{-1}$ of NaCl, 0.14 $g \cdot L^{-1}$ of K_2HPO_4, 0.14 $g \cdot L^{-1}$ of $CaCl_2 \cdot 2H_2O$, 0.25 $g \cdot L^{-1}$ of NHCl, 0.25$g \cdot L^{-1}$ of $NaNO_3$, 4.18 $g \cdot L^{-1}$ of $MgCl_2 \cdot 6H_2O$, 0.33 $g \cdot L^{-1}$ of KCl, 0.5$g \cdot L^{-1}$ of $MgSO_4 \cdot 7H_2O$. 1 $g \cdot L^{-1}$ of Kraft lignin (471003-500G, Lot#MKCK3344, SIGMA-ALDRICH, St.Louis, USA) was added into the artificial seawater medium as the sole carbon and energy source. 1 $g \cdot L^{-1}$ of resazurin as oxygen indicator was added into the medium. Before autoclaving, the mixed medium was sparged with N_2 for 15 min to crowd out O_2 and subsequently sealed with a butyl rubber stopper and plastic cap. After autoclaving, trace elements (SL-10 in DSMZ medium 320) and vitamins (DSMZ medium 141) were added to the medium. The final pH was 7.6. Around 1 % of original enrichment enriched with plant detritus in the deep sea of the South China Sea was added into the medium amended with lignin and incubated under the conditions of no light, 4 °C and 0.2 MPa. After cultivation for three months (label this incubation as G0), 1 ml of incubation liquid was added into two new vials of medium amended with lignin and incubated under the same conditions. After six months and one year, 10 ml liquid was absorbed from the incubations and immediately filtered with a 0.22-μm pore size polycarbonate membrane (Millipore, USA). Here, we labeled the incubated cultures for six months and one year as G1 and G2, respectively.

DNA extraction method is shown in 2.2. Community structure was determined by high-throughput sequencing of V3-V4 region of 16S rRNA gene.

De novo synthesis of cobalamin by MF

Cobalamin (vitamin B12) is an essential enzyme cofactor in the synthesis of nucleotides

and amino acids, in addition to carbon processing within all domains of life (1). Cobalamin producers are therefore very important to various ecosystems; however, only a relatively small subset of bacteria and archaea are capable of producing cobalamin. A recent report showed that cobalamin biosynthesis in marine systems has been inferred in three main groups: select heterotrophic Proteobacteria, chemoautotrophic Thaumarchaeota, and photoautotrophic Cyanobacteria; in the deep sea, only Thaumarchaeota are major producers of cobalamin (2).

Here, we confirmed that MF bacteria are cobalamin producers, but only the members of MF-2 have the entire *de novo* synthesis pathway (**Table S15**), encoding all the genes for the first part, i.e., corrin ring biosynthesis via the anaerobic pathway by the gene cluster of *cysG* and *cbiCDEFGHJKLT*, and the second part, i.e., the final synthesis reaction, in which cobyrinic acid is further modified to form cobalamin by the gene cluster of *cbiA* and *cobAQCUS*, in addition to the transporter complex *cbiMNOQ* involved in cobalt import (**Table S15**) (3). In addition, MAG B8, B9, and B11 have almost the entire *de novo* cobalamin synthesis pathway (**Table S15**). The metatranscriptome data demonstrate the transcriptional expression of related genes of MAG B6, B8, and B9 during in situ degradation of various types of OM in the deep sea (**Table S15**). Our results show that MFs, as cobalamin producers, play distinctive roles in cobalamin-based microbial interdependencies that sustain the community composition and biogeochemical function of OM in the deep sea. With regard to the wide distribution of MF in various marine habitats, especially chemoheterotrophic ecosystems such as wood falls, their contribution as cobalamin producers in the ocean is worth considering.

Environmental adaptation to the deep sea by MF

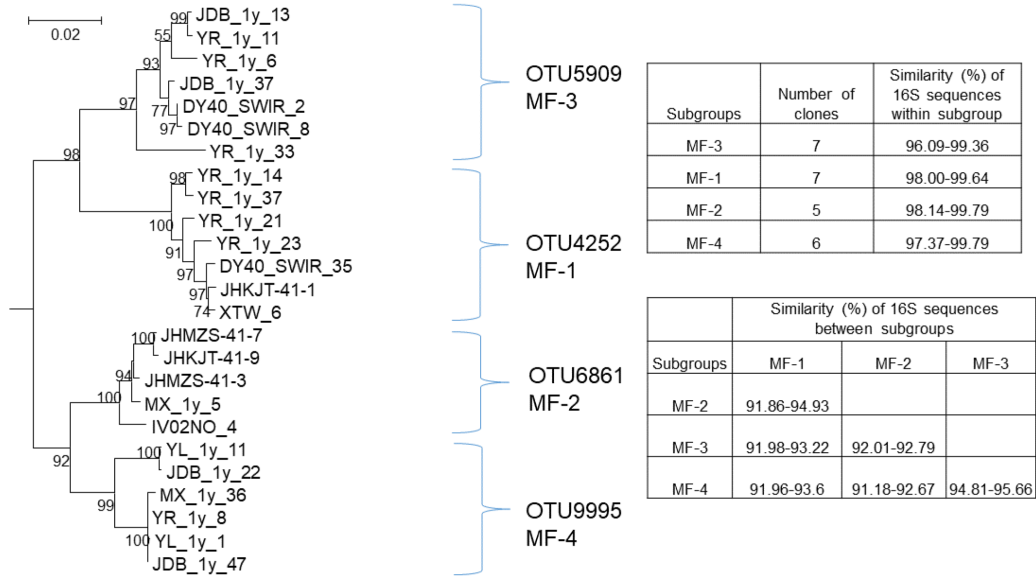
The deep sea is an extreme environment characterized by high hydrostatic pressure and low temperature, where indigenous microbes must thus adapt to the extreme environmental stresses. As a result, genomic features endow MF bacteria with cold adaptation (cold-shock protein *CapAB*). CapAB have been reported to prevent the formation of cold-induced mRNA secondary structures (4). Genes encoding CapAB in MAG B6, B8, and B9 were highly transcribed in polysaccharide/lignin, protein, and/or lipid enrichments (**Table S16**).

For high hydrostatic pressure stress, MF members harbour some genes encoding *OmpH*, and these genes were expressed at high levels in MAG B6, B8, and B9 *in situ* (**Table S16**). The gene *OmpH* has been reported to be induced in response to elevated pressure (5). Recently, a study demonstrated that two potential novel species of MF isolated from sulfidic waters of the Black Sea, tentatively named *Ancylomarina euxinus* and *Labilibaculum euxinus*, relieved the high hydrostatic pressure in the deep sea by changing the fatty acid and lipid composition in the membrane (6).

Since large quantities of hydrogen sulfide are produced in OM-rich environments (7) and subsequently may be oxidized to diverse valence states of reactive sulfur species (8), MF bacteria faced severe oxidative stress *in situ*. Diverse reductases responding to oxidative stress were detected in those MF MAGs and were highly expressed *in situ* in the deep sea, such as thioredoxin, rubrerythrin, peroxidase, and peroxiredoxin (**Table S16**), in addition to these superoxide dismutase mentioned above. Among, the thioredoxin plays an important role in maintaining thiol-disulfide homeostasis to defend against oxidative stress (9). Moreover, peroxiredoxins can remove reactive sulfur species ($RS^{\bullet}$, $RSSR^{-\bullet}$, and RSOOH) and are critical for protecting cellular components from oxidative damage, as described in this literature (10). In conclusion, all above

findings explain the mechanisms how MF members can survive in the extreme deep-sea environment.

Supplementary Figures



Subgroups	Number of clones	Similarity (%) of 16S sequences within subgroup
MF-3	7	96.09-99.36
MF-1	7	98.00-99.64
MF-2	5	98.14-99.79
MF-4	6	97.37-99.79

	Similarity (%) of 16S sequences between subgroups		
Subgroups	MF-1	MF-2	MF-3
MF-2	91.86-94.93		
MF-3	91.98-93.22	92.01-92.79	
MF-4	91.96-93.6	91.18-92.67	94.81-95.66

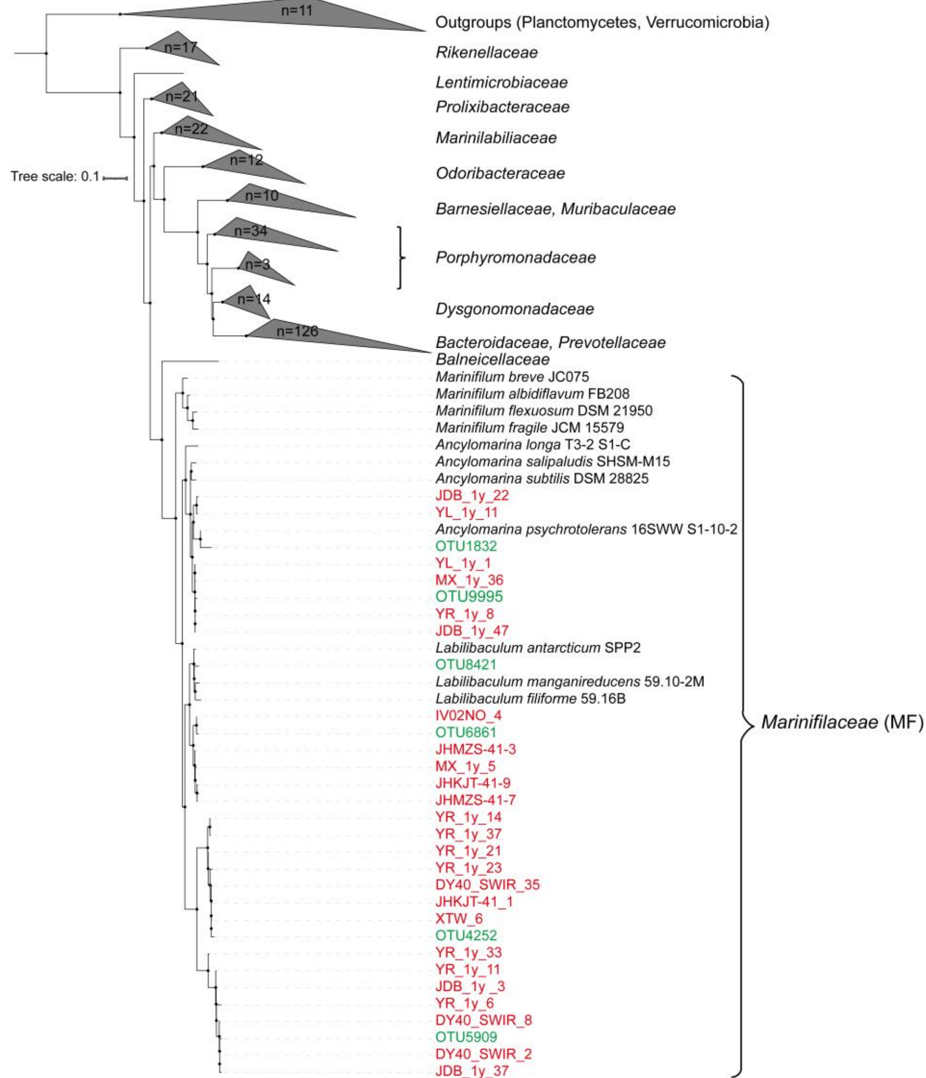


Fig. S1 Phylogenetic relationships for MF based on 16S rRNA gene sequences. (A) Phylogenetic tree based on the 25 clone sequences belonging to MF and obtained in this study and the 16S rRNA gene sequence similarities within and between the groups. A total of 25 near-full-length 16S rRNA gene sequences affiliated with the MF clade were cloned from these enrichments. The lowest similarity among subgroups was 91.2 % between MF-4 and MF-2, so we used the full-length 16S rRNA gene sequences of the four phylogenetic representatives as the query to retrieve the homologues from the NCBI database using Blastn. (B) 16S rRNA gene phylogenetic tree containing 25 clones (marked red) and 6 OTUs (marked green) belonging to the MF family, as well as currently identified strains within *Bacteroidales*. Some strains within the phyla Planctomycetes and Verrucomicrobia were identified as outgroups. This tree shows the phylogenetic placement of MF members within *Bacteroidales*.

A

300
250
200
150
100
50
0

Numer of gene

Number of GH gene
Number of GH gene with signal peptide

MF-2
MF-4
MF-3
MF-1
MF-8

B6
WF05
WF09
WF13
Labilibaculum sp. LM
Labilibaculum sp. 44
Labilibaculum sp. A4
Labilibaculum manganireducens 59.10-2M
Labilibaculum filiform 59.16B
Labilibaculum antarcticum SPP2
B7
B8
B9
Ancylomarina subtilis DSM 28825
Ancylomarina salipaludis SHSM-M15
Ancylomarina psychrotolerans 16SWW S1-10-2
Ancylomarina sp. M3P
Ancylomarina sp. M1P
B10
B11
B12
B13
Marinifilum fragile JCM 15579
Marinifilum breve JC075
Marinifilum flexuosum DSM 21950
Marinifilum sp. JC070
Marinifilum sp. N1E240

B

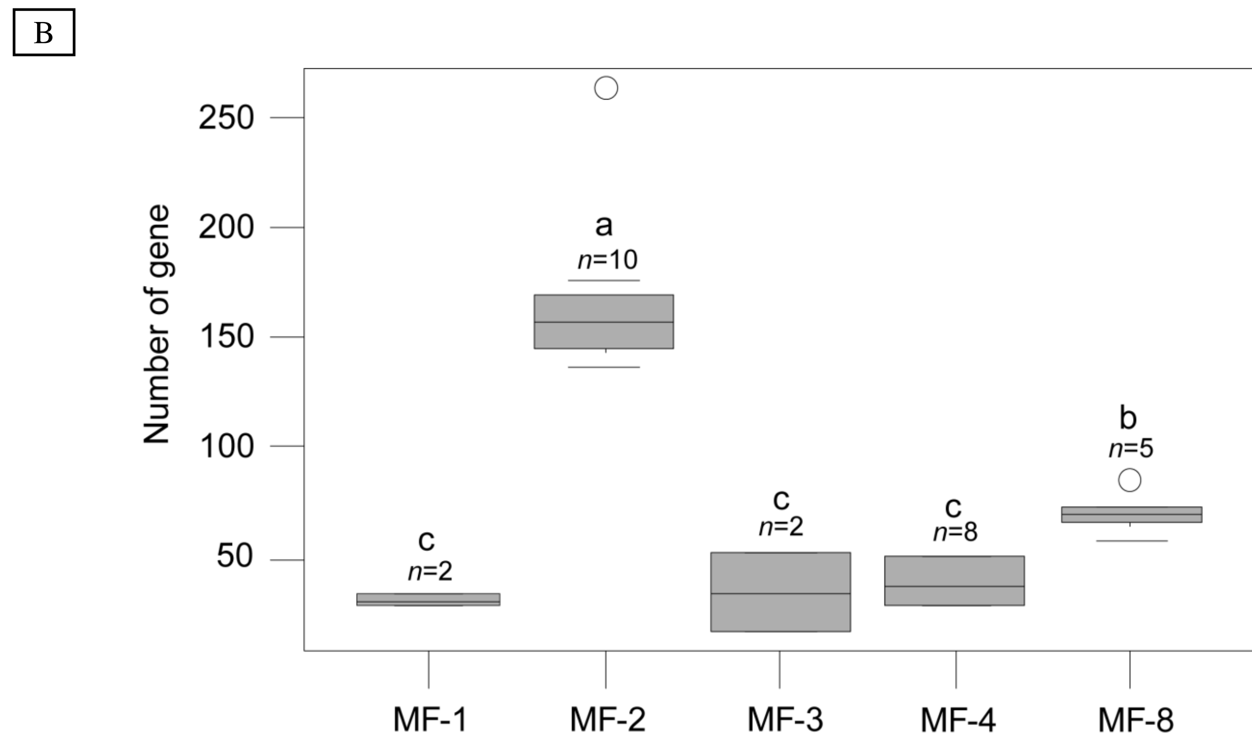


Fig. S2 The number of total GH genes and GH genes containing signal peptides in

each genome (A) and the comparative analysis of GH numbers (B) among the five MF subgroups. This result indicates that MF-2 members have significantly more GH-encoding genes than the other four subgroups (MF-1, 3, 4, and 8). WF13, WF05, and WF09 are from wood falls (19, 38), B6-B13 are from our *in situ* enrichments in this study, and others are cultured strains. There were significant differences ($P < 0.001$) in the number of genes between the groups marked with different letters on the boxes.

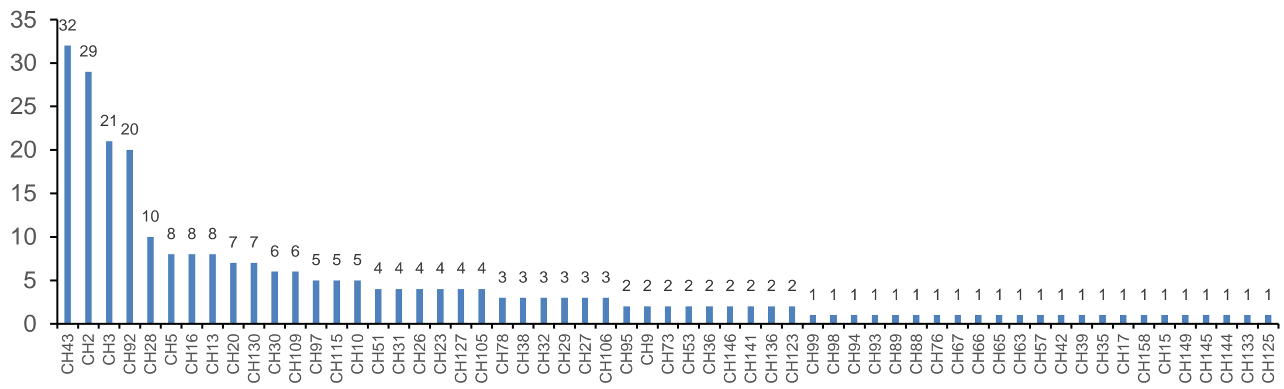


Fig. S3 The gene numbers of different GH families in MAG B6. MAG B6 of MF-2 is the predominant species group in the polysaccharide assemblages.

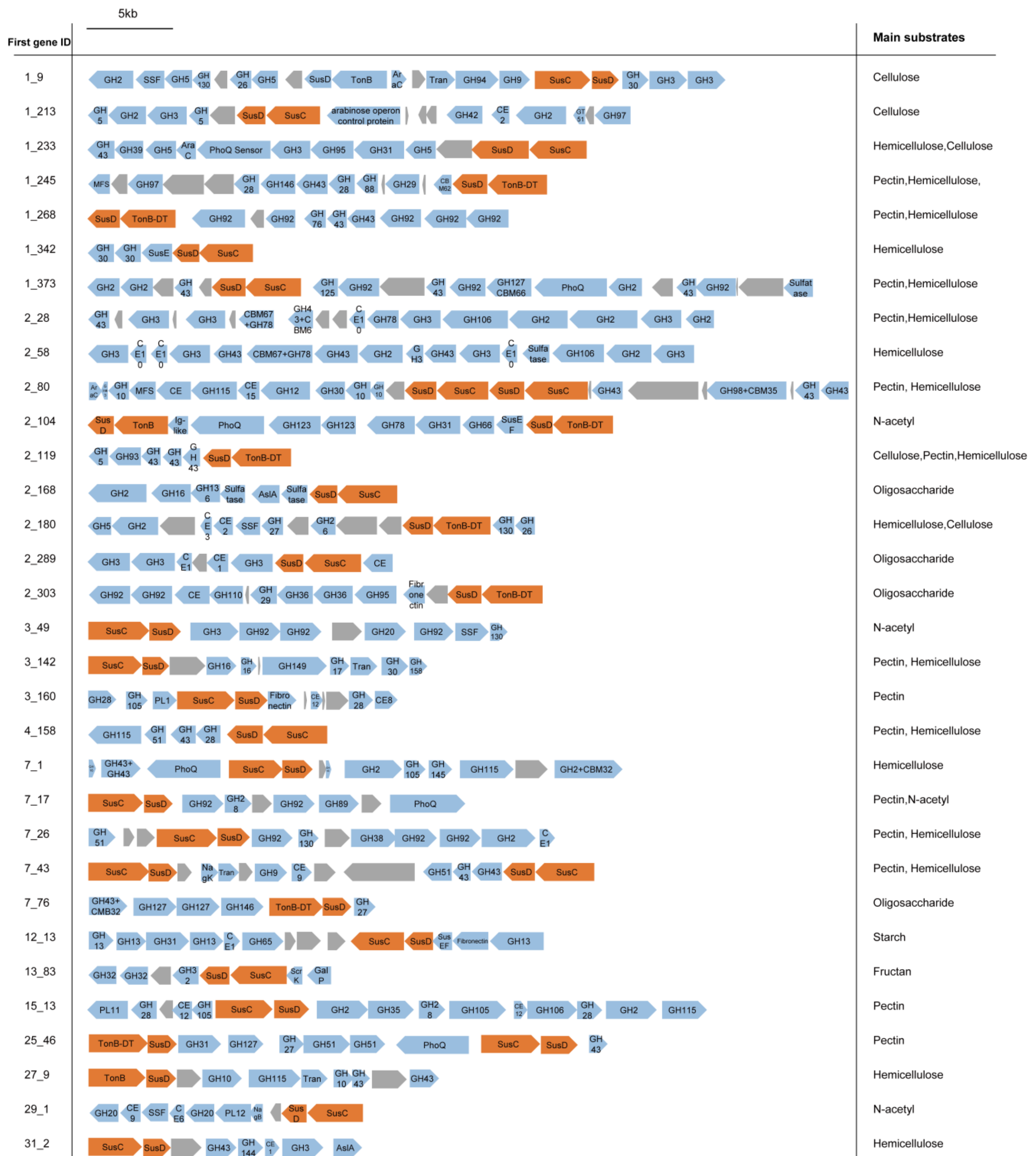


Fig. S4 Polysaccharide utilization loci (PULs) in MAG B6. SusCD-like genes cluster with various GH, CBM, GT, and/or CE genes to form PULs. There are at least 32 PULs present in MAG B6. The first gene ID in each PUL is shown on the left and hydrolytic substrate for each PUL is shown on the right. The genes marked in red are SusCD-like complex genes; the genes marked in blue are GH, CBM, GT, and/or CE genes; and the genes marked grey are of unknown function.

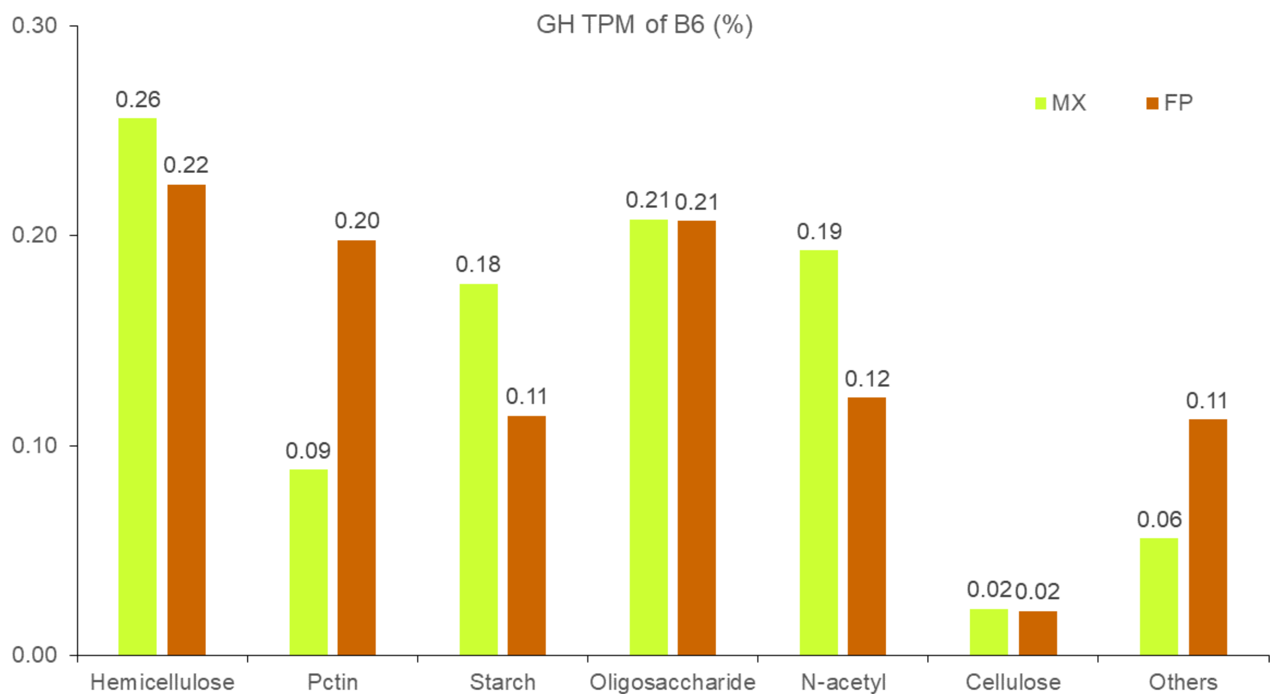


Fig. S5 Bar chart showing the relative transcriptional levels of different GHs in MAG B6 involved in polysaccharide and oligosaccharide hydrolysis in wood chip (MX) and wheat bran (FP) enrichments. Those GHs involved in the hydrolysis of hemicellulose, pectin, starch, N-acetyl-containing polysaccharide, and oligosaccharide are transcribed at high levels and account for > 89 % of the total GH transcripts in MAG B6.

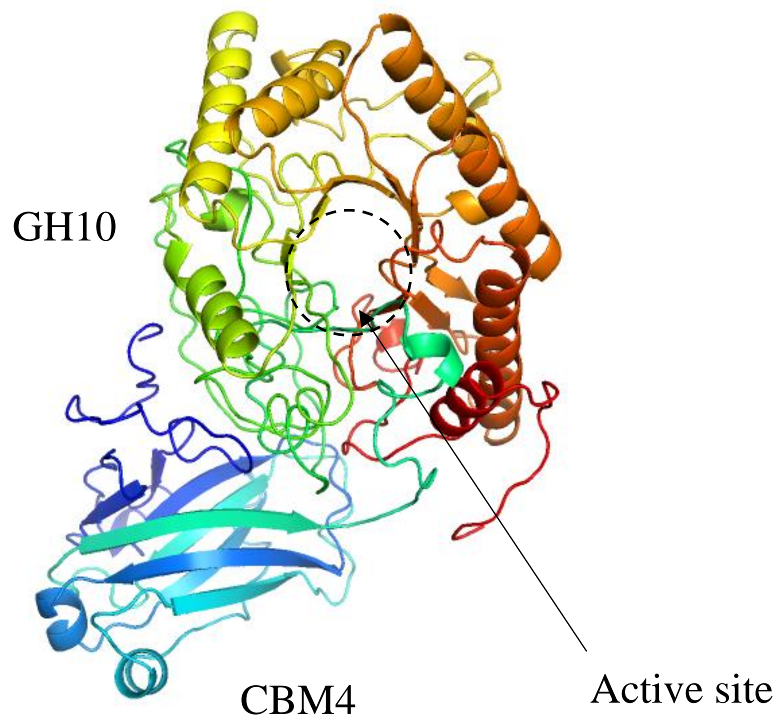


Fig. S6 The protein structure of key enzymes. The three-dimensional protein structure of gene 27_12 in MAG B6 involved in polysaccharide hydrolysis predicted by using Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index).

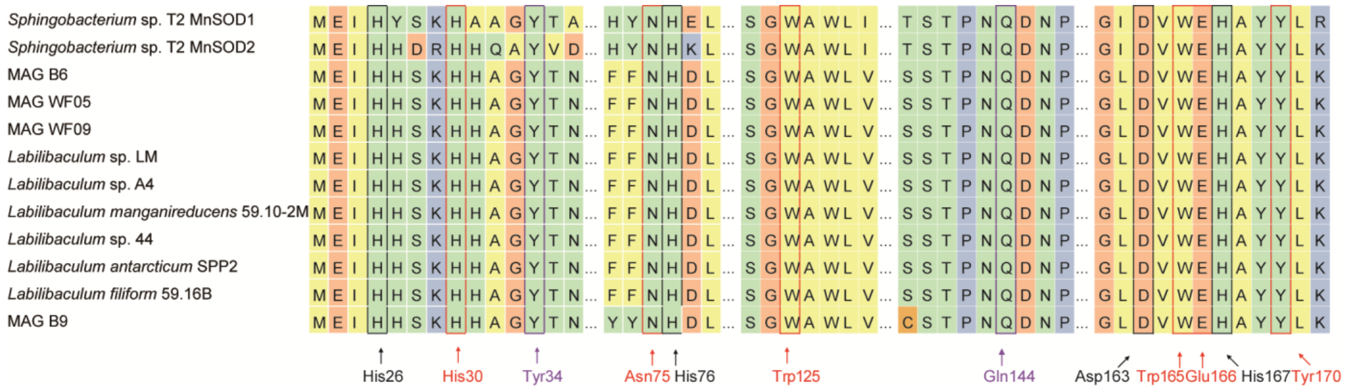


Fig. S7 The conserved amino acids for the key residues in MnSOD. MnSODs of MF members contain the structurally and functionally important residues like MnSOD1 and MnSOD2 both from *Sphingobacterium* sp. T2; Mn(II) ligands His26, His76, Asp163, and His167; gateway residue Tyr34 and catalytic Gln144; other active site residues His30, Asn75, Trp125, Trp165, Glu166, and Tyr170.

Fig. S8 Neighbour-joining tree of amino acid sequences of the group A3 [FeFe]-hydrogenase catalytic subunit (A), and the group B and C1 [FeFe]-hydrogenase catalytic subunit (B). The tree shows sequences of MF members from metagenome-assembled genomes obtained in this study and cultured strains (red) alongside

representative reference sequences (black). The representative reference sequences were retrieved from a previous study (11).

1. Lu X, Heal KR, Ingalls AE, Doxey AC, Neufeld JD. Metagenomic and chemical characterization of soil cobalamin production. The ISME journal. 2020;14(1):53-66.
2. Heal KR, Qin W, Ribalet F, Bertagnolli AD, Coyote-Maestas W, Hmelo LR, et al. Two distinct pools of B_{12} analogs reveal community interdependencies in the ocean. Proceedings of the National Academy of Sciences. 2017;114(2):364-9.
3. Fang H, Kang J, Zhang D. Microbial production of vitamin B12: a review and future perspectives. Microbial Cell Factories. 2017;16(1):15.
4. Higgs PG. RNA secondary structure: physical and computational aspects. Q Rev Biophys. 2000;33(3):199-253.
5. Bartlett DH, Welch TJ. ompH gene expression is regulated by multiple environmental cues in addition to high pressure in the deep-sea bacterium Photobacterium species strain SS9. J Bacteriol. 1995;177(4):1008-16.
6. Yadav S, Villanueva L, Bale N, Koenen M, Hopmans EC, Damsté JSS. Physiological, chemotaxonomic and genomic characterization of two novel piezotolerant bacteria of the family Marinifilaceae isolated from sulfidic waters of the Black Sea. Syst Appl Microbiol. 2020;43(5):126122.
7. Kalenitchenko D, Le Bris N, Dadaglio L, Peru E, Besserer A, Galand PE. Bacteria alone establish the chemical basis of the wood-fall chemosynthetic ecosystem in the deep-sea. The ISME journal. 2017.
8. Mishanina TV, Libiad M, Banerjee R. Biogenesis of reactive sulfur species for signaling by hydrogen sulfide oxidation pathways. Nat Chem Biol. 2015;11(7):457-64.
9. Ezraty B, Gennaris A, Barras F, Collet J-F. Oxidative stress, protein damage and repair in bacteria. Nature Reviews Microbiology. 2017;15:385.
10. Rhee SG. Overview on Peroxiredoxin. Molecules and cells. 2016;39(1):1-5.
11. Greening C, Biswas A, Carere CR, Jackson CJ, Taylor MC, Stott MB, et al. Genomic and metagenomic surveys of hydrogenase distribution indicate H-2 is a widely utilised energy source for microbial growth and survival. Isme Journal. 2016;10(3):761-77.