

Supplementary information:

Genomic and *in situ* investigations of the novel uncultured Chloroflexi associated with 0092 morphotype filamentous bulking in activated sludge

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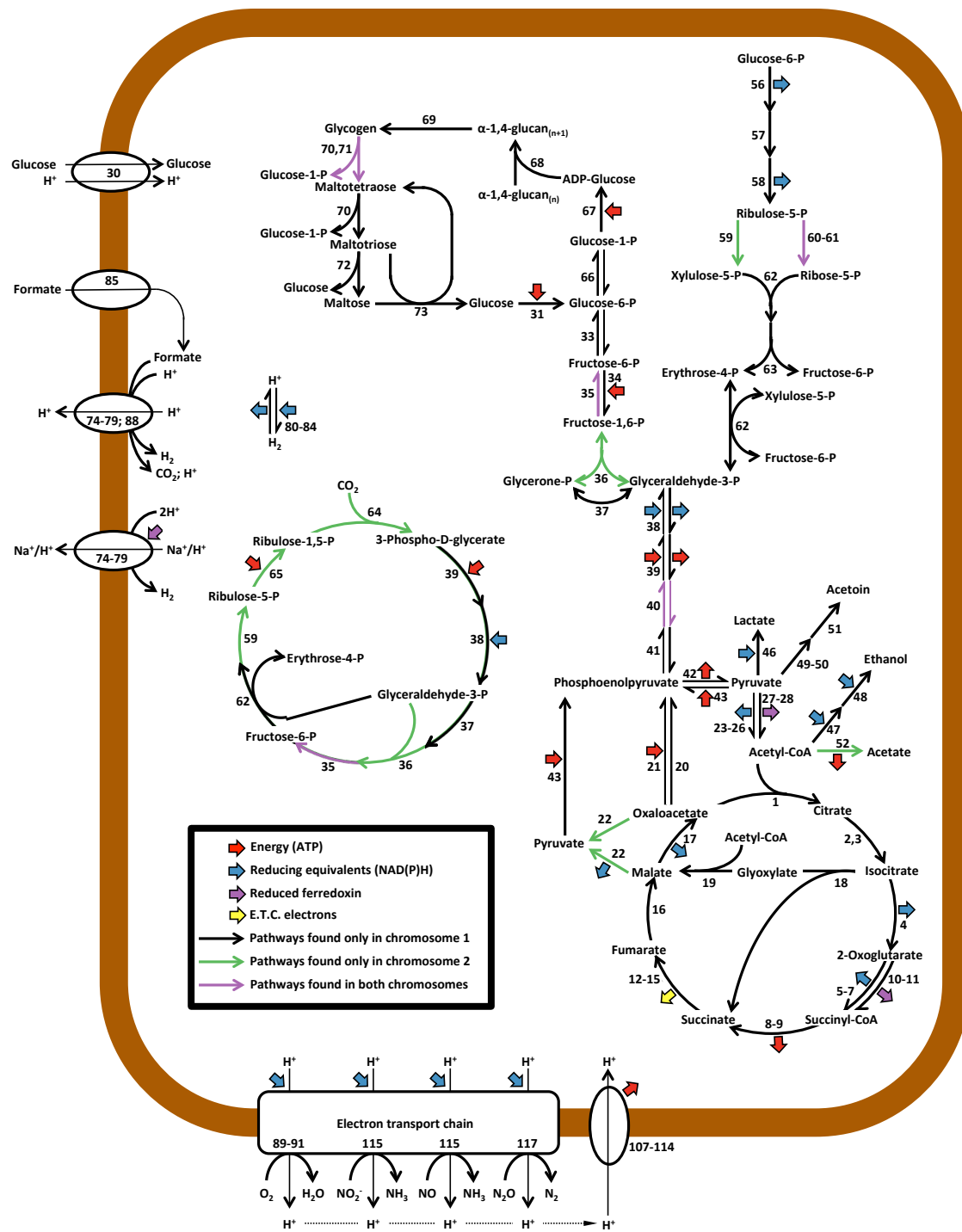


Figure S1. Metabolic summary for Cfx-K in activated sludge. Reference numbers for each reaction relate to the details in **Table S2**.

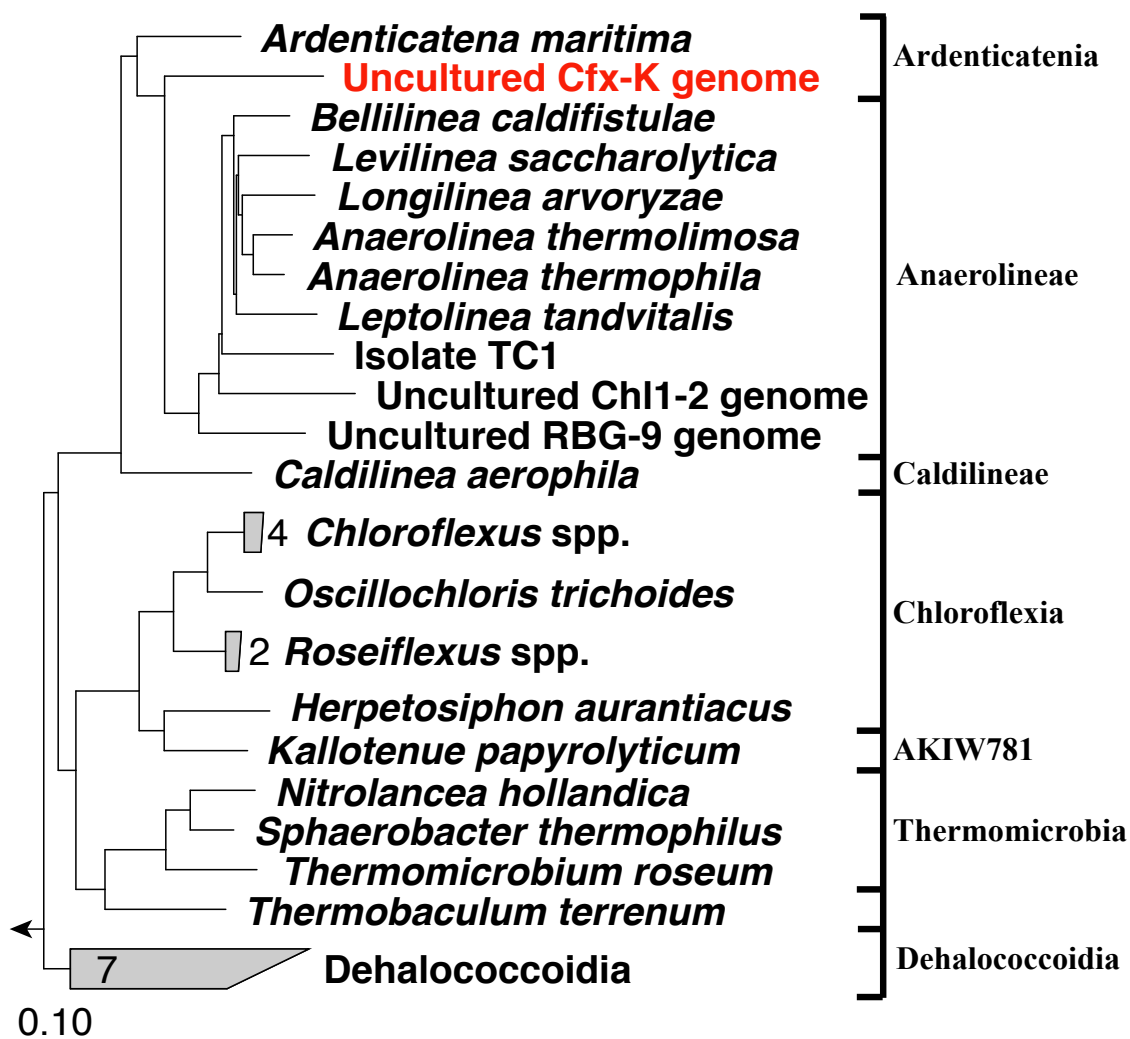


Figure S2. Phylogenetic tree for the available Chloroflexi genomes. The CheckM software (Parks *et al.*, 2015) was used to generate the tree using default settings, which is inferred from the concatenation of 43 conserved marker genes and incorporates 2052 finished and 3604 draft genomes from the IMG database (Markowitz *et al.*, 2012). Generated trees were imported into ARB for visualization and phylogenetic grouping of sequences into clades. Additional genomes include: *Leptolinea tandvitalis* (BBYA00000000), *Longilinea arvoryzae* (BBXY00000000), *Anaerolineaceae* bacterium TC1 (BBYH00000000), *Ardentecatenia maritima* (BBZA00000000), *Anaerolinea thermolimos* (BBXW00000000), *Bellilinea caldifistulae* (BBXX00000000), *Levilinea saccharolytica* (BBXZ00000000), *Nitrolancea hollandica* (CAGS00000000), RBG-9 (PRJNA167727) and Ch11-2 (APVK00000000). The genome obtained in this study is shown in red. Class level classification (right) is based on the MiDAS database (Release 1.20).

			Cell envelope characteristics																M					
Enriched in	Category	PFAM	PFAM description	M	M	M	M	D	DA	DA	DA	DA	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	M
				Actinobacteria	Chloroflexi	Firmicutes	Tenericutes	Deinococcus-Thermus	Thermotogae	Synechistales	Cyanobacteria	Fusobacteria	Bacteroidetes	Chlorobi	Chlamydiae	Verrucomicrobia	Planctomycetes	Spirochaetes	Proteobacteria	Acidobacteria	Aquificae		Chloroflexi 0092 k2	
P	LPS	PF04413	Glycos_transf_N - (kdottransferase)	0	0	0.01	0	0	0.67	0.02	1	0.92	1	0.97	0.88	1	0.22	0.93	1	1				
P	LPS	PF02614	LpxK - Tetraacyl-disaccharide-1-P 4'-kinase	0	0	0.01	0	0	0.67	0.05	1	0.98	1	1	1	1	0.16	0.94	1	1				
P	LPS	PF02684	LpxB - Lipid-A-disaccharide synthetase	0	0	0.02	0	0.17	0	0.67	1	0.96	0.98	1	1	1	1	0.22	0.93	1	1			
P	LPS	PF03331	LpxC - UDP-3-O-acyl N-acetylglucosamine deacetylase	0	0	0.02	0	0.25	0	0.67	0.98	1	0.98	1	1	1	1	0.22	0.93	1	0.92			
P	LPS	PF04613	LpxD - UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase, LpxD	0	0	0.02	0	0	0.33	1	1	0.98	1	1	1	1	1	0.22	0.91	1	0.5			
P	CE	PF02472	ExbD - Biopolymer transport protein ExbD/TolR	0	0	0.02	0	0.75	0	1	0.59	1	0.99	1	1	1	1	0.33	0.96	1	1			
P	CE	PF07244	Surf_Ag_VNR - Surface antigen variable number repeat	0	0	0.02	0	1	1	0.67	1	1	1	1	1	1	1	1	1	1	1			
P	CE	PF03739	YjgP_YjgQ - Predicted permease YjgP/YjgQ family	0	0	0.02	0	1	1	0.67	1	1	0.99	1	1	1	1	1	1	0.95	1	1		
P	CE	PF01103	Bac_surface_Ag - Surface antigen	0	0	0.02	0	1	0.77	0.67	1	1	0.99	1	1	1	1	1	1	1	1			
P	CE	PF00263	Secretin - Bacterial type II and III secretion system protein	0	0	0.01	0	1	1	1	0.8	1	0.08	0.42	1	0.88	1	0.18	0.85	1	1			
P	CE	PF02321	OEP - Outer membrane efflux protein	0	0	0.13	0	0.83	0.62	0.67	1	1	0.98	1	0	1	1	0.87	0.98	1	1			
P	CE	PF03968	OstA - OstA-like protein	0	0	0.02	0	0.92	0.62	1	0.8	0.88	0.23	0.25	0	0.75	0.11	0.91	0.95	0.6	1			
P	CE	PF00593	TonB_dep_Rec - TonB dependent receptor	0.01	0	0.01	0	0	0.67	0.34	0.96	0.98	1	0	1	0.33	0.29	0.87	1	1				
P	CE	PF03544	TonB - Gram-negative bacterial tonB protein	0	0	0.02	0	0	0.67	0.46	0.83	0.98	1	0	0.88	0.33	0.24	0.89	1	1				
P	CE	PF07715	Plug - TonB-dependent Receptor Plug Domain	0	0	0.01	0	0	0.67	0.36	0.96	0.98	1	0	1	0.33	0.31	0.88	1	1				
P	CE	PF04166	PdxA - Pyridoxal phosphate biosynthetic protein PdxA	0.14	0	0.16	0	0	0.08	0.33	0.98	0.5	0.97	1	0	1	1	0.18	0.88	0.4	1			
P	CE	PF06835	Lipopolysaccharide-assembly, LptC-related	0	0	0.01	0	0	0.23	0.33	0.97	0.75	0.95	1	0	0.38	0.11	0.38	0.81	0.4	0.75			
P	CE	PF03740	PdxJ - Pyridoxal phosphate biosynthesis protein PdxJ	0	0	0	0	0	0	0.98	0	0.8	1	0	1	1	0.13	0.9	0	1				
P	CE	PF03548	LolA - Outer membrane lipoprotein carrier protein LolA	0	0	0	0	0.08	0	0	0	0	0.72	0	0	0.25	0.22	0.47	0.97	1	1			
P	CE	PF04052	TolB_N - TolB amino-terminal domain	0	0	0	0	0	0	0	0	0	0	0.83	0	0.13	0	0	0.92	1	0			
P	CE	PF04453	OstA_C - Organic solvent tolerance protein	0	0	0	0	0	0	0	0	0	0	0	0.5	0.33	0.09	0.93	1	0.5				
A+F	CE	PF02645	DegV - Uncharacterised protein, DegV family COG1307	0.65	1	0.96	1	1	1	0	0	0.96	0.01	0.08	0	0	0	0	0.06	0	0		CFX0092_v1_a2545	
A+F	CE	PF05103	DivIVA - DivIVA protein	0.98	0.33	0.91	0.71	0.92	0.38	1	0	0	0.07	0.08	0	0	0	0.02	0.05	0	0.17			
A+F	CE	PF02650	HTH_WhiA - Sporulation Regulator WhiA C terminal domain	0.98	0	0.98	1	0	1	0.67	0	1	0	0	0	0	0	0	0	0	0			
A+F	CE	PF10298	WhiA_N - Sporulation Regulator WhiA N terminal	0.94	0	0.94	0.57	0	0.69	0	0	0.92	0	0	0	0	0	0	0	0	0			
A+F	CE	PF04472	DUF552 - Protein of unknown function (DUF552)	0.99	0	0.97	0.57	0	0	0.33	0.98	1	0	0	0	0	0	0	0	0	0			
A+F	CE	PF04203	Sortase - Sortase family	0.78	0.6	0.84	0	0	0	0	0	0	0	0	0	0	0	0.07	0.4			CFX0092_v1_a1813		
A+F	CE	PF03816	LytR_cpsA_psr - Cell envelope-related transcriptional attenuator domain	0.99	0.6	0.98	0	1	1	0.67	0.82	0.17	0.02	0	0	0	0	0.91	0	0			CFX0092_v1_a0162_a1038_a2212	
A+F	Other	PF09269	DUF1967 - Domain of unknown function (DUF1967)	0.98	1	0.98	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0		CFX0092_v1_a3578	
A+F	Other	PF01424	R3H - R3H domain	0.9	1	0.81	0.07	1	1	1	0.61	0.96	0	0	0	0.38	0	1	0.05	0.8	0		CFX0092_v1_a0550_a3587	

Figure S3. Annotated cell envelope components of the Cfx-K genome. The genome encodes protein families consistent with an archetypical monoderm cell envelope. Protein families substantially enriched or depleted in archetypal monoderm lineages (Actinobacteria and Firmicutes, A+F) relative to an archetypal diderm lineage (Proteobacteria, P), most of which have known roles in cell envelope biosynthesis. Cell envelope classification: Monoderm (M), Diderm (D), Diderm-LPS (DL), Diderm-Atypical (DA). Category: Lipopolysaccharide related (LPS), Cell envelope related (CE).

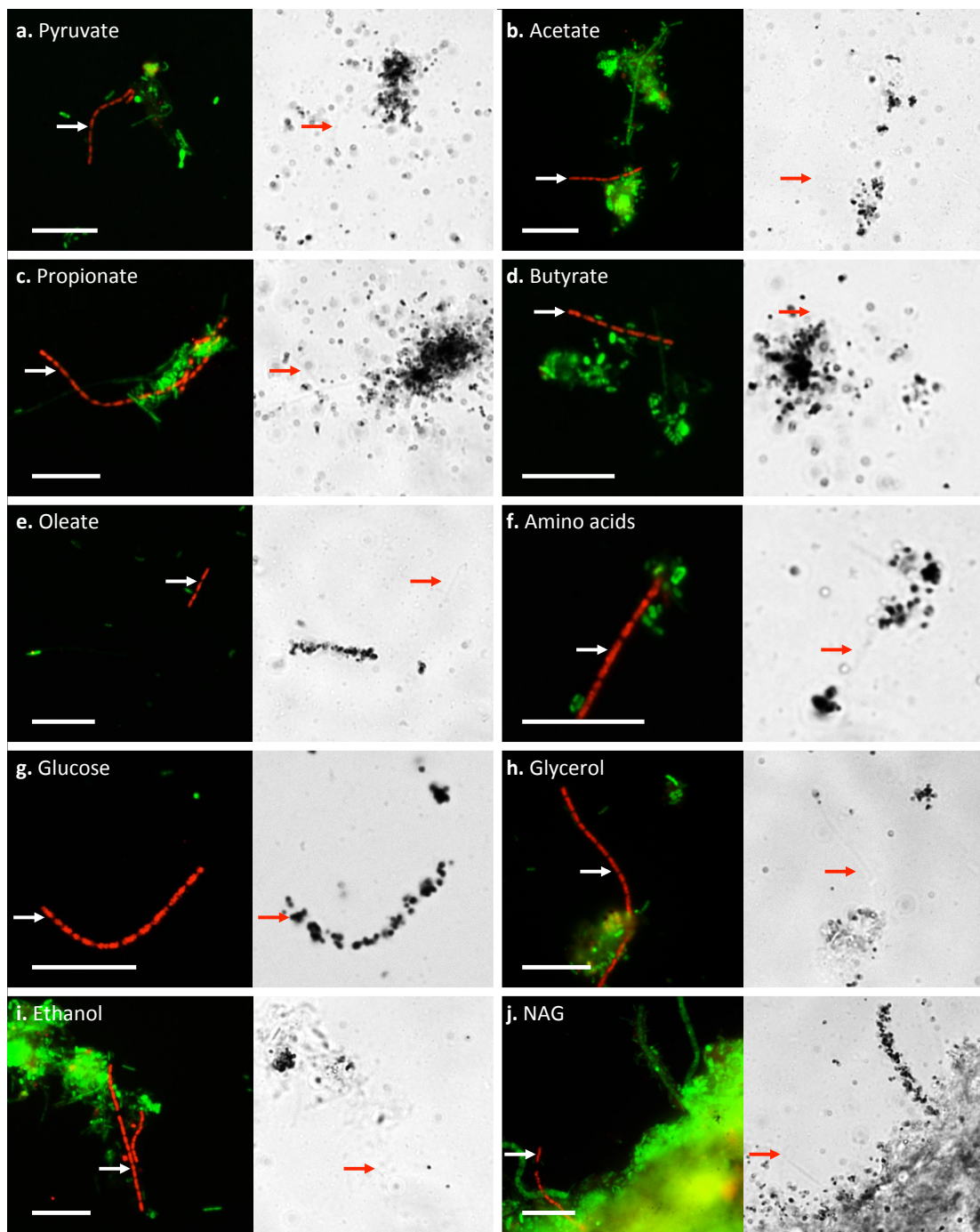


Figure S4. FISH and corresponding bright-field MAR micrographs for B45 taxon members in full-scale sludge. Radiolabeled MAR-substrate is indicated for each image set. Target cells in FISH micrograph overlays appear red [CFX197 (Speirs *et al.*, 2009) (Cy3 = red)] and non-target green [EUBmix (Amann *et al.*, 1990; Daims *et al.*, 1999) only]. Note that the EUBmix probe does not cover B45 members. Black silver granules indicate positive MAR signal. Arrows indicate CFX197 FISH-positive filaments. Scale bars represent 10 μm .

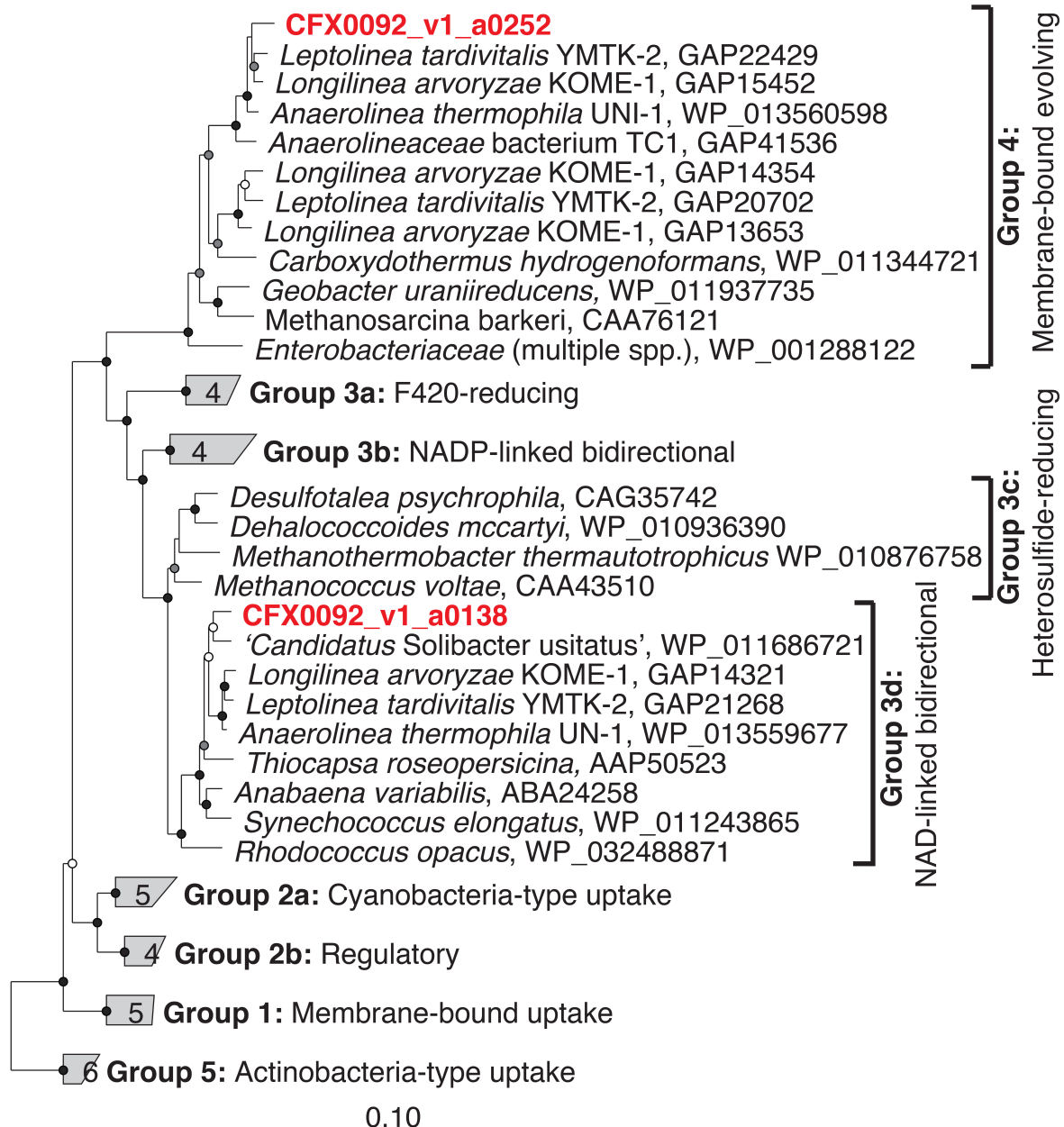


Figure S5. Phylogenetic affiliation of the annotated [NiFe]-hydrogenases of the Cfx-K genome (shown in red). Selected sequences were imported and aligned in the ARB software package using ClustalW and the phylogenetic tree constructed using the maximum-likelihood PHYML method with the Dayhoff amino acid substitution model. Reference sequences for the large hydrogenase sub-unit and classification is based on previous studies into hydrogenase phylogeny (Vignais and Billoud, 2007; Berney *et al.*, 2014; Koch *et al.*, 2014) with the inclusion of selected sequences related to the Cfx-K sequences (GAP22429, GAP15452, WP_013560598, GAP41536, GAP14354, GAP20702, GAP13653, WP_011686721, GAP14321, GAP21268 and WP_013559677). Bootstrap values from 100 resamplings are indicated for branches with >50% (white dot), 50-70% (grey) and >90% (black) support. The scale bar represents estimated sequence divergence.

Table S1. Summary of activated sludge biomass samples

Location	Date	Analyses
Kočevje WWTP, Slovenia	Sept. 24, 2012	Metagenome
Ejby Mølle WWTP, Denmark	Jan. 22, 2014	MAR-FISH
Randers WWTP, Denmark	Jan. 7, 2014	MAR-FISH
Aalborg West WWTP, Denmark	Mar.-Apr. 2015	Exoenzyme activities; MAC

All listed Danish plants are configured for biological nitrogen and phosphorus removal. The Kočevje is configured for nitrogen removal only.

Table S2. Genome assembly statistics

	Metagenome	Chloroflexi Bin	Cfx-K genome
Contigs (no.)	63,776	39	3
Length (Mbp)	181 395 850	5 091 158	5 033 966
GC content (%)	55	64	64
Read coverage* (K1.PE)	22	364	362/375/359**
Read coverage* (K2.PE)	14	160	162/148/249**
Essential genes (duplicates)	3744	113 (7)	113 (7)
rRNA operons	35	1	1

* The average read coverage of nucleotide positions in assemblies estimated by read mapping.

** The average read coverage for chromosome 1/chromosome 2/plasmid.

Table S3. COG classification summary table for the Cfx-K genome

Property	Replicon 1	Replicon 2	Replicon 3
D – Cell control, cell division, chromosome partitioning	1.3	1.3	-
M – Cell wall/membrane/envelope biogenesis	7.1	2.8	-
N – Cell motility	0.5	0.5	1.6
O – Posttranslational modification, protein turnover, chaperones	3.0	2.1	-
T – Signal transduction mechanisms	4.8	7.4	1.6
U – Intracellular trafficking, secretion, and vesicular transport	0.9	2.7	1.6
V – Defence mechanisms	1.8	2.5	-
W – Extracellular structures	>0.1	0.2	-
Z – Cytoskeleton	>0.1	-	-
A – RNA processing and modification	>0.1	-	-
B – Chromatin structure and dynamics	0.1	-	-
J – Translation, ribosomal structure and biogenesis	4.7	2.3	1.6
K – Transcription	5.4	7.5	1.6
L – Replication, recombination and repair	3.9	5.9	6.6
C – Energy production and conversion	6.0	2.3	-
E – Amino acid transport and metabolism	9.5	6.2	1.6
F – Nucleotide transport and metabolism	1.6	1.3	-
G – Carbohydrate transport and metabolism	7.1	6.4	1.6
H – Coenzyme transport and metabolism	2.5	1.0	-
I – Lipid transport and metabolism	3.1	1.7	-
P – Inorganic ion transport and metabolism	5.2	5.4	1.6
Q – Secondary metabolites biosynthesis, transport and catabolism	3.0	1.8	-
R – General function prediction only	15.4	16.6	1.6
S – Function unknown	7.6	8.1	3.3
Unclassified	25.9	35.9	82.0

Table S4. Summary of phenotypic characteristics of members of the classes Ardenticatenia, Anaerolineae and Caldilineae

Species	Isolation source	Temperature optimum	Physiology	Electron acceptor conditions	Carbon sources/electron donors**	Fermentation by-products (from sugars)	References
Class Ardenticatenia***							
<i>Ardenticatena maritima</i> ^T	Coastal hydrothermal field	Thermophile	Chemoheterotroph	<i>Facultative anaerobe:</i> O ₂ ; NO ₃ ⁻ (to NH ₃ or N ₂ *); Fe(III)	Carbohydrates; proteins; fatty acids ^w	-	(Kawaichi <i>et al.</i> , 2013, 2015)
Cfx-K (B45)	Activated sludge	Mesophile	Chemoheterotroph; Autotroph?; Fermenter	<i>Facultative anaerobe:</i> O ₂ ; NO ₂ ⁻ (to NH ₃ *); N ₂ O*	Carbohydrates	Acetate*; lactate*; ethanol*; acetoin*; H ₂ *	This study
Class Anaerolineae***							
<i>Bellilinea caldifistulae</i> ^T	Thermophilic anaerobic digester	Thermophile	Chemoheterotroph; Fermenter	<i>Obligate anaerobe</i>	Carbohydrates; proteins ^w	Acetate; lactate; formate; H ₂ ; propionate ^w ; pyruvate ^w	(Yamada <i>et al.</i> , 2007)
<i>Levilinea saccharolytica</i> ^T	Mesophilic anaerobic digester	Mesophile	Chemoheterotroph; Fermenter	<i>Obligate anaerobe</i>	Carbohydrates; proteins	Acetate; formate; H ₂ ; lactate ^w	(Yamada <i>et al.</i> , 2006)
<i>Longilinea arvoryzae</i> ^T	Rice paddy soil	Mesophile	Chemoheterotroph; Fermenter	<i>Obligate anaerobe</i>	Carbohydrates; proteins	Acetate; lactate; H ₂	(Yamada <i>et al.</i> , 2007)
<i>Leptolinea tardivitalis</i> ^T	Mesophilic anaerobic digester	Mesophile	Chemoheterotroph; Fermenter	<i>Obligate anaerobe</i>	Carbohydrates; proteins	Acetate; lactate; pyruvate; H ₂ ; succinate ^w ; formate ^w	(Yamada <i>et al.</i> , 2006)
<i>Pelolinea submarina</i> ^T	Marine sediment	Mesophile	Chemoheterotroph; Fermenter	<i>Obligate anaerobe</i>	Carbohydrates	Acetate; lactate; ethanol; H ₂ ; pyruvate ^w ; propionate ^w	(Imachi <i>et al.</i> , 2014)
<i>Ornatilinea apprima</i> ^T	Hot water bath microbial mat	Mesophile	Chemoheterotroph; Fermenter	<i>Obligate anaerobe</i>	Carbohydrates; proteins	Acetate; ethanol; H ₂ ; lactate ^w ; formate ^w	(Podosokorskaya <i>et al.</i> , 2013)
<i>Anaerolinea thermophila</i> ^T	Thermophilic anaerobic digester	Thermophile	Chemoheterotroph; Fermenter	<i>Obligate anaerobe</i>	Carbohydrates; proteins ^w	Acetate; H ₂ ; lactate ^w ; succinate ^w ; formate ^w	(Sekiguchi <i>et al.</i> , 2003)
<i>Anaerolinea thermolimos</i> ^T	Thermophilic anaerobic digester	Thermophile	Chemoheterotroph; Fermenter	<i>Obligate anaerobe</i>	Carbohydrates; proteins	Acetate; lactate; H ₂	(Yamada <i>et al.</i> , 2006)
<i>Thermomarinilineae lacunofontalis</i> ^T	Hydrothermal vent	Thermophile	Chemoheterotroph; Fermenter	<i>Obligate anaerobe</i>	Proteins	Not reported	(Nunoura <i>et al.</i> , 2013)
' <i>Thermanaerotherix daxensis</i> ' ^T	Deep hot aquifer	Thermophile	Chemoheterotroph; Fermenter	<i>Obligate anaerobe</i>	Carbohydrates	Lactate; acetate; CO ₂ ; H ₂ ^w	(Grégoire, Fardeau, <i>et al.</i> , 2011)
Class Caldilineae***							
<i>Caldilinea aerophila</i> ^T	Hot spring biomat	Thermophile	Chemoheterotroph; Fermenter	<i>Facultative anaerobe:</i> O ₂	Carbohydrates; proteins; fatty acids	Not reported	(Sekiguchi <i>et al.</i> , 2003)
<i>Caldilinea tarbellica</i> ^T	Deep hot aquifer	Thermophile	Chemoheterotroph; Fermenter	<i>Anaerobe</i> (tolerates <1.5% O ₂)	Carbohydrates; proteins	Succinate; lactate; acetate ^w ; CO ₂ ^w ; H ₂ ^w	(Grégoire, Bohli, <i>et al.</i> , 2011)
<i>Litorilinea aerophila</i> ^T	Intertidal hot spring	Thermophile	Chemoheterotroph	<i>Aerobe:</i> O ₂	Carbohydrates; proteins	-	(Kale <i>et al.</i> , 2013)

All listed species have a filamentous morphology; *Not empirically demonstrated; ** Proteins = protein based substrates rich in amino acids and peptides; *** Phylogeny based on SILVA version 119 (Quast *et al.*, 2013); ^wRelatively weak growth observed/trace amounts produced

Table S5: List of curated annotations for the Cfx-K genome

No.	Gene	Protein	EC No.	Chromosome 1	Chromosome 2
TCA cycle related					
1	<i>citA/Z</i>	Citrate (Si)-synthase	2.3.3.1	CFX0092_v1_a0240	-
2	<i>acnA</i>	Aconitate hydratase	4.2.1.3	CFX0092_v1_a0806	-
3	<i>acnB</i>	Bifunctional aconitate hydratase 2 and 2-methylisocitrate dehydratase	4.2.1.3, 4.2.1.99	-	-
4	<i>icd</i>	Isocitrate dehydrogenase (NADP(+))	1.1.1.42	CFX0092_v1_a1590	-
5	<i>ipd</i>	Dihydrolipoyl dehydrogenase	1.8.1.4	CFX0092_v1_a1697 CFX0092_v1_a0400	-
6	<i>sucA</i>	Oxoglutarate dehydrogenase (succinyl-transferring)	1.2.4.2	CFX0092_v1_a3633	-
7	<i>sucB</i>	Dihydrolipoyllysine-residue succinyltransferase	2.3.1.61	CFX0092_v1_a3632	-
8	<i>sucC</i>	Succinate--CoA ligase (ADP-forming), beta subunit	6.2.1.5	CFX0092_v1_a0656	-
9	<i>sucD</i>	Succinate--CoA ligase (ADP-forming), alpha subunit	6.2.1.5	CFX0092_v1_a0655	-
10	<i>korA</i>	2-oxoglutarate synthase, alpha sub-unit	1.2.7.3	CFX0092_v1_a1541 CFX0092_v1_a1544*	-
11	<i>korB</i>	2-oxoglutarate synthase, beta sub-unit	1.2.7.3	CFX0092_v1_a1542 CFX0092_v1_a1545*	-
12	<i>sdhA</i>	Succinate dehydrogenase/fumarate reductase, flavoprotein subunit	1.3.5.1	CFX0092_v1_a0554	-
13	<i>sdhB</i>	Succinate dehydrogenase/fumarate reductase, iron sulphur subunit	1.3.5.1	CFX0092_v1_a0555	-
14	<i>sdhC</i>	Succinate dehydrogenase/fumarate reductase, cytochrome B subunit	1.3.5.1	CFX0092_v1_a0552	-
15	<i>sdhD</i>	Succinate dehydrogenase, hydrophobic membrane anchor subunit	1.3.5.1	CFX0092_v1_a0553	-
16	<i>fumC</i>	Fumarate hydratase	4.2.1.2	CFX0092_v1_a1397	-
17	<i>mdh</i>	Malate dehydrogenase	1.1.1.37	CFX0092_v1_a1699	-
Glyoxylate shunt					
18	<i>aceA</i>	Isocitrate lyase	4.1.3.1	CFX0092_v1_a2006	-
19	<i>aceB</i>	Malate synthase	2.3.3.9	CFX0092_v1_a2005	-
Glycolysis/gluconeogenesis					
20	<i>ppc</i>	Phosphoenolpyruvate carboxykinase	4.1.1.31	CFX0092_v1_a1393	-
21	<i>pckA</i>	Phosphoenolpyruvate carboxykinase (ATP)	4.1.1.49	CFX0092_v1_a0089	-
22	<i>maeB</i>	Malate dehydrogenase	1.1.1.40	-	CFX0092_v1_b0357

23	<i>pdhA</i>	Pyruvate dehydrogenase, decarboxylase (E1) alpha sub-unit	1.2.4.1	CFX0092_v1_a0233	-
24	<i>pdhB</i>	Pyruvate dehydrogenase, decarboxylase (E1) alpha sub-unit	1.2.4.1	CFX0092_v1_a0232	-
25	<i>pdhC</i>	Dihydrolipoamide acetyltransferase (E2)	2.3.1.12	CFX0092_v1_a0231	-
26	<i>pdhD</i>	Dihydrolipoyl dehydrogenase	1.8.1.4	CFX0092_v1_a0400 CFX0092_v1_a1697	-
27	<i>porA</i>	Pyruvate:ferredoxin oxidoreductase, alpha subunit	1.2.7.1	CFX0092_v1_a1544*	-
28	<i>porB</i>	Pyruvate:ferredoxin oxidoreductase, beta subunit	1.2.7.1	CFX0092_v1_a1545*	-
29	<i>nifJ</i>	Pyruvate-flavodoxin oxidoreductase	1.2.7.-	CFX0092_v1_a0442	-
30	<i>glcP</i>	Glucose/mannose: H ⁺ symporter	-	CFX0092_v1_a1631*	-
31	<i>glk</i>	Glucokinase	2.7.1.2	CFX0092_v1_a1479	-
32	<i>ppgK</i>	Polyphosphate glucokinase	2.7.1.63	CFX0092_v1_a2623	-
33	<i>pgi</i>	Bifunctional glucose-6-phosphate isomerase/transaldolase	5.3.1.9, 2.2.1.2	CFX0092_v1_a3597	-
34	<i>pfkA</i>	6-phosphofructokinase	2.7.1.11	CFX0092_v1_a0941 CFX0092_v1_a0942 CFX0092_v1_a1389	-
35	<i>glpX</i>	Fructose-bisphosphatase	3.1.3.11	CFX0092_v1_a0722	CFX0092_v1_b0338
36	<i>fba</i>	Fructose-bisphosphate aldolase	4.1.2.13	-	CFX0092_v1_b0547
37	<i>tpiA</i>	Triose-phosphate isomerase	5.3.1.1	CFX0092_v1_a1277	-
38	<i>gapA</i>	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	CFX0092_v1_a0726	-
39	<i>pgk</i>	Phosphoglycerate kinase	2.7.2.3	CFX0092_v1_a1960	-
40	<i>gpm</i>	Phosphoglycerate mutase	5.4.2.12	CFX0092_v1_a0373 CFX0092_v1_a0535	CFX0092_v1_b0369*
41	<i>eno</i>	Phosphopyruvate hydratase	4.2.1.11	CFX0092_v1_a3618	-
42	<i>pyk</i>	Pyruvate kinase	2.7.1.40	CFX0092_v1_a1836	-
43	<i>pps</i>	Pyruvate, water dikinase	2.7.9.1	CFX0092_v1_a0441	-
Entner-Doudoroff pathway					
44	<i>edd</i>	Phosphogluconate dehydratase	4.2.1.12	-	-
45	<i>eda</i>	Multifunctional 2-keto-3-deoxygluconate 6-phosphate aldolase and 2-keto-4-hydroxyglutarate aldolase and oxaloacetate decarboxylase	4.1.2.14, 4.1.3.16	-	-
Fermentation					
46	<i>ldh</i>	Lactate dehydrogenase	1.1.1.27	CFX0092_v1_a0479	-
47	<i>eutE</i>	Aldehyde dehydrogenase	1.2.1.10	CFX0092_v1_a2131	-
48	<i>adh</i>	Alcohol dehydrogenase	1.1.1.1	CFX0092_v1_a0379*	-

					CFX0092_v1_a2782*	
49	<i>ilvH</i>	Acetolactate synthase, small sub-unit	2.2.1.6	CFX0092_v1_a0225	-	
50	<i>ilvI</i>	Acetolactate synthase, large sub-unit	2.2.1.6	CFX0092_v1_a0226	-	
51	<i>ald</i>	Acetolactate decarboxylase	4.1.1.5	CFX0092_v1_a2994	-	
52	<i>yfiQ</i>	Acetyl-transferase	6.2.1.13	-		CFX0092_v1_b0110 CFX0092_v1_b0132
53	-	Aldehyde dehydrogenase	1.2.1.3	CFX0092_v1_a0733* CFX0092_v1_a1380*		CFX0092_v1_b0470*
54	<i>aor</i>	Aldehyde ferredoxin oxidoreductase	1.2.7.5	CFX0092_v1_a2072	-	
55	<i>acsA</i>	Acetyl-coenzyme A synthetase	6.2.1.1	CFX0092_v1_a0396	-	
Pentose phosphate pathway						
56	<i>zwf</i>	Glucose-6-phosphate dehydrogenase	1.1.1.49	CFX0092_v1_a0169	-	
57	<i>pgl</i>	6-phosphogluconolactonase	3.1.1.31	CFX0092_v1_a1478	-	
58	<i>gnd</i>	Phosphogluconate dehydrogenase	1.1.1.44	CFX0092_v1_a0212	-	
59	<i>rpe</i>	Ribulose-phosphate isomerase	5.1.3.1	-		CFX0092_v1_b0337
60	<i>rpiA</i>	Ribose-5-phosphate isomerase	5.3.1.6	CFX0092_v1_a2219	-	
61	<i>rpiB</i>	Ribose-5-phosphate isomerase	5.3.1.6, 5.3.1.-	CFX0092_v1_a2137*		CFX0092_v1_b0773*
62	<i>tkt</i>	Transketolase	2.2.1.1	CFX0092_v1_a0909	-	
63	<i>tal</i>	Transaldolase	2.2.1.2	CFX0092_v1_a0910 CFX0092_v1_a3597	-	
Calvin cycle						
64	<i>cbbL</i>	Ribulose biophosphate carboxylase, large sub-unit	4.1.1.39	-		CFX0092_v1_b0544
65	<i>prk</i>	Phosphoribulokinase	2.7.1.19	-		CFX0092_v1_b0545
Glycogen metabolism						
66	<i>pgm</i>	Phosphoglucomutase	5.4.2.2	CFX0092_v1_a2722 CFX0092_v1_a0533* CFX0092_v1_a0683*		-
67	<i>glgC</i>	Glucose-1-phosphate adenylyltransferase	2.7.7.27	CFX0092_v1_a3183	-	
68	<i>glgA</i>	Starch (bacterial glycogen) synthase	2.4.1.21	CFX0092_v1_a3245	-	
69	<i>glgB</i>	1,4-alpha-glucan-branching enzyme	2.4.1.18	CFX0092_v1_a0318 CFX0092_v1_a1326*		-
70	<i>glgP,</i> <i>glgY</i>	Glycogen phosphorylase	2.4.1.1	CFX0092_v1_a1797 CFX0092_v1_a3214		-

71	<i>glgX</i>	Glycogen debranching enzyme	3.2.1.-	CFX0092_v1_a2220*	CFX0092_v1_b0489
72	<i>malZ</i>	Alpha-glucosidase	3.2.1.20	CFX0092_v1_a2594 CFX0092_v1_a0054* CFX0092_v1_a3350*	-
73	<i>malQ</i>	4-alpha-glucanotransferase	2.4.1.25	CFX0092_v1_a3243	-
Hydrogenase					
74	<i>echA</i>	Hydrogenase, membrane subunit (echA-like)	-	CFX0092_v1_a0257*	-
75	<i>echB</i>	Hydrogenase, membrane subunit (echB-like)	-	CFX0092_v1_a0251*	-
76	<i>echC</i>	Ni,Fe-hydrogenase III small subunit (echC-like)	-	CFX0092_v1_a0254*	-
77	<i>echE</i>	Ni,Fe-hydrogenase III, large subunit (echE-like)	1.12.7.2	CFX0092_v1_a0252*	-
78	<i>echF</i>	4Fe-4S ferredoxin, iron-sulphur binding (echF-like)	-	CFX0092_v1_a0250*	-
79	<i>hyfB</i>	Hydrogenase, membrane subunit (hyfB-like)	-	CFX0092_v1_a0256*	-
80	<i>hoxF</i>	NAD reducing hydrogenase sub-unit	1.12.1.2	CFX0092_v1_a0135	-
81	<i>hoxU</i>	NAD reducing hydrogenase sub-unit	1.12.1.2	CFX0092_v1_a0136	-
82	<i>hoxY</i>	NAD reducing hydrogenase sub-unit	1.12.1.2	CFX0092_v1_a0137	-
83	<i>hoxH</i>	NAD reducing hydrogenase sub-unit	1.12.1.2	CFX0092_v1_a0138	-
84	<i>hoxE</i>	NAD reducing hydrogenase sub-unit	1.12.1.2	CFX0092_v1_a0134	-
85	<i>focA</i>	Formate transporter	-	CFX0092_v1_a0448	-
86	<i>fdhD</i>	Formate dehydrogenase formation protein FdhD	-	CFX0092_v1_a2095	-
87	<i>fdnH</i>	Formate dehydrogenase, beta subunit	1.2.1.2	CFX0092_v1_a2096*	-
88	<i>fdhA/F</i>	Formate dehydrogenase, alpha subunit	1.2.1.2	CFX0092_v1_a0447 CFX0092_v1_a2092	-
Respiration					
89	<i>ctaD</i>	Cytochrome c oxidase subunit 1	1.9.3.1	CFX0092_v1_a3385	-
90	<i>ctaC</i>	Cytochrome c oxidase subunit 2	1.9.3.1	CFX0092_v1_a3384	-
91	<i>ctaE</i>	Cytochrome c oxidase subunit 3	1.9.3.1	CFX0092_v1_a3387	-
92	<i>ctaG</i>	Cytochrome c assembly factor	-	CFX0092_v1_a3388	-
93	<i>nuoA</i>	NADH-quinone oxidoreductase subunit A	1.6.5.3	CFX0092_v1_a1770 CFX0092_v1_a2674	-
94	<i>nuoB</i>	NADH-quinone oxidoreductase subunit B	1.6.5.3	CFX0092_v1_a1771	-
95	<i>nuoC</i>	NADH-quinone oxidoreductase subunit C	1.6.5.3	CFX0092_v1_a1773	-
96	<i>nuoD</i>	NADH-quinone oxidoreductase subunit D	1.6.5.3	CFX0092_v1_a1774	-

97	<i>nuoE</i>	NADH-quinone oxidoreductase subunit E	1.6.5.3	CFX0092_v1_a1775	-
98	<i>nuoF</i>	NADH-quinone oxidoreductase subunit F	1.6.5.3	CFX0092_v1_a1776	-
99	<i>nuoG</i>	NADH-quinone oxidoreductase subunit G	1.6.5.3	CFX0092_v1_a1778	-
100	<i>nuoH</i>	NADH-quinone oxidoreductase subunit H	1.6.5.3	CFX0092_v1_a1779 CFX0092_v1_a2675	-
101	<i>nuoI</i>	NADH-quinone oxidoreductase subunit I	1.6.5.3	CFX0092_v1_a1780	-
102	<i>nuoJ</i>	NADH-quinone oxidoreductase subunit J	1.6.5.3	CFX0092_v1_a1781 CFX0092_v1_a2676	-
103	<i>nuoK</i>	NADH-quinone oxidoreductase subunit K	1.6.5.3	CFX0092_v1_a1782 CFX0092_v1_a2677	-
104	<i>nuoL</i>	NADH-quinone oxidoreductase subunit L	1.6.5.3	CFX0092_v1_a1783 CFX0092_v1_a2678	-
105	<i>nuoM</i>	NADH-quinone oxidoreductase subunit M	1.6.5.3	CFX0092_v1_a1784 CFX0092_v1_a2679 CFX0092_v1_a2680	-
106	<i>nuoN</i>	NADH-quinone oxidoreductase subunit N	1.6.5.3	CFX0092_v1_a1795 CFX0092_v1_a2681	-
107	<i>atpB</i>	ATP synthase F0, subunit A	3.6.3.14	CFX0092_v1_a2683	-
108	<i>atpF</i>	ATP synthase F0, subunit B	3.6.3.14	CFX0092_v1_a2685	-
109	<i>atpE</i>	ATP synthase F0, subunit C	3.6.3.14	CFX0092_v1_a2684	-
110	<i>atpA</i>	ATP synthase F1, alpha subunit	3.6.3.14	CFX0092_v1_a1718	-
111	<i>atpD</i>	ATP synthase F1, beta subunit	3.6.3.14	CFX0092_v1_a1716	-
112	<i>atpG</i>	ATP synthase F1, gamma subunit	3.6.3.14	CFX0092_v1_a1717	-
113	<i>atpH</i>	ATP synthase F1, delta subunit	3.6.3.14	-	-
114	<i>atpC</i>	ATP synthase F1, epsilon subunit	3.6.3.14	CFX0092_v1_a1715	-
Nitrogen metabolism					
115	<i>nirfA</i>	Nitrite reductase (cytochrome; ammonia-forming)	1.7.2.2	CFX0092_v1_a1256	-
116	<i>nirfH</i>	Cytochrome c-type protein NrfH	-	CFX0092_v1_a1255	-
117	<i>nosZ</i>	Nitrous oxide reductase	1.7.2.4	CFX0092_v1_a0104	-
118	<i>nosD</i>	Nitrous oxide reductase family maturation protein	-	CFX0092_v1_a0102	-
119	<i>nosF</i>	ABC transporter ATP-binding protein nosF	-	CFX0092_v1_a0101	-
120	<i>nosY</i>	Nitrous oxide reductase maturation transmembrane protein	-	CFX0092_v1_a0100	-
121	<i>nosL</i>	Nitrous oxide reductase accessory protein	-	CFX0092_v1_a0099 CFX0092_v1_a0098	-

122	<i>amtB</i>	Ammonium transporter	-	CFX0092_v1_a1028	-
<i>Storage lipids</i>					
123	<i>aftA</i>	Diacylglycerol O-acyltransferase	2.3.1.20	CFX0092_v1_a0084 CFX0092_v1_a0211 CFX0092_v1_a2593	-
124	<i>phaC</i>	PHA synthase	2.3.1.-	-	-

* = Substantial evidence for an alternative gene assignment or low evidence.

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