

## GENOME SEQUENCING OF DIVERSE CHLOROFLEXI

Draft genome of *Leptolinea tardivitalis* YMTK-2, a mesophilic anaerobe from the Chloroflexi class *Anaerolineae*

Ward LM, Hemp J, Pace LA, Fischer WW. 2015. Draft genome sequence of *Leptolinea tardivitalis* YMTK-2, a mesophilic anaerobe from the Chloroflexi class *Anaerolineae*.

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**Abstract**

We present the draft genome of *Leptolinea tardivitalis* YMTK-2, a member of the Chloroflexi phylum. This organism was initially characterized as a strictly anaerobic non-motile fermenter; however, genome analysis demonstrates that it encodes for a flagella and might be capable of aerobic respiration.

**Genome announcement**

*Leptolinea tardivitalis* YMTK-2 was originally isolated from sludge granules of an upflow anaerobic sludge blanket (UASB) reactor used in wastewater processing (1). Closely related stains have been reported from other anaerobic wastewater treatment systems, soils (2), and aquatic moss pillars from Antarctica (3). *L. tardivitalis* is a filamentous, non-

sporulating organism that can ferment a number of sugars and fatty acids (1). It grows optimally at 37 °C (range 25-50 °C) and pH 7.0 (range pH 6.0-7.2).

The genome of *Leptolinea tardivitalis* YMTK-2 (DSM 16556) was sequenced as part of a project to expand the phylogenetic breadth of Chloroflexi genomes. Genome sequencing was performed at Seqmatic using the Illumina MiSeq sequencing platform. SPAdes 3.1.1 (4) was used to assemble the genome. The genome was screened for contaminants based on sequence coverage, GC composition, and BLAST hits of conserved single copy genes. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline. The draft genome is 3.74 Mb in size, assembled into 15 contigs. It encodes 3349 genes, 2939 CDSs, 1 16sRNA, 46 tRNAs, and 5 CRISPR arrays. It is estimated to be ~96% complete based on conserved single copy genes (107/111).

All *Anaerolineae* strains isolated to date have been classified as strictly anaerobic fermenters (1). However genome analysis suggests that *L. tardivitalis* has a richer physiology than previously recognized. It encodes for both Complex I (NADH dehydrogenase) and quinol *bd* oxidase (5), suggesting that it might be capable of microaerobic respiration. *L. tardivitalis* is missing genes for LPS biosynthesis and known outer membrane proteins, suggesting that it does not have an outer membrane (6). Furthermore *L. tardivitalis* encodes for gram-positive flagella, making it likely that it is motile under certain conditions.

**References:**

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Draft genome of *Bellilinea caldifistulae* reveals capacity for aerobic respiration and phototrophy in the *Chloroflexi* class *Anaerolineae*

**Abstract**

We report the draft genome of *Bellilinea caldifistulae*, a member of the *Anaerolineae* class of the bacterial phylum *Chloroflexi*. This genome contains genes for aerobic respiration and proteorhodopsin-based phototrophy that were not revealed by culture-based studies, and expands the known metabolic potential of *Anaerolineae*.

**Genome Announcement**

The bacterial phylum *Chloroflexi* (previously referred to as the Green Non-sulfur Bacteria) is characterized by filamentous organisms with gliding motility; its studied members are dominantly anoxygenic photoheterotrophs (1). The majority of cultured *Chloroflexi* belong to the class *Chloroflexi*, which includes many anoxygenic phototrophs and facultative aerobes. In contrast, the class *Anaerolineae* is less well characterized and only contains members described as anaerobic fermenters (1,2). But this group makes up more than 70% of all *Chloroflexi* sequences present in 16S gene datasets (3).

We report here the draft genome sequence for *Bellilinea caldifistulae*, isolated from a propionate-degrading consortium of thermophilic digester sludge in Japan, and described in pure culture (4). *B. caldifistulae* was characterized as obligately anaerobic, nonmotile

filamentous, and capable of growth on a range of carbohydrates when supplemented with yeast extract under circumneutral pH and temperatures between 45°C and 65°C (4).

The genome of *Bellilinea caldifistulae* was sequenced as part of a project to expand the phylogenetic breadth of Chloroflexi genomes and reconstruct their metabolic evolution. Genome sequencing was performed at Seqmatic using the Illumina MiSeq sequencing platform. SPAdes 3.1.1 (5) was used to assemble the genome. The genome was screened for contaminants based on sequence coverage, GC composition, and BLAST hits of conserved single copy genes. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline. The draft genome is 4.41 Mb in size, assembled into 45 contigs. It encodes 3846 genes, 3347 CDSs, 2 16sRNA, 50 tRNAs, and 3 CRISPR arrays. It is estimated to be ~96% complete based on conserved single copy genes (107/111).

Interestingly, analysis of the annotated *B. caldifistulae* genome revealed genes encoding for traits not observed in culture studies. This includes the capacity for aerobic respiration marked by a complete high-potential electron transport chain (using alternative complex III), and terminal cytochrome *c* oxidase (A-family). The genome also contains a *bd* oxidase. This study continues the first description of the capacity for aerobic respiration in *Anaerolineae* (6), a class previously defined by its obligate anaerobic lifestyle (3). The genome also revealed the capacity for proteorhodopsin-based phototrophy; however no genes associated with phototrophic chlorin-based reaction centers were observed. Furthermore the *B. caldifistulae* genome contains genes for flagellar motility and chemotaxis, in contrast to its description as nonmotile.

This draft genome helps expand the genomic coverage of *Anaerolineae*—an understudied class within the *Chloroflexi*. Additionally, it reveals the potential capacity for metabolic traits common in other *Chloroflexi* that were previously undiscovered in *Anaerolineae*, providing valuable new data for phylogenetic investigations into the evolution of high-potential metabolisms in this phylum.

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Draft genome of *Herpetosiphon geysericola* GC-42, a non-phototrophic member of the  
Chloroflexi class *Chloroflexia*

Ward LM, Hemp J, Pace LA, Fischer WW. 2015. Draft genome sequence of *Herpetosiphon geysericola* GC-42, a nonphototrophic member of the Chloroflexi class *Chloroflexia*.

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### **Abstract**

We report the draft genome of *Herpetosiphon geysericola* GC-42, a predatory non-phototrophic member of the class *Chloroflexia* in the Chloroflexi phylum. This genome provides insight into the evolution of phototrophy and aerobic respiration within the Chloroflexi.

### **Genome announcement**

The majority of cultured members of the bacterial phylum Chloroflexi belong to the class *Chloroflexia*, which are prominently anoxygenic phototrophs (1). However the most basal *Chloroflexia*, members of the orders *Herpetosiphonales* and *Kallotenuales*, are non-phototrophic (2). This makes these clades of central importance to understanding the evolution of phototrophy within the Chloroflexi. The *Herpetosiphonales* order currently contains only two species, *Herpetosiphon geysericola* and *Herpetosiphon aurantiacus*, which are pigmented filamentous organoheterotrophs that exhibit gliding motility (3). In culture *Herpetosiphon* strains are obligate aerobes that prefer microaerobic conditions (3). The physiology and ecology of these organisms is not well understood, but it has been



suggested that members of the *Herpetosiphon* genus might be capable of predation via a “wolf pack” strategy (4). *H. geysericola* was isolated from a biofilm at a hot spring in Baja California, Mexico (5).

The genome of *Herpetosiphon geysericola* GC-42 (DSM 7119) was sequenced as part of a project to expand the phylogenetic breadth of Chloroflexi genomes. Genome sequencing was performed at Seqmatic using the Illumina MiSeq sequencing platform. SPAdes 3.1.1 (6) was used to assemble the genome. The genome was screened for contaminants based on sequence coverage, GC composition, and BLAST hits of conserved single copy genes. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline. The draft genome is 6.24 Mb in size, assembled into 46 contigs. It encodes 5335 genes, 4688 CDSs, 2 16sRNA, 47 tRNAs, and 6 CRISPR arrays. It is estimated to be ~99% complete based on conserved single copy genes (111/111).

Analysis of the *H. geysericola* genome revealed genes for a branched aerobic respiratory chain including two different Complex I (NADH dehydrogenase), Complex II (succinate dehydrogenase), Complex III (cytochrome *bc* complex), an A-family heme-copper oxygen reductase, and a quinol *bd* oxidase. No genes for phototrophy were found. In addition no genes associated with the 3-hydroxypropionate cycle—a CO<sub>2</sub> fixation pathway present in photosynthetic members of Chloroflexi (7)—were found in *H. geysericola*; this is consistent with its predicted heterotrophic ecology.

The sequencing of *H. geysericola*, along with *Herpetosiphon aurantiacus* (4), completes the genomic knowledge of the cultured members of the genus *Herpetosiphon*. These data provide important constraints that help ordinate the acquisition of phototrophy, aerobic respiration, and carbon fixation within the Chloroflexi.

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Draft genome of *Levilinea saccharolytica* KIBI-1, a member of the Chloroflexi class

*Anaerolineae*

Hemp J, Ward LM, Pace LA, Fischer WW. 2015. Draft genome sequence of *Levilinea saccharolytica* KIBI-1, a member of the Chloroflexi class *Anaerolineae*. *Genome Announc* 3(6):e01357-15. DOI: 10.1128/genomeA.01357-15

**Abstract**

We report the draft genome of *Levilinea saccharolytica* KIBI-1, a facultative anaerobic member of the Chloroflexi class *Anaerolineae*. While *L. saccharolytica* was characterized as an obligate anaerobe, genome analysis provides evidence for the presence of both aerobic respiration and partial denitrification pathways.

**Genome Announcement**

*Levilinea saccharolytica* KIBI-1 was isolated from sludge granules of a mesophilic wastewater reactor (1). A closely related strain was detected in a trichlorobenzene-transforming microbial consortium (2). *L. saccharolytica* was characterized as an obligately anaerobic, non-motile filamentous microbe capable of growth on a range of carbohydrates when supplemented with yeast extract (1). It grows optimally at 37 °C and pH 7.0.

The genome of *Levilinea saccharolytica* KIBI-1 (DSM 16555) was sequenced as part of a project to expand the phylogenetic breadth of Chloroflexi genomes. Genome sequencing was performed at Seqmatic using the Illumina MiSeq sequencing platform. SPAdes 3.1.1

(3) was used to assemble the genome. The genome was screened for contaminants based on sequence coverage, GC composition, and BLAST hits of conserved single copy genes. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline. The draft genome is 4.30 Mb in size, assembled into 65 contigs. It encodes 3672 genes, 3173 CDSs, 1 16sRNA, and 46 tRNAs. It is estimated to be ~99% complete based on conserved single copy genes (110/111).

The *Anaerolineae* described to date have all been classified as strict anaerobes, however *L. saccharolytica* encodes for a branched aerobic respiration pathway. It has a Complex I (NADH dehydrogenase), Complex II (succinate dehydrogenase), an Alternative Complex III (ACIII) (4), and both an A-family heme-copper oxygen reductase and a *bd* oxidase. It also encodes for two nitrite reduction pathways; a NirS nitrite reductase that reduces nitrite into nitric oxide, and a NrfA protein that reduces it into ammonia. The presence of aerobic respiration genes in *L. saccharolytica* and other recently sequenced *Anaerolineae* suggests that this Chloroflexi class is substantially more physiologically diverse than previously recognized (5).

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Draft genome of *Thermanaerotherix daxensis* GNS-1, a thermophilic facultative anaerobe  
from the Chloroflexi class *Anaerolineae*

*Pace LA, Hemp J, Ward LM, Fischer WW. 2015. Draft genome of Thermanaerotherix  
daxensis GNS-1, a thermophilic facultative anaerobe from the Chloroflexi class  
Anaerolineae. Genome Announc 3(6):e01354-15. DOI: 10.1128/genomeA.01354-15*

### **Abstract**

We present the draft genome of *Thermanaerotherix daxensis* GNS-1, a thermophilic member of the Chloroflexi phylum. This organism was initially characterized as a non-motile strictly anaerobic fermenter; however, genome analysis demonstrates that it encodes genes for a flagellum and multiple pathways for aerobic and anaerobic respiration.

### **Genome Announcement**

*Thermanaerotherix daxensis* GNS-1 was isolated from a deep groundwater aquifer (149 m) housed within sedimentary strata of the large Mesozoic and Tertiary Aquitaine Basin in southwestern France (1). Closely related strains have been reported from a hot spring in Yellowstone National Park (2), a hot spring in southwestern Taiwan, geothermal soil, a thermophilic anaerobic digestive sludge, and a thermophilic electrochemical cell (3). *T. daxensis* is a filamentous, non-sporulating organism that can ferment a number of sugars and organic acids (1). It grows optimally at 65 °C (range 50-73 °C) and pH 7 (range pH 5.8-8.5) (1).

The genome of *Thermanaerotherix daxensis* GNS-1 (DSM 23592) was sequenced as part of a project to expand the phylogenetic breadth of Chloroflexi genomes. Genome sequencing was performed at Seqmatic using the Illumina MiSeq sequencing platform. SPAdes 3.1.1 (4) was used to assemble the genome. The genome was screened for contaminants based on sequence coverage, GC composition, and BLAST hits of conserved single copy genes. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline. The draft genome is 3.06 Mb in size, assembled into 6 contigs. It encodes 2798 genes, 2395 CDSs, 1 16sRNA, 47 tRNAs, and 4 CRISPR arrays. It is estimated to be ~95% complete based on conserved single copy genes (106/111).

Genome analysis of *T. daxensis* detected the presence of aerobic and anaerobic respiration pathways, hinting at a richer physiology than previously recognized. It encodes for Complex I (NADH dehydrogenase), Complex II (succinate dehydrogenase), and an aerobic CO dehydrogenase. It also has two aerobic respiration modules; an A-family heme-copper oxygen reductase coupled to an alternative complex III (ACIII) (5), and a quinol *bd* oxidase (6). In addition *T. daxensis* has two respiratory nitrite reductases; NirS, which reduces  $\text{NO}_2^-$  to NO, and NrfA that reduces  $\text{NO}_2^-$  to  $\text{NH}_4^+$ . The genome provides no evidence for the presence of LPS biosynthesis genes or outer membrane proteins, suggesting that this organism has only one membrane (7). Furthermore it encodes for a gram-positive flagella and is likely motile under certain physiological conditions.

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Draft genome of *Ornatilinea apprima* 3PM-1, an anaerobic member of the Chloroflexi class *Anaerolineae*

Hemp J, Ward LM, Pace LA, Fischer WW. 2015. Draft genome sequence of *Ornatilinea apprima* P3M-1, an anaerobic member of the Chloroflexi class *Anaerolineae*. *Genome Announc* 3(6):e01353-15. DOI: 10.1128/genomeA.01353-15

### **Abstract**

We report the draft genome of *Ornatilinea apprima* 3PM-1, a strictly anaerobic member of the Chloroflexi class *Anaerolineae*. This genome provides insight into the diversity of metabolism within the *Anaerolineae*, and the evolution of respiration within the Chloroflexi.

### **Genome Announcement**

*Ornatilinea apprima* 3PM-1 was isolated in Siberia from a microbial mat in a wooden bathtub sourced with water from a 2775 m well (1). Closely related strains have been reported from fresh water lakes and rice paddy soils. *O. apprima* was physiologically characterized as a filamentous, non-motile, obligately anaerobic organotroph. It can ferment a wide range of polypeptides and carbohydrates, including microcrystalline cellulose. It grows optimally at 42-45 °C and pH 7.5-8.0 (1).

The genome of *Ornatilinea apprima* 3PM-1 (DSM 23815) was sequenced as part of a project to expand the phylogenetic breadth of Chloroflexi genomes. Genome sequencing was performed at Seqmatic using the Illumina MiSeq sequencing platform. SPAdes 3.1.1

(2) was used to assemble the genome. The genome was screened for contaminants based on sequence coverage, GC composition, and BLAST hits of conserved single copy genes. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline. The draft genome is 4.41 Mb in size, assembled into 45 contigs. It encodes 3846 genes, 3347 CDSs, 2 16sRNA, 50 tRNAs, and 3 CRISPR arrays. It is estimated to be ~96% complete based on conserved single copy genes (107/111).

The majority of cultured Chloroflexi belong to the class *Chloroflexia*, which is composed of anoxygenic phototrophs and facultative aerobes (3). Even though > 70% of all Chloroflexi sequences present in 16S datasets belong to the *Anaerolineae* class (4), they are less well characterized. To date all described members are anaerobic fermentative organisms (5). Consistent with its description as an obligate anaerobe, *O. apprima* contains no genes for O<sub>2</sub> respiration. Even though it was classified as non-motile the genome does encode genes for flagella and chemotaxis, two traits not previously observed in *Anaerolineae*, suggesting that it is capable of motility. In addition no genes for LPS biosynthesis or outer-membrane proteins were found, which is consistent with the hypothesis that Chloroflexi have only one membrane (6).

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Draft genome of *Ardenticatena maritima* 110S, a thermophilic nitrate and iron-reducing member of the Chloroflexi class *Ardenticatenia*

Hemp J, Ward LM, Pace LA, Fischer WW. 2015. Draft genome sequence of *Ardenticatena maritima* 110S, a thermophilic nitrate- and iron-reducing member of the Chloroflexi class *Ardenticatenia*. *Genome Announc* 3(6):e01347-15. DOI: 10.1128/genomeA.01347-15

### **Abstract**

We report the draft genome of *Ardenticatena maritima* 110S, the first sequenced member of the *Ardenticatenia* class of the Chloroflexi phylum. This thermophilic organism is capable of a range of physiologies, including aerobic respiration and iron reduction. It also encodes a complete denitrification pathway with a novel nitric oxide reductase.

### **Genome Announcement**

*Ardenticatena maritima* 110S was originally isolated from an iron-rich coastal hydrothermal field in the Kirishima Volcanic Belt of Japan (1). Closely related strains have been reported from hot springs (2) and hydrothermal vents (3). *A. maritima* is a filamentous, non-motile organism that can facultatively reduce nitrate and iron (1). It grows optimally at 50-70 °C and pH 7.0 (range pH 5.5-8.0).

The genome of *Ardenticatena maritima* 110S (DSM 23922) was sequenced as part of a project to expand the phylogenetic breadth of Chloroflexi genomes. Genome sequencing was performed at Seqmatic using the Illumina MiSeq sequencing platform. SPAdes 3.1.1

(4) was used to assemble the genome. The genome was screened for contaminants based on sequence coverage, GC composition, and BLAST hits of conserved single copy genes. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline. The draft genome is 3.62 Mb in size, assembled into 12 contigs. It encodes 3041 genes, 2578 CDSs, 2 16sRNA, 46 tRNAs, and 10 CRISPR arrays. It is estimated to be ~99% complete based on conserved single copy genes (111/111).

Analysis of the *A. maritima* genome revealed the presence of many genes responsible for its physiological breadth. *A. maritima* encodes for a branched aerobic respiratory chain, including: Complex I (NADH dehydrogenase), Complex II (succinate dehydrogenase), Complex III (cytochrome *bc* complex) and three oxygen reductases (A and B-family heme-copper oxygen reductases and *bd* oxidase). B-family heme-copper oxygen reductases are commonly found in aerobic thermophiles, enabling growth with the low oxygen levels found in thermal systems (5). *A. maritima* also encodes a complete denitrification pathway composed of nitrate reductase (NapA), nitrite reductase (NirK), a novel nitric oxide reductase (eNOR) (6), and nitrous oxide reductase (NosZ). Interestingly the eNOR is found in an operon with NirK, suggesting that these genes are co-regulated. No genes were found for either LPS biosynthesis or outer membrane proteins, consistent with the proposal that Chloroflexi only have one membrane (7).

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