Isolation, characterization, and genome sequence of the first representative of a novel class within the Chloroflexi that is abundant in some US Great Basin hot springs and may play important roles in N and C cycling

Jeremy A. Dodsworth 1, Senthil K. Murugapirain 1, Jonathan Georgewick 1, James Han 2, Tanja Woyke 2, Susan M. Lucas 2, Sam Pitlack 2, Len Pennacchio 2, Lynne Goodwin 2, and Brian P. Hedlund 1

1University of Nevada-Las Vegas, Las Vegas, NV 89154
2US DOE Joint Genome Institute, Walnut Creek, CA, 94598

Abstract

A thermophilic, facultatively microaerophilic, heterotrophic bacterium, designated strain JAD2, was isolated from sediments of Great Boiling Spring (GBS), an 80°C, circumneutral hot spring in the US Great Basin (GB). The strain grew anaerobically on yeast extract or peptone with an optimal growth temperature of 70-75°C. Growth was stimulated by addition of 0.01 atm O2 to the culture vessel headspace, but was inhibited by higher concentrations (0.2 atm). Cells of JAD2 formed non-motile filaments ranging from 30 to 300 μm in length, which typically decreased in length during stationary phase. 16S rRNA gene-targeted pyrotag sequencing and clone library data suggest that close relatives of this isolate are prominent members of the sediment communities in GBS. Shotgun sequencing of the JAD2 genome produced an assembly consisting of 3.2 Gbp with an average G+C content of 67.9%. Phylogenetic analysis inferred from the 16S rRNA gene and predicted amino acid sequences of various conserved proteins indicate that JAD2 is the first cultivated representative of the GAL35 group, a new class within the Chloroflexi. Predicted genes in the draft genome encode a putative carbon monoxide dehydrogenase (comXKL), nitrite reductase (nirHA) and nitrous oxide reductase (nox2) suggest that this isolate may play important roles in N and C cycling in GBS sediments.

Introduction

Recent studies have shown that novel phylum- and class-level bacterial and archaeal lineages are abundant in various hot springs in the US Great Basin (GB) (Costa et al, 2009, Dodsworth et al, 2010; Dodsworth et al, 2011; Vick et al, 2010). Among these, Great Boiling Spring (GBS), a circumneutral, 80°C, hot spring, has been shown to have a robust biogeochemical nitrogen cycle (Doddsworth et al, 2011, Hedlund et al, 2011). Our overarching goal of our studies of hot springs in the GB and elsewhere is to understand the metabolic capabilities of microbial novel lineages and how they contribute to biogeochemical and energy cycling in these environments. Here we describe the isolation, initial characterization and draft genome sequence of strain JAD2, the first cultivated representative of the “GAL35” group (Hugenholtz phylogeny, Greengenes), which likely represents a new class within the phylum Chloroflexi.

Methods

Ultrafiltered spring water or a synthetic medium mimicking GBS water chemistry, supplemented with NaCl, NH4Cl, and a trace element mixture, was made anaerobic by sparging with N2,CO2 (80/20) for 1 hour, pretreated in an anaerobic chamber, and autoclaved. Solid medium was supplemented with gelrite (0.6%) and MGC (0.4%). Subsequent addition of a vitamin mixture, phosphate, and complex organics from sterile, anaerobic stocks were made by needle and syringe. Liquid cultures (5 ml) were grown in 18x150 mm tubes sealed with butyl rubber stoppers with 5 mm screw caps. For anaerobic cultivation, microaerobic 16S rRNA gene pyrotag sequencing and genomic sequencing (Illumina paired end, assembled using Velvet) were performed at the United States Department of Energy Joint Genome Institute (US DOE-JGI).

Isolation and characterization

- Isolated from GBS sediment using solid media designed to enrich for heterotrophic nitrate reducers (5 mM NO3 and complex organics, anaerobic conditions)
- Samples of colonies were observed by microscopy; those containing filaments were streaked for isolation (5x)
- Anaerobic growth on GBS spring water extract and/or peptone as C and energy sources, no added electron acceptor
- Growth stimulated by 0.01 atm O2, inhibited by 0.2 atm.
- Generation time of 1 day with yield of 10^10 filaments/ml.
- Pretreatment of butyl rubber stoppers by boiling in 1% Na2S5 was necessary for consistent growth in liquid culture.
- Filamentous morphology (Fig. 1); no obvious motility.
- Draft genome sequence is 3.2 Gbp in ~80 contigs with N50 of 139,873, GC-content of 67.9%.
- Growth on synthetic medium with added FeCl3; most other trace elements appeared to prevent growth in this synthetic medium.

Figure 1. Phase contrast microscope [400x] of a late log-phase culture of JAD2.

Figure 2. Close relatives of JAD2 in the GAL35 lineage (Fig. 4) are abundant in GBS sediments; DNA was extracted from sediment collected at various locations in GBS in Feb 2010 (corresponding to sites A, B, C, and E in Guy et al, 2011). 16S rRNA gene fragments from bacteria and archaea were amplified by PCR using primers 939F and 1392R and the products were sequenced at GB on a Roche 454 FLX pyrosequencer using Titanium chemistry. Resulting sequences were classified using PyroTagger (Kuxin and Hugenholtz, 2010). Members of the GAL35 group are prominent in 16S rRNA gene clone libraries in a variety of GB hot springs (Costa et al, 2009; Dodsworth et al, 2011; Vick et al, 2010).

Figure 3. Strain JAD2 is a member of the phylum Chloroflexi. The cladogram shows the phylogeny inferred from concatenated amino acid sequences of 31 housekeeping genes from taxa representing major phyla within the Bacteria (Woese and Fox, 2008). Strain JAD2 and other members of the Chloroflexi form a monophyletic lineage with high bootstrap support (100% of 500 replicates). Sequences were aligned using ClustalW and the tree was constructed and visualized using the MEGAS software package (http://www.megasoftware.net).

Phylogeny of strain JAD2 and the GAL35 group

Potential roles of JAD2 in N and C cycling in GBS

Figure 3. Strain JAD2 is a member of the phylum Chloroflexi. The cladogram shows the phylogeny inferred from concatenated amino acid sequences of 31 housekeeping genes from taxa representing major phyla within the Bacteria (Woese and Fox, 2008). Strain JAD2 and other members of the Chloroflexi form a monophyletic lineage with high bootstrap support (100% of 500 replicates). Sequences were aligned using ClustalW and the tree was constructed and visualized using the MEGAS software package (http://www.megasoftware.net).

Figure 4. Phylogenies of 16S rRNA genes demonstrate that JAD2 is the closest relative (40% identity) in the GAL35 group form a distinct clade within the phylum Chloroflexi. Phylogeny was inferred using distance matrix and neighbor joining methods (Felsenstein, 2004) with near-full-length sequences from named classes and class-level groups within the Chloroflexi (Hugenholtz et al, 2004), using F. coli, B. subtilis, and C. dyerophilum as outgroups. Named species or isolates are in bold. Regions where GAL35 clones were obtained are indicated. GB, Great Basin.

Summary and future directions

Strain JAD2 is a member of the GAL35 group, which likely represents a new class-level group within the phylum Chloroflexi (Figs. 1,4). Close relatives of JAD2 are abundant in GBS and other GB hot springs (Fig. 3) and have been found in terrestrial geothermal systems worldwide (Fig. 4), and may play important roles in N and C cycling in these environments (Figs. 5,6).

Further characterization of JAD2 will include:
- Full physiological characterization, formal proposal of class- through species-level taxonomic groups.
- Compare cell envelope structure to that of other members of the Chloroflexi and other bacterial phyla.
- Determine whether predicted pathways for CO oxidation, N2O reduction and nitrite reduction to ammonium are functional and linked to respiration, and their importance in GBS and other hot springs.
- Assess the physicochemical habitat and biogeography of the GAL35 lineage in hot springs globally by qPCR.

Figure 5. Loci in the JAD2 genome encoding a putative N2O reductase (A) and CO dehydrogenase (B). CysDE are elsewhere in the genome. Homologs of the NB-30 flavin nitrogen reductase (nirHA), catalyzing respiratory nitrogen ammoxinification in W. succinogenes, are also present.

Figure 6. Groups potentially catalyzing steps in the N cycle in GBS: ammonia (blue) and nitrite (green) oxidation, denitrification (red), and dissimilatory nitrate reduction to ammonium (orange). Based on JAD2 genome and other studies (Dodsworth et al, 2011; Hedlund et al, 2011).

Acknowledgments: The authors thank David and Sandy Harmon for access to GBS. This work was supported by NSF grants EPS-9979807, MCB-0548685, and OCE-1046611. Work conducted at GB was supported by the Office of Science of the US Department of Energy under contract No. DE-AC02-05CH11231.

References: