


Aug 9th, 10:15 AM - 12:00 PM

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

Jeremy A. Dodsworth
University of Nevada, Las Vegas

Senthil K. Murugapiran
University of Nevada, Las Vegas

Jonathan Gevorkian
Follow this and additional works at: https://digitalscholarship.unlv.edu/cs_urop

 Part of the [Biochemistry Commons](#), [Ecology and Evolutionary Biology Commons](#), and the [Genetics and Genomics Commons](#)
US DOE Joint Genome Institute

Tanja Woyke
Repository Citation *Joint Genome Institute*

Dodsworth, Jeremy A.; Murugapiran, Senthil K.; Gevorkian, Jonathan; Han, James; Woyke, Tanja; Lucas, Susan M.; Pitluck, Sam; Pennacchio, Len; Goodwin, Lynne; and Hedlund, Brian P., "Isolation, characterization, and genome sequence of the first representative of a novel class within the Chloroflexi that is abundant in some U.S. Great Basin hot springs and may play important roles in N and C cycling" (2011). *Undergraduate Research Opportunities Program (UROP)*. 8.
https://digitalscholarship.unlv.edu/cs_urop/2011/aug9/8

This Event is protected by copyright and/or related rights. It has been brought to you by Digital Scholarship@UNLV with permission from the rights-holder(s). You are free to use this Event in any way that is permitted by the copyright and related rights legislation that applies to your use. For other uses you need to obtain permission from the rights-holder(s) directly, unless additional rights are indicated by a Creative Commons license in the record and/or on the work itself.

This Event has been accepted for inclusion in Undergraduate Research Opportunities Program (UROP) by an authorized administrator of Digital Scholarship@UNLV. For more information, please contact digitalscholarship@unlv.edu.

Presenters

Jeremy A. Dodsworth, Senthil K. Murugapiran, Jonathan Gevorkian, James Han, Tanja Woyke, Susan M. Lucas, Sam Pitluck, Len Pennacchio, Lynne Goodwin, and Brian P. Hedlund

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. Dodsworth¹, Senthil K. Murugapiran¹, Jonathan Gevorkian¹, James Han², Tanja Woyke², Susan M. Lucas², Sam Pitluck², Len Pennacchio², Lynne Goodwin², and Brian P. Hedlund¹
Contact: brian.hedlund@unlv.edu; jeremy.dodsworth@unlv.edu

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 10 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 Mbp with an average G+C content of 67.3%. Phylogenies 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 encoding a putative carbon monoxide dehydrogenase (*coxMSL*), nitrite reductase (*nrfHA*) and nitrous oxide reductase (*nosZ*) 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 spring, has been shown to have a robust biogeochemical nitrogen (N) cycle (Dodsworth *et al.*, 2011; Hedlund *et al.*, 2011). One 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 (Hugenholz 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 NaCO₃, NH₄Cl, and a trace element mixture, was made anaerobic by sparging with N₂/CO₂ (80:20) for 1 hour, portioned in an anaerobic chamber, and autoclaved. Solid medium was supplemented with gelrite (0.8%) and MgCl₂ (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 1 atm overpressure of N₂/CO₂ in the headspace. Growth was monitored by phase contrast microscopy. 16S rRNA gene pyrotag sequencing and genome 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 medium with yeast extract and/or peptone as C and energy sources, no added e⁻ acceptor.
- Growth stimulated by 0.01 atm O₂, inhibited by 0.2 atm.
- Generation time of ~1 day with yield of 10⁶-10⁷ filaments/ml.
- Pretreatment of butyl rubber stoppers by boiling in 1% Na₂S was necessary for consistent growth in liquid culture.
- Filamentous morphology (Fig. 1); no obvious motility.
- Draft genome sequence is ~3.2 Mbp in ~80 contigs with N₅₀ of 139873, G+C content of 67.3%.
- Growth on synthetic medium with added FeCl₃; most other trace elements appeared to prevent growth in this synthetic medium.

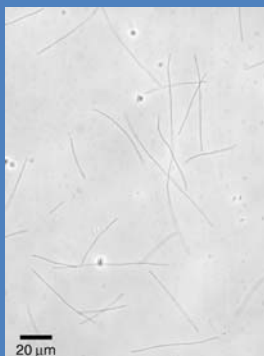


Figure 1. Phase contrast micrograph (400x) of a late log-phase culture of JAD2.

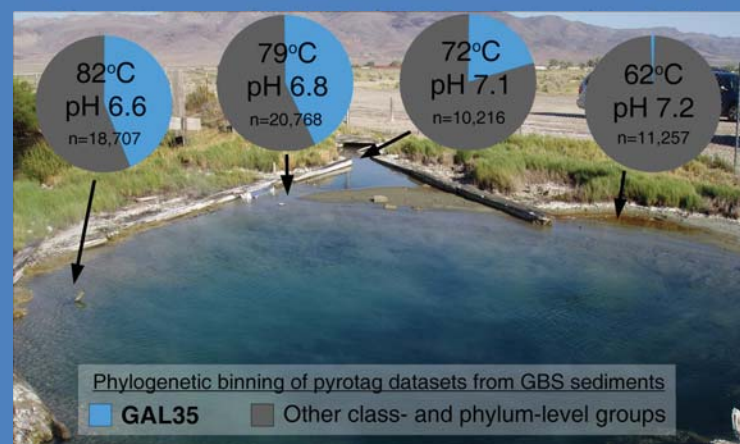


Figure 2. Close relatives of JAD2 in the GAL35 lineage (Fig. 4) are abundant in GBS sediments. DNA was extracted from sediment collected at various locations in GBS in Feb. 2010 (corresponding to sites A, B, C and E in Guy *et al.*, 2011). 16S rRNA gene fragments from bacteria and archaea were amplified by PCR using primers 926F and 1392R and the products were sequenced at JGI on a Roche 454 FLX pyrosequencer using Titanium chemistry. Resulting sequences (n) were classified using PyroTagger (Kunin and Hugenholz, 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).

Phylogeny of strain JAD2 and the GAL35 group

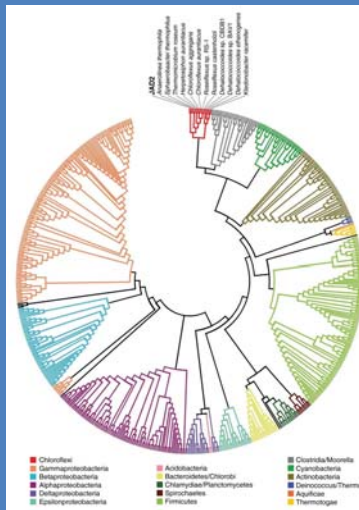


Figure 3. Strain JAD2 is a member of the phylum *Chloroflexi*. The cladogram shows the phylogeny inferred from concatenated amino acid sequences of 31 housekeeping genes from taxa representing major phyla within the Bacteria (Wu and Eisen, 2008). Strain JAD2 and other members of the *Chloroflexi* form 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 MEGA5 software package (<http://www.megasoftware.net>).

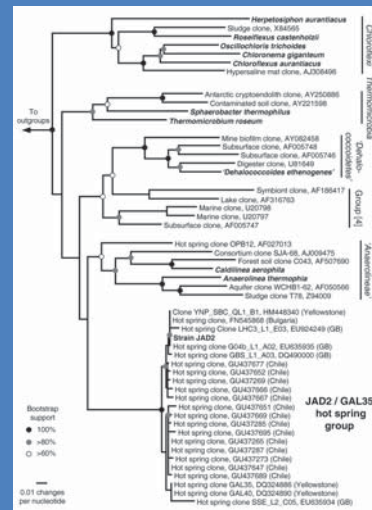


Figure 4. Phylogeny of 16S rRNA genes demonstrate that strain JAD2 and relatives (>90% identity) in the GAL35 group form a distinct clade within the phylum *Chloroflexi*. Phylogeny was inferred using distance matrix and neighbor joining methods (Felsenstein, 2004) with near-full-length sequences from named classes and class-level groups within the *Chloroflexi* (Hugenholz *et al.*, 2004), using *E. coli*, *B. subtilis*, and *C. diptheriae* as outgroups. Named species or isolates are in bold. Regions where GAL35 clones were obtained are indicated. GB, Great Basin.

Potential roles of JAD2 in N and C cycling in GBS

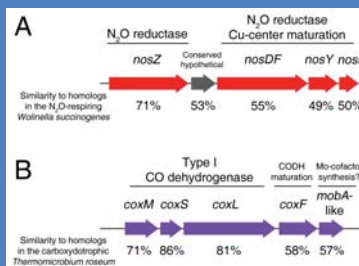


Figure 5. Loci in the JAD2 genome encoding a putative N₂O reductase (A) and CO dehydrogenase (B). *CoxD* are elsewhere in the genome. Homologs of the NH₄⁺-forming nitrite reductase (*nrfHA*), catalyzing respiratory nitrite ammonification in *W. succinogenes*, are also present.

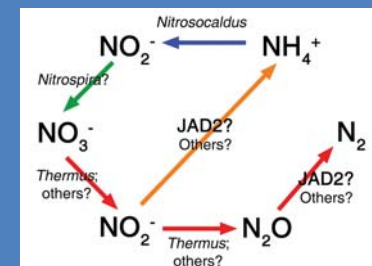


Figure 6. Groups potentially catalyzing steps in the N cycle in GBS: ammonia (blue) and nitrite (green) oxidation, denitrification (red), and dissimilatory nitrite reduction to ammonium (orange). Based on JAD2 genome and other studies (Dodsworth *et al.*, 2011; Hedlund *et al.*, 2011).

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. 3,4). Close relatives of JAD2 are abundant in GBS and other GB hot springs (Fig. 2) 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 proposed 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.

Acknowledgements: The authors thank Dave and Sandy Jamieson for access to GBS. This work was supported by NSF grants EPS-9977809, MCB-0546865, and OISE-0968421. Work conducted at JGI was supported by the Office of Science of the US Department of Energy under contract No. DE-AC02-05CH11231.

References
Costa, K.C., Navarro, J.B., Shock, E.L., Zhang, C.L., Soukup, D., and Hedlund, B.P. (2009) *Extremophiles* 13:447-59.
Dodsworth, J.A., and Hedlund, B.P. (2010) *Journal of Environmental Microbiology* 21(Sp):315-8.
Dodsworth, J.A., Hungate, B.A., and Hedlund, B.P. (2011) *Environmental Microbiology*, in press.
Felsenstein, J. (2004) PHYLIP version 3.6. Distributed by the author. Dept. of Genome Sciences, UW-Seattle.
Guy *et al.*, 2011, is in the current poster session; J. Peacock presenting.
Hedlund, B.P., McDonald, A.L., Lam, J., Dodsworth, J.A., Brown, J.R., and Hungate, B.A. (2011). *Geobiology*, in review.
Hugenholz, P., and Stackenbrandt, E. (2004) *ISME* 54:2049-51.
Kunin, Y., and Hugenholz, P. (2010) *The Open Journal* 1:1-8.
Vick, T.J., Dodsworth, J.A., Costa, K.C., Shock, E.L., and Hedlund, B.P. (2010) *Geobiology* 8:140-54.
Wu, M., and Eisen, J.A. (2008) *Genome Biology* 9:R151.