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Genomic foundations of carbon fixation in bacteria living in hot springs

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Rachel Skinner
Mentor - Brian Hedlund

Photosynthesis does not occur above 73°C, so organisms living above this temperature must obtain useable carbon by some other mechanism. It is generally assumed that carbon is fixed by thermophiles through the process of chemolithoautotrophy; however, primary production has never been demonstrated to occur in hot springs >73°C. We have shown that two organisms, Thermocrinis and Pyrobaculum, make up more than 90% of the cells in an 80°C Great Basin hot spring, Great Boiling Spring. We hypothesize that these organisms fix carbon in the hot spring via the reverse tricarboxylic acid (rTCA) cycle. To test this hypothesis we will: i) confirm that Thermocrinis and Pyrobaculum dominate in water from the spring; ii) determine whether key genes for the rTCA cycle, citryl co-A lyase (ccl), 2-oxoglutarate:ferredoxin oxidoreductase (korA), pyruvate:ferredoxin oxidoreductase (porA), are present and expressed in the spring; and iii) measure rates of carbon fixation in the spring. Linkage of the genetic data with carbon fixation rate data may help to provide an image of carbon fixation and cycling in Great Basin hot springs.

Genomic foundations of carbon fixation in bacteria living in hot springs

Rachel K. Skinner, Brian P. Hedlund, and Jeremy A. Dodsworth

Introduction

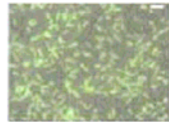

Figure 1. Epifluorescent micro-graph of organisms from GBS stained with SYBR Green.

Photosynthesis is not known to occur at temperatures above ~73°C, thus organisms living above these temperatures must obtain usable carbon by some other mechanism. It is generally assumed that organisms in these springs (Fig. 1) fix carbon by chemolithoautotrophy, but primary production has never been demonstrated in hot springs >73°C. Analysis of 16S ribosomal RNA (Fig.) 2 has shown a member of the *Aquificaceae* related to *Thermocrinis ruber* to be the dominant organism in a two-member microbial community in the bulk water of an 80°C Great Basin hot spring called Great Boiling Spring (GBS)(our unpublished data). We hypothesized that this organism plays an important part in the ecology of this spring by fixing carbon via the reductive TCA cycle (Fig. 3), as do other *Aquificaceae* including closely related *Hydrogenobacter thermophilus* (Shiba et al., 1985). To test this hypothesis, we attempted to amplify and sequence three genes necessary for the reductive TCA cycle and their transcriptional products.

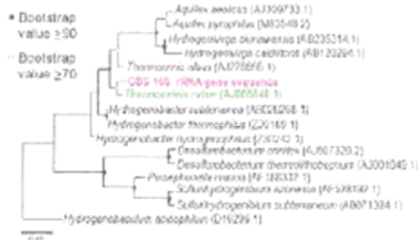

Figure 2. Phylogenetic tree created using 16S rRNA gene sequences and PHYLIP software maximum likelihood application. This analysis revealed the sequence from GBS (pink) to be closely related to *Thermocrinis ruber* (green).

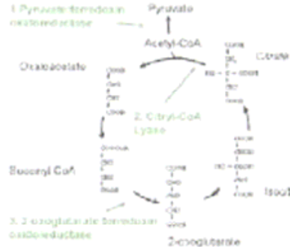

Figure 3. Reductive TCA Cycle. Three genes are distinctive to the reductive TCA cycle
1. Pyruvate: ferrodoxin oxidoreductase (*por*)
2. Citryl-CoA lyase (*ccl*)
3. 2-oxoglutarate: oxidoreductase (*kor*).
(Figure modified from Raymond, 2005)

Results

DNA Amplification

Figure 4. Gradient PCR of *ccl* gene. Gene products containing approximately 500 base pairs (highlighted in pink) were obtained by PCR. Lane 2 contains the positive control *T. ruber*. Lanes 5-8 contain appropriate gene products amplified from water

Figure 5. Gradient PCR of *korA* gene Positive control *T. ruber* is in lane 2 of both rows. The top row contains samples from GBS water; samples in the bottom row are from sediment. This gradient PCR produced products close to the expected size of 716 base pairs.

Figure 6. *T. ruber* (positive control) is shown in lane 2. A suspected *porA* clone is shown in lane 7. The product from *porA* primers was expected to be around 800 base pairs, but both *T. ruber* and environmental samples produced bands of 900-1000 base pairs. Sequencing of *porA* clones revealed that the gene had not been isolated.

Citryl-CoA Lyase Phylogeny

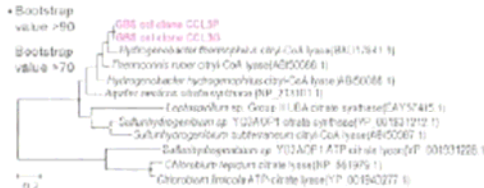

Figure 7. Phylogenetic tree of citryl-coA lyase clones and related genes. Clones of *ccl* sequences isolated from GBS water are shown in pink. Cloned *ccl* sequences were 80-87% similar to top BLASTx hit *T. ruber ccl*.

2-Oxoglutarate:ferredoxin oxidoreductase phylogeny

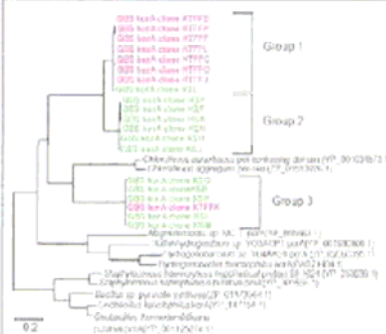

Figure 8. Phylogenetic tree of *korA* clones and related genes. *korA* sequences isolated from GBS water are shown in pink. *korA* sequences isolated from GBS sediment are shown in green. Cloned sequences fell into 3 distinct groups. *Bootstrap values were not significant (<70).* The putative *korA* gene isolated from *T. ruber* is currently being sequenced but is not yet available. Difference in similarity of Group 1 water clones top BLASTx hits and *Hydrogenobacter thermophilus korA* hit was 4 percentage points. Top BLAST hits for all sequences were pyruvate flavodoxin/ferredoxin oxidoreductase domain-containing protein from *Chloroflexus aurantiacus* (YP_001634573.1) or pyruvate flavodoxin/ferredoxin oxidoreductase-like from *Chloroflexus aggregans* (ZP_01513926.1) 2

Results

RNA Amplification

Figure 9. Amplification of probable citryl-CoA lyase transcripts from DNA isolated from GBS sediment by reverse transcriptase PCR (RT-PCR). Lane 2 contains the *ccl* gene from *T. ruber*. Lanes 2 and 3 contain cDNA synthesized from RNA isolated from GBS sediment. Lane 4 contains the negative control, which contained no reverse transcriptase during RT-PCR.

Discussion and Further Directions

Citryl Co-A Lyase (*ccl*)

Newly designed degenerate primers targeting the gene encoding citryl-CoA lyase were successful in amplifying the *ccl* gene from *Thermocrinus* inhabiting GBS (Fig. 4, 7). In addition, reverse transcriptase results (Fig. 9), suggest that this gene is being expressed, which would be consistent with the hypothesis that *Thermocrinis* fixes carbon via the rTCA cycle in GBS. The small discrepancy in size observed between the *T. ruber* DNA and the environmental DNA may be a result of highly degenerate primers or genetic variation in the ccl allele between *T. ruber* and the species found in GBS. Future sequencing of DNA amplified from cDNA is required to resolve this issue.

2-Oxoglutarate ferredoxin oxidoreductase (*korA*)

PCR with highly degenerate primers for the 2-oxoglutarate:ferredoxin oxidoreductase large subunit gene yielded amplicons of the expected size at a variety of annealing temperatures (Fig. 5). PCR products were cloned and sequenced, yielding three phylogenetic groups that generally corresponded to origin from sediment or water (Fig. 8). Group 3 may derive from relatives of *Chloroflexus* species, which use the 3-hydroxypropionate cycle for carbon fixation (Herter et al., 2002). Of note, novel *Chloroflexi* are the dominant clones in 16S rRNA gene clone libraries from GBS sediment (Costa et al., unpublished data). At this point, the exact sources and functions of the three genes amplified with these primers are uncertain. We will address whether any of these genes derive from *Thermocrinus* by cloning and sequencing the putative *korA* PCR product from *T. ruber*. Sequencing of this gene will also allow us to design more specific primers that may yield more accurate PCR products. In addition, future studies may attempt to isolate and sequence genes necessary for the 3-hydroxypropionate cycle.

Pyruvate:ferredoxin oxidoreductase (*porA*)

PCR primers designed to amplify *porA* amplified two products, the stronger of which was close to the expected size (data not shown). That product was cloned, however, the clone library was dominated by the smaller product. One clone that appeared to be the correct size (Fig. 6) was sequenced, yielding a putative tRNA gene. We will sequence more clones and, in addition, sequence the PCR product from *T. ruber* to determine whether the *porA* PCR primers amplify the intended gene.

Measurement of carbon fixation rate in GBS

Carbon fixation activity will be measured in GBS by measuring $^{14}CO_2$ incorporation. Correlation of carbon fixation data with genomic evidence of carbon fixation may help to provide a clearer idea of primary production in GBS.

Methods and Materials

Collection of Samples

Water and sediment samples were from Great Boiling Spring (also known as GBS) near Gerlach, Nevada and frozen immediately. Samples were stored at -80°C until use.

Extraction of DNA and RNA from hot spring water and sediment

DNA was extracted from sediment and water samples using the Qbiogene FastDNA® SPIN kit for soil. RNA was extracted from sediment and water using the Qbiogene FastRNA® Pro Soil-Direct Kit. DNA and RNA extracts were stored at -20°C.

Amplification and Cloning of DNA from sediment

Citryl-CoA lyase (ccl) was amplified by PCR using the newly designed primers ccl2F (5'-GAY CAY GGN ATH GCN CCN CC-3') and ccl2r (5'-GCD ATD ATH AAR AAN CCY TTN CC-3') at annealing temperature 55°C. The alpha subunit of 2-oxoglutarate ferredoxin oxidoreductase (korA) were amplified by PCR using the newly designed primers korA-F(5'-CCN GCN GAR ATH AAR GGN GG-3') and kor-R(5'-CCN GGN CCN WSN GTN GTN GTC AT-3') at annealing temperature 60°C. PCR primers for the alpha subunit of pyruvate ferredoxin oxidoreductase (porA) porA-F(5'-TAY CCN ATH CAN CCN GAR-3') and porA-R1(5'-GGC TTN GTC ATN CCN CC-3') or porA-R2(5'-AAA NGG NCK DAT NGG YTT-3') were used at annealing temperature 44°C or 30°C. PCR products were cloned with the Invitrogen TOPO TA Cloning Kit. Clones were sequenced by Functional Biosciences (Madison, WI) and analyzed using BLASTx from the National Center for Biotechnology Information.

Amplification of RNA from sediment

RNA extracts were treated prior to Reverse Transcriptase (RT) PCR with Promega RQ1 RNase-Free DNase following the manufacturer's instructions. RT-PCR was performed using Quanta Biosciences qScript cDNA Synthesis Kit according to manufacturer's directions. PCR of synthesized cDNA was performed using primers and conditions used in PCR amplification of hot spring DNA.

Construction of Phylogenetic Trees

Trees were constructed using the maximum likelihood application of PHYLIP software.

References

Raymond, Jason. The Evolution of Biological Carbon and Nitrogen Cycling—a Genomic Perspective. 2005. Reviews in Mineralogy and Geochemistry 59:211-231.
Shiba, Hirotaka, Toshiyuki Kawasumi, Yasuo Igarashi, Tohru Kodama, and Yasuji Minoda. 1985. The CO2 assimilation via the reductive tricarboxylic acid cycle in an obligately autotrophic, aerobic hydrogen-oxidizing bacterium, *Hydrogenobacter thermophilus*. Archives of Microbiology. 141:198-203.
Herter, Sylvia, Georg Fuchs, Adelbert Bacher, and Wolfgang Eisenreich. 2002. A Bicyclic Autotrophic CO2 Fixation Pathway in *Chloroflexus aurantiacus*. Journal of Biological Chemistry. 277:20277-20283.

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