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Genomic foundations of carbon fixation in bacteria living in hot springs

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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.
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Introduction

Figure 1. Phylogenetic position of organisms from GBS presented in the Bacteria domain. The placement of this group suggests that they are likely to be a novel branch of the Bacteria phylogenetic tree.

Figure 2. DNA Amplification.

Results

DNA Amplification

Figure 4. Gradient PCR of DNA fragments amplified in pairs from the 16S rRNA gene.

Figure 5. Gradient PCR of DNA fragments amplified in pairs from the 16S rRNA gene.

Figure 6. F. thermocyclus strain in a growth curve.

Figure 7. Phylogenetic tree of GBS strains, including the sequences obtained from the 16S rRNA gene.

Cytol-CoA Lyase

Figure 8. Phylogenetic tree of GBS strains, including the sequences obtained from the 16S rRNA gene.

2-Oxoprotutaric Ferredoxin oxidoreductase

RNA Amplification

Figure 8. Amplification of soluble RNA (S) type transcript from GBS strain isolated from the 16S rRNA gene.

Discussion and Further Directions

Cytol-CoA Lyase

Newly designed degenerate primers targeting the gene encoding cytol-CoA lyase were successful in amplifying the 16S rRNA gene from GBS. The amplified fragments were sequenced using high-throughput sequencing methods.

2-Oxoprotutaric Ferredoxin oxidoreductase

Pomarinokferredoxin oxidoreductase was present in the GBS strains, which was confirmed by sequencing the 16S rRNA gene.

Methods and Materials

Culture of bacteria

GBS were cultured on nutrient broth plates at 37°C.

Amplification and Sequencing from DNA

DNA was extracted from GBS strains using the FastDNA SPIN kit for soil. PCR was performed using degenerate primers specific for the 16S rRNA gene.

References


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