Diversity of Sulfur Reducing Prokaryotes Using the Dissimilatory Sulfite Reductase (DSR) Pathway in Complex Microbial Communities

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Abstract

The diversity of sulfur reducing microorganisms in the environment can give researchers a better understanding of biogeochemical changes that take place in a given area. Sulfur reduction or sulfur metabolism plays a vital role in the sulfur cycle. Monitoring the amount of sulfur reduction taking place in the environment can estimate the size and diversity of the microbial communities taking part. The Dissimilatory Sulfite Reductase Pathway (DSR) is used to reduce sulfur (S) to hydrogen sulfide (H₂S). To be able to estimate the amount of sulfur reduction in environmental samples DNA primers had to be designed to amplify the dsr A and B genes, which are both key factors in the DSR pathway. In order to test these primers, samples were obtained from the bottom layers of a Winogradsky column. The dsrA gene fragment was amplified and a clone library was created and sequenced. 16S primers were similarly used to look at the overall microbial population in the Winogradsky column, and determine the proportion of the population that was utilizing the dissimilatory sulfite reductase gene.

Introduction

The sulfur cycle is an important process in nature. Its importance lies in its diversity of use: e.g. mineralization of organic sulfur to sulfate, production of sulfide and sulfate compounds, reduction of sulfate to sulfide and the creation of essential amino acids, vitamins and hormones. The amount of sulfur readily available for living organisms in soil is significant especially for oceanic and freshwater ecosystems. Between the lithosphere and the ocean lies an anaerobic environment that is the home of many sulfate reducing bacteria. These bacteria can convert sulfate to sulfide, which can then be used by many other organisms. The study focuses on dissimilatory sulfate reduction in prokaryotes. Dissimilatory sulfate reduction is one of the most primitive pathways for energy production. It occurs in a variety of environmental stresses such as temperature, salinity, and acidity. It can provide a better understanding of how life may have survived and diversified on primitive earth.(1)

The dissimilatory sulfate reduction to hydrogen sulfide consists of three primary steps. The first step starts with activation of sulfate to adenosine-5-phosphosulfate by ATP sulfurylase at the expense of ATP. Next, adenosine-5-phosphosulfate is hydrolyzed and converted into sulfite and AMP by adenosine-5-phosphosulfate reductase, and the sulfite created is finally reduced to hydrogen sulfide by the dissimilatory sulfite reductase (dsr) in a one-electron transfer reaction.(1)

In the absence of oxygen this pathway allows organisms to use sulfur as a terminal electron acceptor for anaerobic respiration. This type of respiration is important for the survival of microbial communities found those found at the bottom layers of a Winogradsky column (figure 1).

Methods

Dissimilatory Sulfite Reductase/DSR Primers

Primers were created based on known sulfate reducers retrieved from JGI’s IMG database(2). The sequences were aligned and organized into these phylogenetic groups. For each group, different forward and reverse primer pairs were designed. Refer to table one below for list of primers.

DNA Extraction

Using a Midori Laboratories, Inc. Ultra Clean Soil DNA Isolation kit DNA was extracted from sediment samples collected from a Winogradsky column. A dsrA gene was used to isolate the samples and break the cells. Then the DNA was re-isolated in water.

PCR Amplification

The extracted DNA from the environmental samples was then confirmed for use in this experiment, by using primers designed to amplify 16S rDNA on each sample. In figure two is the top down thermocycling protocol used for amplification. The positive control used for each reaction was genomic DNA from Desulfovibrio vulgaris.

Microscopy and PCR

Dapi stain revealed a majority of rod and cocci shapes (figure 1A). Mainly Gram negative organisms were found after staining (figure 1B). In gel electrophoresis, faint bands with mild streaking were observed in lanes four and five of the dsrA gel (figure 2). Bright bands were observed in the 16s gel also in lanes four and five (figure 3).

Results

Diversity

10% of the 16s sequences obtained from the bottom sample were potential sulfur reducers (figure 4). Of the 16s sequences obtained from top sample 21% were potential sulfur reducers (figure 5). Over ninety percent of the dsr sequences were 5-proteobacteria and of that the main species found was Desulfovibrio ahrensenii (figure 6). dslab was found to represent a larger diversity of 16s genes in the bottom sample than in the top sample (figure 7).

Discussion

As bacteria oxidize the sulfur in the upper layers of the Winogradsky column they provide the sulfate needed for the reducing bacteria in lower layers. The samples from the bottom of the Winogradsky column proved to contain a high diversity of prokaryotes containing the dissimilatory sulfite reductase gene. Of these organisms many Gram negative rods and cocci were found, which is characteristic of dsr-containing prokaryotes. The lack of dsr sequence in top samples was proof that sulfate reduction was not taking place in the presence of high amounts of oxygen even though the percentage of phyla with 16s sequences that were potential sulfur reducers was higher in the top sample than in the bottom sample. Contrary to the BLAST diversity, the Shannon diversity of the top sample was higher than in the bottom sample than in the top sample. This relationship between the top and bottom layers of the Winogradsky column is a key factor to the diversity and survival of complex microbial communities.

References


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