The Diversity of Sulfur Oxidizing Bacteria Utilizing the Sox Pathway in a Winogradsky Column

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Abstract

Analyzing the diversity of microbial communities allows researchers the opportunity to gain insight into the biogeochemical processes taking place in a particular environment. Sulfur metabolism in microorganisms is one such process that can be monitored with molecular techniques. The diversity of sulfur oxidizing organisms can estimate the amount and type of sulfur oxidation occurring in a specific environment. The Sox pathway is used by sulfur oxidizing bacteria to oxidize thiosulfate (S_{2}O_{3}^{2-}) to sulfite (SO_{3}^{2-}), in order to oxidize thiosulfate to sulfite (4). This pathway is comprised of eight protein complexes encoded in fifteen genes (2). SoxI is the sulfite oxidoreductase of the Sox system (1). It is reported to have a similar structure to the Ni(II) reductase of E. coli (3). The SoxI gene has been suggested to be an ideal candidate for environmental studies in which sulfur oxidation is key (4).

Methods

SoxB Primer Design

Primers were designed using the primer analysis tool from JGI's IMG database of known sulfur oxidizing bacteria. The amplified sequences were aligned and organized into five phylogenetic groups. For each group, unique sets of primers were designed.

DNA Extraction

DNA was extracted from soil samples collected from the Winogradsky column using the MoBio Laboratories, Inc. Ultra Clean Soil DNA Isolation kit.

DNA Amplification

PCR amplification of ssob was performed using equimolar mixtures of each primer. The PCR product was sequenced using the Thermo polimerase chain reaction protocol for use with SoxB primers. A step-down protocol was designed in which the first ten cycles were performed with a higher annealing temperature than subsequent cycles.

Cloning

DNA was ligated into pCR-4TOPO vector and transformed into OneShot chemically competent E. coli cells using the Invitrogen TOPO cloning kit. Plasmid containing cells were selected for. DNA-Products were purified and sent to Polymorphic DNA Technologies Inc. for sequencing.

Phylogeny

SoxB sequences were chosen to best represent major groups of SOBs. OTUs were manually aligned using ClustalW. Reference sequences from GenBank were added and manually aligned to the soxB sequences. A maximum parsimony tree with bootstrap values was constructed using Mega.

Diversity Calculations

OTUs were formed using 97% similarity in Sequencher. The number of each OTU was estimated to calculate the Shannon diversity index with EstimateS 8.0.

Results

DNA Amplification

Design of the primers to amplify soxb gene sequences was successful. Mixtures of all primers were required to achieve bands on the gel. The desired band was very faint and could only be made out under UV light with the naked eye. Several undesirable bands also appeared.

Phylogeny

Although many of the species included in the tree gave a BLAST result as the same organism, this relationship was only partially retained. The majority of bootstrap values were under 50.

References