Direct PCR Detection, Cloning, and Characterization of Bacterial RubisCO Genes from New Jersey Soils

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Abstract

Ribulose-1,5-bisphosphate carboxylase/oxygenase, commonly known by the abbreviation **RubisCO**, is an enzyme involved in the first major step of carbon fixation, a process by which atmospheric carbon dioxide is converted by bacteria to energy-rich molecules such as glucose. Microbial DNA was extracted from temperate soils using the Zymo Microbe DNA MiniPrep protocol. RubisCo gene sequences were amplified by PCR using degenerate primers *cbbL*G1F and *cbbL*G1R. DNA fragments of approximately 800 base pair were detected in all positive soil samples. Clone libraries were constructed with the amplified DNA fragments by ligating the detected fragments with vector pCR®4-TOPO. Transformations were performed using competent Mix and Go *Escherichia coli* cells. Plasmids were isolated from each clone using the Zyppy Plasmid Miniprep and inserts were screened by PCR using M13 DNA primers. More than 100 clones were screened for the presence of RubisCo genes with 52 clones showing a positive reaction. DNA sequencing and BLAST analysis determined the identity of the cloned fragments. DNA sequencing of clone libraries showed that 88% of the sequences were related to Proteobacteria and 12% to Actinobacteria. The number one bacterial species detected were *Variovorax paradoxus* with 17% of clones. Other bacterial species detected were, *Bradyrhizobium elkanii, Pseudonocardia dioxanivorans, Rhodopseudomonas palustris,* and *Starkeya novella.*

Objectives

- To extract DNA from soil.
- To amplify bacterial RubisCO genes from extracted DNA using PCR.
- To clone the amplified genes and build libraries to screen and identify the inserted fragments.
- To sequence the individual clones to determine the gene structure and identity of bacterial RubisCO genes in soils.

Materials and Methods

DNA extractions

DNA was extracted from 300 mg of soils using the ZR Soil Microbe DNA MiniPrep protocol (Zymo Research, Irvine, CA)

PCR reactions

The reactions were targeting a fragment of approximately 800 base pair (bp) of the RubisCO gene using degenerate primers *cbbL*G1F and *cbbL*G1R. The reaction conditions were 95ºC for 4 minutes, and 32 cycles of 95ºC /1 min., 62ºC/1min., 72ºC/1min. with a final extension of 10 minutes at 72ºC. Ready-To-Go (RTG) PCR beads (GE Healthcare, Buckinghamshire, UK) were used for each PCR reaction.

Materials and Methods

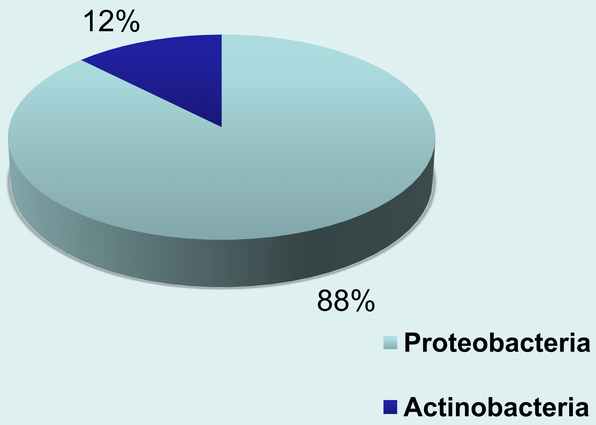
Cloning libraries

The DNA fragments from the PCR amplification of RubisCO genes were cloned using plasmid pCR®4-TOPO (Life Technologies, Thermo Fisher Scientific, Grand Island, NY) according to the manufacturer's instructions. Transformations were performed using Mix and Go Competent *E. coli* strains (Zymo Research, Irvine, CA). White colonies grown on Luria Bertani (LB) Agar with ampicillin (50 ug/ml) were transferred to LB broth containing ampicillin (50 ug/ml). Samples were incubated overnight at 37°C.

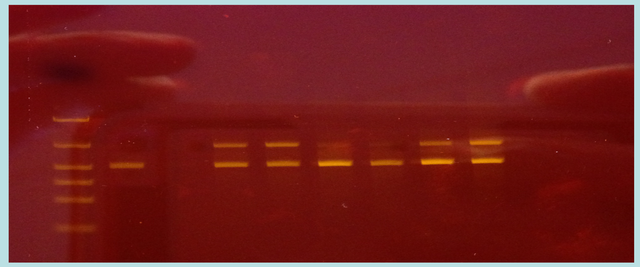
Plasmids were isolated from each clone using the Zyppy Plasmid Miniprep Kit (Zymo Research, Irvine, CA). Cloned inserts were reamplified using M13 DNA primers. DNA sequencing reactions of the amplified PCR fragments was performed by Genewiz, Inc. (South Plainfield, New Jersey). Homology searches were performed using the GenBank server of the National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the BLAST algorithm.

Results

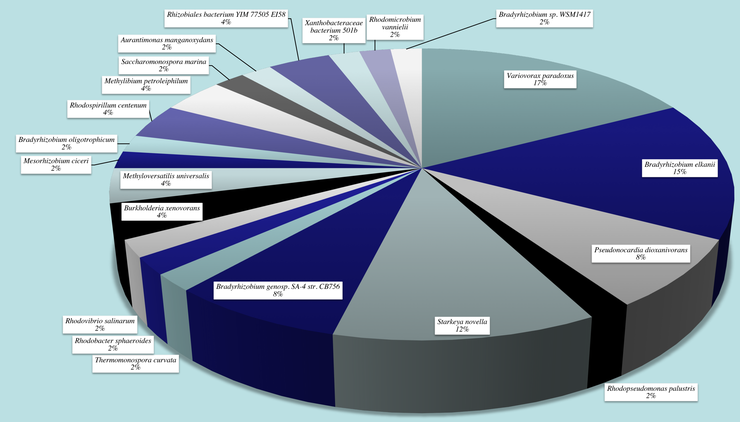
Bacterial phyla in Soil with RubisCO genes



PCR detection of bacterial RubisCO genes in soil



Bacterial species with RubisCO genes in clone libraries



Conclusions

- Analysis of clone libraries showed the Proteobacteria phylum with the highest numbers of RubisCO genes (88%).
- Other bacteria were found to belong to the phylum Actinobacteria (12%).
- The predominant Proteobacteria species with RubisCo genes were *Variovorax paradoxus*.

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