Direct PCR Detection, Cloning, and Characterization of 16S rRNA from Archaea Species in New Jersey Soils

Stephanie Perez, Arianna Pinto, Sibora Peca, and Luis Jimenez*
Department of Biology and Horticulture,
Bergen Community College, Paramus, New Jersey, USA

Abstract

The Domain Archaea wasn't recognized as a major domain of life until quite recently. The structure and diversity of Archaea in soils is poorly understood. DNA was extracted from Bergen Community College soils using the Zymo Microbe DNA MiniPrep protocol. Archaea gene sequences were amplified by PCR using degenerate primers A21F and A958R. DNA fragments of approximately 950 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 50 clones were screened for the presence of Archaea genes with 39 clones showing a positive reaction. DNA sequencing and BLAST analysis determined the identity of the cloned fragments. DNA sequencing of clone libraries showed that 72% of sequences were unknown Archaea sequences, 15% *Thaumarchaeota,* and 13% *Crenarchaeota*. Phylogenetic analyses of clones showed some similaritites with the genera *Nitrocosmicus* and *Nitrosophaera* that are associated with oxidation of ammonia in soils.

Objectives

- To amplify Archaea genes from extracted soil 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 identity of Archaea in Bergen Community College (BCC) soils and determine the phylogenetic relationships between clones and known Archaea Genera.

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 950 base pair (bp) of the 16S rRNA gene using degenerate primers A211F and A958R. The reaction conditions were 94ºC for 5 min, and 35 cycles of 94ºC /30 sec, 58ºC/45 sec, 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.

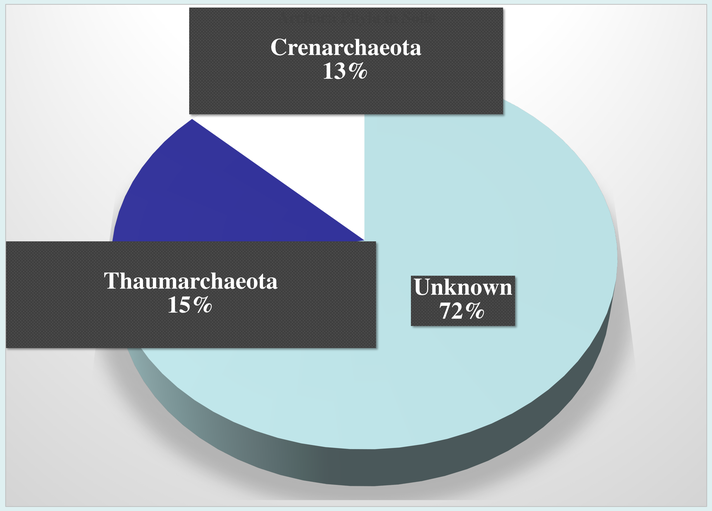
Materials and Methods

Cloning libraries

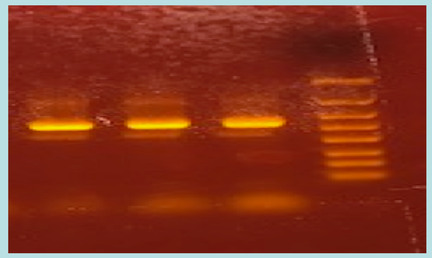
The DNA fragments from the PCR amplification of Archaea 16S RNA genes were cloned using plasmid pCR®4-TOPO (Life Technologies, Thermo Fisher Scientific, Grand Island, NY) according to the manufacturer's instructions. Transformations were performed using Mix and Go Competent *E. coli* strains (Zymo Research, 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. Phylogenetic analysis were performed using Clustal Omega from the European Bioinformatics Institute (EMBL-EBI) (https://www.ebi.ac.uk/Tools/msa/clustalo/)

Results

Archae phyla in BCC Soil

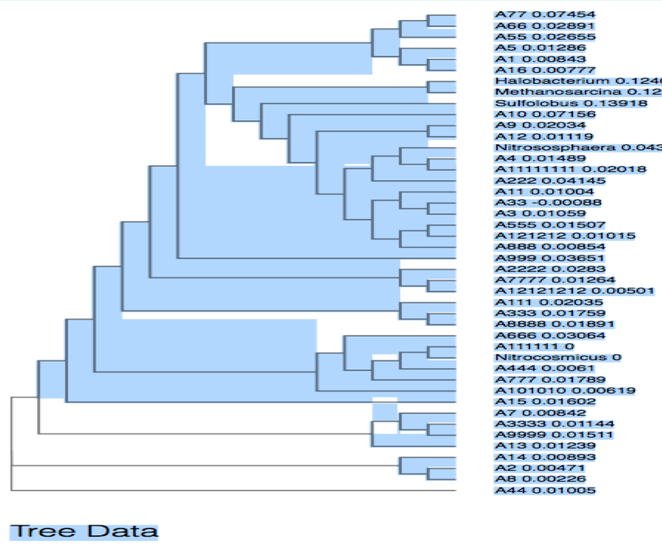


PCR detection of Archaea 16S rRNA genes in soil



←950 bp

Phylogenetic tree of Archaea clones in BCC soils



Conclusions

- Analysis of clone libraries showed that unknown Archaea 16S rRNA sequences accounted for 72% of the community in soils.
- *Thaumarchaeota* were the second most abundant sequences with 15% followed by the *Crenarchaeota* with 13%.
- Phylogenetic analyses revealed several genetic clusters related to known Archaea genera such as *Nitrocosmicus* and *Nitrososphaera* that are associated with the oxidation of ammonia in soils.

The contents of this poster were developed under a grant from the Department of Education. However, those contents do not necessarily represent the policy of the Department of Education, and you should not assume endorsement by the Federal Government.